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Nutritional phenotyping in COPD

by

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ABSTRACT

FACULTY OF MEDICINE

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NUTRITIONAL PHENOTYPING IN COPD

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Chronic obstructive pulmonary disease (COPD) is a collective term for a condition associated with irreversible airways obstruction causing breathlessness and risk of recurring chest infections, termed exacerbations. Malnutrition has been shown to play an important role in many chronic diseases, but in COPD the extent that differences in nutritional wellbeing determine the patient's risk of exacerbation, resilience to infection or response to treatment, has not been adequately addressed and so nutritional aspects of care have not been considered primary concerns in the management of the disease.

COPD exacerbations are assumed a major driver for the progression of COPD and of healthcare utilisation. Current respiratory variables measured in routine clinical practice are insufficient to identify patients at risk of exacerbation in the short- to medium-term and hence interventions to ameliorate exacerbation risk are limited. There is a real need to explore other non-respiratory markers for risk assessment in COPD patients. Whilst nutritional assessment has been established as an important tool in understanding risk of long-term outcomes, especially mortality, its role in assessing patient's risk of and response to exacerbations is less well understood. This thesis sought to explore the nutritional status in COPD patients with different disease presentations and clinical outcomes, in order to identify nutritional markers of exacerbation risk. Analysis focused on exploring the relative importance of disease history on nutritional status at baseline (how does previous disease impact on nutritional status), associations between disease status and nutritional status in stable COPD and relationship between nutritional status and clinical outcomes like time to first exacerbation (TTFE) and exacerbation rate over 12 month.

Systematic review of the literature demonstrated variability in methods used to assess structural and functional markers of lean mass of COPD patients, which limited the conclusions that could be made. To address this a comparison of methods used in COPD patients was devised and conducted, showing comparability of the results of these methods.

To explore the relationship between the nutritional status and clinical outcomes in COPD, a longitudinal, 12 months prospective cohort study, comprised of 127 COPD patients with a previous history of exacerbations was carried out. Nutritional status was assessed using markers

of body composition, appetite and functional capacity (grip strength, grip endurance, walk test) at baseline and quarterly basis. These nutritional markers were used to explore their relationship with past medical history, baseline disease markers (spirometry, inflammation) and clinical outcomes, with further assessment of their predictive value for clinical outcomes measured as TTFE.

In the studied cohort, 7 to 25% men and approximately 33% women had lean depletion (depending on applied criteria), and unexpectedly, low lean mass was not associated with a shorter time to first exacerbation. Those who had poor appetite were at higher risk of exacerbation in the following month (HR 1.6, p=0.023), which was independent of the exacerbations history and disease severity. Functional limitations including stopping during the 6-minute walk test (HR 1.90, p=0.001) and distance of less than 350m (HR 1.95, p=0.002) were related to TTFE. As none of the domains was sufficient to independently identify patients at high risk of exacerbation with sufficient accuracy in the next month, a multicomponent approach was taken by combining appetite score, body composition and walk test. Poor nutritional status marked by two or more components showed a higher risk of exacerbation, compared with those identified by any single component (HR 3.47 p<0.001). Use of history of exacerbation as additional component did not improve relevance to clinical outcomes and appetite score appeared to be of the greatest relevance to time to first exacerbation.

In summary, the findings of this thesis demonstrate that nutritional status is not determined by the history of exacerbations, yet relates to markers of disease status in stable COPD and clinical outcomes in the future. Multicomponent nutritional assessment showed potential to identify patients with high risk of exacerbation in the near future, which was not possible when using standard respiratory measures alone. This thesis emphasises the role of assessing nutritional status in COPD patients and the relevance and need for recognizing different nutritional phenotypes in the disease management.

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DECLARATION OF AUTHORSHIP

I, MALWINA MARIA NAGHIBI

declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

NUTRITIONAL PHENOTYPING IN COPD

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help[1] ;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. None of this work has been published before submission

Signed:

Date:

[1]AERIS study (ClinicalTrials.gov: NCT01360398) was a prospective, single centre, observational cohort study involving participants recruited from patient screening clinics, primary care, community services and outpatient departments and wards. The study was conducted in Southampton General Hospital between June 2011 and June 2014. The AERIS study was a large investigator-led industry-sponsored project, with Dr Tom Wilkinson as a Principal Investigator. The author of this thesis had an opportunity to join the research team and explore the nutritional component in the AERIS cohort. The AERIS study was conducted by a large research team consisting of respiratory consultants, medical doctors, research nurses, laboratory technicians, scientists and nutritionist (the author), who were responsible for different parts of the study delivery and study visits. For details, see Chapter 3.

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Firstly, I would like to express my sincere gratitude to my mentor, Dr Steve Wootton, for his support during my PhD and wider research training, for his patience, motivation, and boundless knowledge. His guidance has helped me through the last four years and I could not have wished for a better mentor.

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Definitions and Abbreviations

6MWT	6-minute walking test
6MWTui	6-minute walking test uninterrupted (no break)
6MWTs	6-minute walking test stopped during test (with break)
6MWW	6-minute walking work
AE	Acute exacerbation
AER	Acute exacerbation rate
AERIS	Acute exacerbation and respiratory infections in COPD Study
AS	Appetite score (based on CNAQ)
ATS	American Thoracic Society
BIA	Bioelectrical Impedance Analysis
BMI	Body mass index
BODE	Body mass index, airflow Obstruction, Dyspnoea and Exercise capacity score
CAT	COPD assessment tool (score 0 – 40)
CI	Confidence interval
CNAQ	Council on Nutrition Appetite Questionnaire (score 0 – 40)
COPD	Chronic obstructive pulmonary disease
CRP	c-reactive protein
D2O	Deuterium dilution
DXA	Dual-energy x-ray absorptiometry
ERS	European Respiratory Society
FEV_1	Forced expiratory volume in one second
FM	Fat mass
FMi	Fat mass index
FFM	Fat-free mass
FFMi	Fat-free mass index
FVC	Forced vital capacity
GOLD	Global initiative for chronic obstructive pulmonary disease
GE	Grip endurance
GS	Grip strength
HAE	History of acute exacerbations (12 months prior enrolment)
MUAC	Mid-upper arm circumference
MUAMA	Mid-upper arm muscle area
MUAMC	Mid-upper arm muscle circumference
MFAC	Mid forearm circumference
MID	Minimal important difference
NERS	Nutrition exacerbation risk score
NS	Not significant
Q1, Q2, Q3, Q4	Quartiles, where 1 is the lowest, 4 is the highest values
R	Pearson's Correlation for normally distributed data
Rho	Spearman's correlation coefficient for not normally distributed data
ROC	Receiver operating characteristic
SCBR	Southampton Centre for Biomedical Research
SD	Standard deviation
T1, T2, T3	Tertiles, where 1 is the lowest, 3 is the highest values
TLCO	Carbon monoxide transfer factor
TSF	Triceps skinfold
TTFE	Time to first exacerbation
TBW	Total body water
Wt	Weight
WHO	World Health Organisation

Poor appetite (low appetite score)	Appetite was assessed using a questionnaire with maximum 40 points score. Results lower or equal to 28 points were considered as a low appetite.
Poor physical capability	The physical capability was assessed using grip strength, grip endurance and 6-minute walk test. There are multiple criteria, which indicate 'poor' physical capability; therefore, depending on test and analysis, various thresholds were used, in all cases lower results were categorised as 'poor'.
Unfavourable body composition	Body composition was measured, but no criteria were used to define 'poor' body composition. Instead, unfavourable body composition was determined as an amount of lean, fat or proportion between the two compartments that predispose to worse outcomes.
Clinical outcomes	Many variables can be used as a clinical outcome, but for the purpose of this thesis, clinical outcomes were understood as a time to first exacerbation (TTFE) and acute exacerbation rate (AER).

Prologue

I walked into the clinical waiting area to see an elderly, good-looking man cheerfully smiling in my direction. A little overweight, he stood up energetically and shook my hand firmly as he introduced himself. We started to chat about the fine weather we were having. He was happy it was summer, as his COPD gets worse during the wintertime. Usually an active man, he was out last week playing golf with friends, and he takes his dog for long walks every day. He reassured me he never leaves home without his inhaler, but in the summer, he feels like COPD is not restricting him as much as it does in the winter months. From October onwards, he feels the need to minimise the higher risk of chest infections, by spending most of the week at home.

When the grandchildren go to school in September, he sees them less often, for when one gets a cold or a runny nose; it can trigger John's* chest infection. Regardless of his cautiousness, he had five infections last wintertime alone, and was hospitalised for a week. Hospitalisations worry his wife Mary*, who is in her 60's and still works, but they also cause practical problems for the family, as she helps with transporting the grandchildren to school. When he is in the hospital, she brings him his favourite dishes to entice him, but he feels bad that he cannot eat them and sometimes has only managed some toast and a glass of water all day. At that point, a research nurse arrived and invited him for a spirometry test. I watched him walk away, and at that moment, it was difficult to imagine him suffering from severe COPD in the winter months. This made me think of Annie*.

The previous week, I was called to the nutritional assessment of a COPD patient on a study. I walked into the clinical room to a patient who was very thin, sitting in her chair, breathing heavily. I could easily see her cheekbones, clavicles, and ribs under her top. Her name was Annie, and she was 67. Her measurements confirmed the obvious; her BMI was 18kg/m^2, and her handgrip was weak. Her family worried about her lack of appetite and persistent weight loss. She was more concerned about being unable to walk up a flight of stairs without stopping for a few puffs of an inhaler, than she was about her poor appetite and weight loss. She said she is one of those who eats to live, not lives to eat. She did not feel any worse than usual, and she told me she had been free from a chest infection for nearly a year. We scheduled a full dietetic review for her, and I left her with the research nurse.

John and Annie were so different; they had nothing in common except for poor spirometry results and a diagnosis of severe COPD. As a nutritionist, I could not miss the fact that they were at opposite ends of the body composition spectrum, yet paradoxically it was the overweight John who seemed to be doing better on an everyday basis. However, it was also John who experienced

exacerbations more often. I was aware there was more to it than merely weight and BMI. I had seen overweight COPD patients who were not doing as well as John. The question that had arisen in my mind was: “what is it about John that means he is able to cope better with the disease on a daily basis, yet his resilience to exacerbation appears to be lower than Annie’s?”.

**Names have been changed to maintain patients’ anonymity*

1 Introduction and background

Malnutrition has been shown to play an important role in many diseases but it has not been extensively studied in chronic obstructive pulmonary disease (COPD) in the context of disease mechanisms. Slow progress has been made in developing our understanding of the disease pathomechanism and identifying the triggers for exacerbations. There is a need to determine whether differences in nutritional state might contribute to the variance in the aetiology and pathophysiology of the disease and whether markers of nutritional state might help to identify patients at risk of exacerbation. Two obvious nutritional features are evident in clinical practice, the loss of weight, lean tissue in particular, and a loss of appetite, especially during exacerbations. This thesis sought to explore differences in nutritional status – markers of lean tissue (structural and functional) and appetite – and the extent to which differences between patients might be related to the lack of resilience to exacerbations seen in some but not all patients. As a background to the work, the following literature review has focused on the role of nutritional status in COPD, current evidence and the extent to which different markers of nutritional status might aid in differentiating between COPD phenotypes and serve as an existing measures in COPD management.

The background review of the existing literature will start by describing the condition and the important role played by exacerbations in determining the course of the disease process. Next, immunonutrition and role of nutritional status in disease processes will be considered, followed by review of methods of nutritional status assessment. This section will also reflect on the role of skeletal muscle in COPD and potential mechanisms involved in muscle wasting. The introduction will conclude with theoretical perspective on the role of nutrition in COPD and direction of those relationships.

1.1 Chronic Obstructive Pulmonary Disease

COPD is a complex disease and an umbrella term for various conditions affecting both the lung and other organs. The Global initiative for Chronic Obstructive Pulmonary Disease (GOLD) has published a general definition of COPD. COPD is ‘characterized by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases’ [3]. This generic definition reflects difficulties in determining the mechanisms leading to the disease development and progression. What is common for all COPD patients is lung tissue damage, which evolves over time. This begins with factors initiating the tissue damage (most commonly smoking), progression due to persistent

damage and insufficient or abnormal tissue repair, leading to failure in maintenance of tissue structure and function.

COPD is one of the most common diseases among adults, being one of the top five causes of mortality worldwide [4]. The WHO estimates that 210 million people are affected by COPD, but the disease is commonly underdiagnosed and so, underestimated [5]. Based on UK data from 2013 – 2016, British Lung Foundation reported that over 1.2 million people (2% of the whole population) live with diagnosed COPD, and the number of people with COPD diagnosis has increased by 27% over the last decade [6]. Based on 17 sites from two international studies, COPD prevalence (GOLD stage 2 or higher – see section 3.3.3.1, page 73, for explanation on differences in disease severity) ranged from approximately 2% to 17% in women, and from 4% to 22% in men, even when the same methodology and post-bronchodilator spirometry results were used [7]. Diagnosed cases constitute just a small proportion of the true prevalence. In 13 of 17 sites from previously mentioned studies, prevalence was higher in men than in women, except US, Austria, Australia and Iceland [7]. Evidence so far has not been sufficient to explain. There is some evidence suggesting a sex difference in clinical outcomes in COPD patients in that women generally do less well than men and have poorer clinical outcomes [8-10], which may be explained by differences in body composition, hormonal status and metabolism.

Mortality data in COPD should be analysed with caution due to the high likelihood of misclassification or omissions in medical records, as European Respiratory Society has raised [11]. COPD is the third leading cause of death in the UK with a trend showing a significant increase in mortality rate in the last 20 years[5]. The UK has the sixth highest death rate from lung and respiratory diseases in Europe (data from 2001-2010) [12] , with one person dying from the condition every 20 minutes (23000 deaths per year) [13, 14].

The costs of COPD management and loss due to lost working days carry a significant economic burden. The ERS highlighted that COPD is the leading cause of lost working days with annual costs of €28.5billion in EU. Moreover, COPD annually generates the cost of €2.9 billion due to inpatient care and €2.7 billion for pharmaceuticals [11]. According to the Department of Health COPD costs the NHS more than £800 million each year. A significant proportion of the high cost of managing COPD is driven by the management of acute exacerbations.

The increasing prevalence of COPD, high personal and economical burden coupled with poor clinical outcomes necessitates an improvement in the disease management and introduction of individualised therapy. Limited understanding of the pathophysiology restricts that, however some of the explored pathways of disease progression open potential for more successful treatment.

1.1.1 COPD pathophysiology

The diagnosis of COPD includes emphysema (parenchymal destruction) and chronic bronchitis (airway inflammatory disease) and small airways disease. It is predominantly characterised by persistent airflow limitation, which is not fully reversible but is both preventable and treatable [15, 16]. The main factors that increase the risk of developing COPD are tobacco smoking and environmental pollution. Occupational dust and chemicals, childhood lung illness and recurrent bronchopulmonary infections as well as a genetic factor, deficiency of α1-antitrypsin, also predispose to COPD [15-17].

The process of lung tissue damage induced by cigarette smoke progresses due to multifaceted interactions among oxidative stress, inflammation, extracellular matrix proteolysis, and apoptotic and autophagic cell death [18]. This process however varies significantly between patients. This variability in an individual's ability to maintain lung structure and promote repair, may explain why not all smokers develop COPD. This could also be one of the reasons for different clinical phenotypes among COPD patients.

Cigarette smoke activates nitric oxide synthase, causing increase in levels of oxidants, such as peroxynitrite (ONOO–), which has been linked to alveolar injury [19]. This however, appears to be genetically moderated to some extent. Mice lacking an RTP801 (a stress-induced mediator of apoptosis through enhanced oxidative stress) [20], exposed to cigarette smoke for 6 months, were protected against cigarette smoke–induced acute inflammation, apoptosis, or emphysema development [20].

Long-term exposure to cigarette smoke and amplification of destructive processes results in irreversible changes to lung structure and function, similar to exaggerated lung aging. In addition, there is some evidence suggesting that COPD can progress despite smoking cessation. There is no consensus on the mechanism by which cigarette smoke initiates changes in the lung, and various pathways have been described, alveolar cell apoptosis, along with oxidative stress and inflammation, were shown to be involved in self-amplifying loops resulting in tissue damage [21], even when smoke exposure was not present. There is some evidence suggesting an imbalance of pro- and anti-inflammatory cells in the airways of patients with COPD [22]. T helper 17 cells are responsible for maintaining mucosal barriers and contributing to pathogen clearance at mucosal surfaces. In COPD patients the T helper 17 cells had higher levels than T regulatory cells, which down regulate induction and proliferation of effector T cells [23]. This suggest a defect in anti-inflammatory homeostasis in COPD. There is also evidence suggesting accelerated cellular senescence in COPD patients, represented by shortening of telomere length, a biomarker of cellular aging [23].

Chronic inflammation has been shown to play a central role in the pathophysiology of COPD. It has been demonstrated thorough increased numbers of goblet cells, mucus gland hyperplasia, fibrosis, narrowing and reduction in the number of small airways and airway collapse and alveolar wall destruction in emphysema [24]. Oxidative stress and inflammation attract macrophages to the site. The activated macrophages release various inflammatory mediators and chemotactic factors (tumour necrosis factor-a, interleukin IL -6, IL -8, monocyte chemotactic peptide, leukotriene LTB4), as well as reactive oxygen species, and secrete proteolytic enzymes like MMP. In turn, all cytokines and chemokines attract and activate immune cells at the site of inflammation [25, 26].

These are only few of many pathways and potential pathomechanism, which are expected to play a role in development and progression of COPD. Historically, lower respiratory tract was considered sterile, however recent evidence suggest that both, healthy and diseased subjects, have diverse communities of bacteria [27]. Therefore, both baseline microbiota, and change in bacteria profile at infection, may play a significant role in the immune response and host defence in COPD.

1.1.2 COPD diagnosis

There are multiple factors that might contribute to the development of the disease, but the dominant feature of disease and its progression is limitation of lung function, which traditionally has been used for the diagnosis of COPD. Spirometry is used to confirm the diagnosis based on a percentage of predicted Forced Expiratory Volume in one second (FEV_1), Forced Vital Capacity (FVC) and FEV_1/FVC ratio. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines based the clinical diagnosis of COPD on dyspnoea, chronic cough or sputum production and/or exposure to risk factors in the past [28]. GOLD categories use FEV1 cut-off of >80% predicted (mild, GOLD 1), ≥50% predicted (moderate, GOLD 2), ≥30% predicted (severe, GOLD 3) and <30% predicted (very severe, GOLD 4). The American Thoracic Society (ATS) and European Respiratory Society (ERS) introduced a classification system different to GOLD (additional category of moderately severe), but both definitions focus only on lung function. In practice, in Europe various criteria are used, which was recently summarised by Miravitlles et al. [29].

For decades, lung function was used as a marker of COPD severity, but over the years, it has become increasingly apparent that FEV_1, even though relatively easy to obtain in clinical practice with good reliability does not inform about underlying processes that determine disease activity [30, 31]. A single measure of lung function does not give any understanding of a patient's journey. At any given time in adulthood, FEV1 is determined by three major factors. First is the maximally

attained level of lung function in early adulthood, which is not immediately assessable in practice. Next is the onset of decline of lung function, which is very individual and depends on a patient's general health, lifestyle and environmental factors. Last, is the rate of decline of lung function, which varies between people, but also the same person may have periods of slower and faster decline. The rate of decline in lung function increases with age [32]. There is some evidence of FEV decline in COPD patients, but results vary a lot depending on the population [33, 34]. The limitations of using FEV_1 and FVC as the only diagnostic criteria for COPD have been highlighted, yet no other, more reliable and accurate measure was identified to diagnose COPD.

Like in many diseases, there is a need to differentiate between the severity of the disease and the disease activity [35]. There is a consensus that airflow limitation and dyspnoea level determine severity of the disease, but there is no consensus how disease activity should be measured. At any stage of COPD severity, patients can present with different disease activity. This can be understood as how 'active' the underlying pathomechanism are and as the rate at which the disease progresses. By some, rate of lung function decline could be a measure of COPD activity, but also frequency of exacerbation could mark the activity of COPD [35, 36].

Therefore, there is an urgent need to go beyond respiratory markers to diagnose, monitor and evaluate COPD progression, in a way that reflects the complexity of COPD and adequately differentiates between the disease severity and activity.

1.1.3 COPD and risk of exacerbations

The clinical course of COPD is frequently interrupted by periods of clinical instability, which are called exacerbations. Almost all COPD patients experience exacerbations, irrespective of the disease severity, however the frequency and severity of those episodes differs significantly between the individuals. Individual resilience to exacerbations is one of the factors determining disease progression, as detrimental effect of acute exacerbations (AE) on health and quality of life has been reported both by patients and clinicians [28, 37]. Therefore, knowing patient's lack of resilience or lack of predisposition to AE could help manage the disease and provide more individualised treatment.

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) defines an acute exacerbation of COPD as "an event in the natural course of the disease characterized by a change in a patient's baseline dyspnoea, cough, and/or sputum that is beyond normal day-to-day variations, is acute in onset, and may warrant a change in regular medication in a patient with underlying COPD" [16]. The definition of exacerbation does not reflect the pathomechanism that underlies the exacerbation or the variance in resilience to it. A patient exposed to an exacerbation trigger may

or may not respond with an exacerbation. Patients with high resilience will be able to activate protective mechanisms and manage the response without obvious changes in symptoms, i.e. not recognisable as an exacerbation. However, patients with a low resilience may not be able to minimise the effect of the response to the trigger and will respond with a number of symptoms identified as an acute exacerbation.

The large burden of AE is related to both short-term effects (e.g. emergency hospital admissions), long-term effects (subsequent readmissions, lung function decline), as well as increased mortality (Figure 1)[2]. Questions regarding mechanisms, triggers and prevention, are now of the great interest of clinicians, patients and scientists.

The patient with COPD generally recognises the AE as a deterioration in respiratory symptoms. None of the markers and grades in COPD currently used in clinical practice has the ability to predict AE frequency or severity. Currently, the best predictor of the risk of AE is the history of exacerbation [38]. It only indicates overall exacerbation risk and gives no indication of time to next exacerbation, which limits any preventative actions.

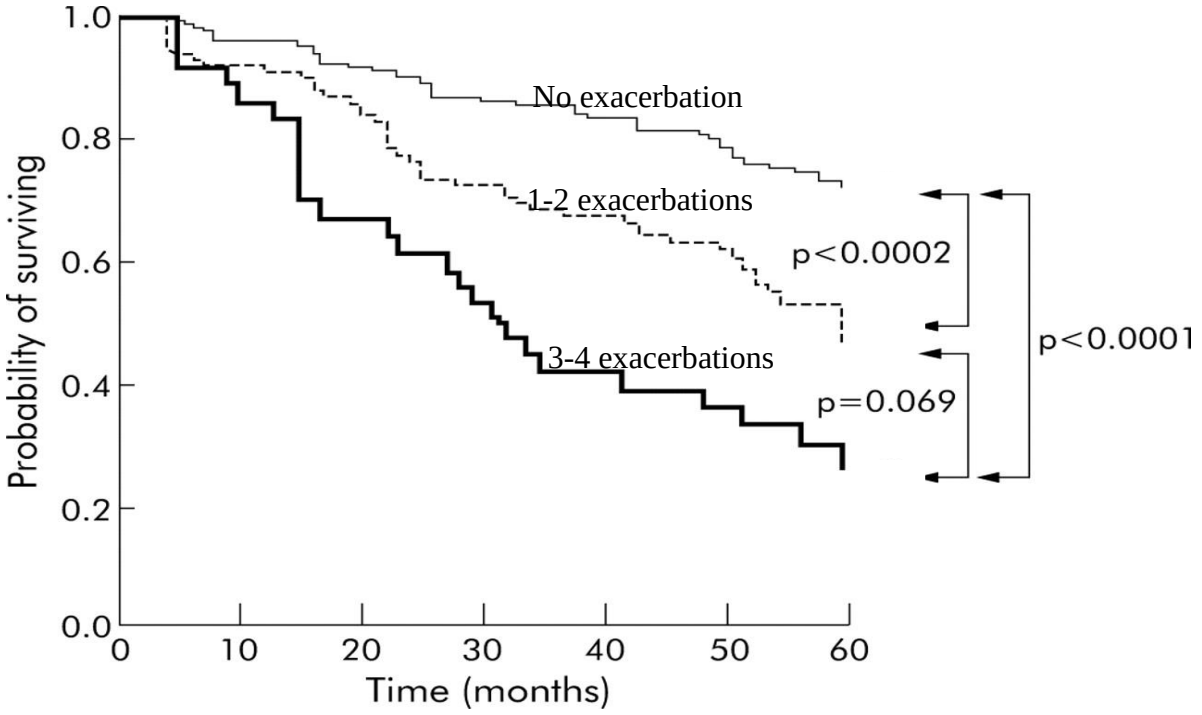


Figure 1 Probability of survival over 5 years for patients with different average exacerbation frequency at baseline, based on [2]

Exacerbations are caused or triggered by a variety of factors including viruses, bacteria, and air pollutants [39, 40], yet the most likely cause of an exacerbation is multifactorial. The biologic response to infection is increased inflammation seen in sputum, serum and peripheral eosinophilia [40, 41]. Changes in lung function prior to exacerbation were shown to be small [42], however, those with greater decline in FEV1 in time had higher airway inflammation (IL-8), which

was also seen in those with higher upper-airways bacteria load [41]. Various studies report that systemic inflammation is up-regulated during exacerbations, as marked by blood CRP [43, 44], IL-8 [45], TNF-a [43, 45](26), leptin [43] and IL-6 [43, 45].There was also evidence of local inflammation during an exacerbation, demonstrated by increased cytokine levels in sputum [46]. Therefore, considering bacterial and viral infection triggering majority of the exacerbations, the current approach to disease management introduces antibiotics and/or steroids to overcome infection and facilitate recovery from exacerbation.

Acute exacerbation of COPD have been shown to have a detrimental effect on patients' health, quality of life and disease progression, but AE is not a homogenous event. It varies in severity, recovery time, frequency and causes. The same set of symptoms can be perceived as an exacerbation by one individual, but not another. Even though there is a definition of AE, it must be remembered that defining the start and end date of AE in clinical practice is almost impossible. Regardless of the importance of AE, there is no mechanism to prevent all AE development. The AE are still unpredictable, there are no clearly defined causes or triggers of AE, all of which makes it difficult to protect patients from AE. There is an increase in AE prevalence in the cold season, which suggests seasonal predisposition in some patients [28, 47]. However, it is not certain, if AE seasonality is related to cold weather, decreased physical activity, change in diet (decreased intake of fresh fruits and vegetables), or higher exposure to viral and bacterial infections or, most likely, a combination of the above. However, predisposition to a larger number of exacerbations per year seems to be evident in some groups of patients. Frequent exacerbators would on average experience 2-3 exacerbations per year, but some patients may present significantly higher prevalence of exacerbations of over a dozen episodes per year [48].

Exacerbation however is not only a formal change of disease status, it is a life changing event for each patient, and includes a whole range of effects like poor appetite, low mood, feeling exhausted and need to remain home, most likely in bed. Therefore, ability to ameliorate effects that exacerbation has is not only to improve status of the disease markers, but more importantly, enabling patients to have control over their lives and ability to live a normal day-to-day life.

1.1.4 Exacerbation management

According to NICE guidelines, COPD patients should be encouraged to self-manage the exacerbation, by starting oral corticosteroid therapy if their increased breathlessness interferes with activities of daily living; and starting antibiotic therapy if sputum is purulent [49]. Most severe patients have "just in case" medication boxes at home. Recent findings show a positive effect of self-management on respiratory symptoms and hospitalisation within 12 months,

however, it is less overt when focusing on the quality of life and appears to have no effect on mortality [50]. Lack of long-term effect could be related to over- or under-treatment, the risk of developing an antibiotic resistance if medications are misused, which would also cause high management costs. To avoid that, Hull and East Yorkshire Hospitals NHS Trust explored potential for exacerbation self-management supported by timely clinical management in order to prevent avoidable hospital admissions [51]. Physiotherapy Acute Respiratory COPD service was introduced to deliver specialist physiotherapy to exacerbating COPD patients within their own home. The program was found to be beneficial and led to the conclusion that use of 'just in case' medication could be improved if guided in a timely fashion [51]. Such novel and more responsive approach could help in exacerbation management and ameliorate AE severity or length, yet lacks potential for AE prevention.

Exacerbations have a detrimental effect on COPD patients' life and health, but could also be seen as a window of opportunity to improve COPD patients' life. Exacerbations are seen as an event while could be considered as a three-stage process, a pre-exacerbation (pre-AE), the exacerbation (AE) and post-exacerbation (post-AE). Such approach could potentially aid in minimising the risk and the effect of an AE. Currently, there is no mechanism to identify a pre-AE period, no tests that would help to recognise those at risk of AE, or interventions that would decrease the risk of AE. Exacerbation is recognised at the stage of symptomatic AE, followed by a recovery period with gradual improvement of health. There is no evidence, but it seems plausible that systemic changes leading to AE do not appear overnight, but balance is slowly shifted until resistance to external stressor such as infection is lost, which is followed by sudden deterioration of health. There is an urgent need to identify the pre-AE period, which might offer an opportunity to intervene to limit the development of an AE. Nutritional status could potentially be helpful in identification of the pre-AE stage, as some of the nutritional changes may be likely to appear before the lung symptoms deterioration.

Considering the complexity of AE, heterogeneity of COPD patients and lack of mechanisms to predict and prevent AE, there is a need to find markers of pre-exacerbation, in order to improve COPD patients' experience. It should be considered that different disease phenotypes, e.g. emphysematous and bronchitic, could present with different pre-exacerbation symptoms, different timelines of exacerbation, as well as different underlying mechanisms, which would require different interventions to improve patients' experience.

1.1.5 COPD – the need to clinically phenotype

COPD is an umbrella term with wide reaching clinical manifestations. Patients with this diagnosis present with different symptoms, severity and disease progression. Given this heterogeneity within the COPD population, various classification of sub-typing have been proposed, including endotypes or phenotypes.

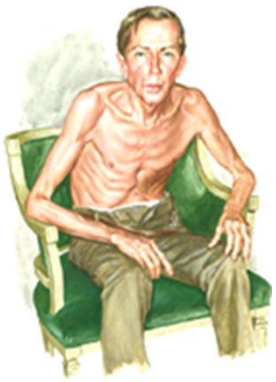
Endotypes are subtypes of a condition defined by a distinctive pathomechanism [52]. Treatments that target particular mechanism are likely to have a greater chance of achieving a better outcome in the patient group that share that mechanism. The challenge for COPD is that to date not many pathomechanisms have been identified, and hence this has not translated into endotype specific treatments [53].

Phenotypes are subtypes of a condition resulting from the end result of interaction between the patients' genes and the environment, leading to subtle or profoundly recognizable characteristics. The assumption is that patients who present with similar recognizable characteristics (symptoms and clinical outcomes) may also have a similar response to therapies [54], allowing for optimization.

In COPD, a phenotype could be based on a single or combination of disease attributes, with the focus being on clinically important outcomes such as exacerbations, response to therapy, rate of disease progression or death [54]. The challenge in COPD populations is that different phenotypes may overlap in the same patient or the same phenotype could result from different biological mechanisms. Therefore, phenotyping can aid clinical decision making, prognostication and inform both patients and clinicians regarding the disease process.

Historically, COPD patients were categorised into two broad phenotypes – the so-called 'pink puffer' and 'blue bloater' - based on symptoms and difference in patients' appearance [55]. As much as these historical phenotypes have limitations in recognising underlying disease pathology [56], the two phenotypes represent the extremes of the variance in nutritional status of COPD patients Table 1. Those with the more overtly emphysematous disease (historically classified as pink puffer) are more likely to be underweight, experience appetite loss and weight loss, and have worse outcomes [56], than those who are more overtly bronchitic (blue bloater), who typically present with a much higher weight and who may continue to remain stable between bouts of acute exacerbations [57].

Table 1 Difference in nutritional status between two COPD phenotypes - pink puffers and blue bloaters (graphics from www.medics4medics.com)



	Pink puffer	Blue bloater
BMI	Low	Normal or high
Visual malnutrition	Overt	Not visible
Lean mass	Low	Low or normal
Fat mass	Low	Normal or high
Oedema	Infrequent	Frequent
Exacerbations frequency	Low	High

Since this early dichotomisation of patients into either pink puffers or blue bloaters, various approaches to capture the differences in phenotypic heterogeneity in COPD [58] and differing COPD phenotypes have been defined using respiratory markers, clinical symptoms, CT, inflammation and comorbidities [54, 59, 60]. A systematic review of eight European and USA studies has shown two phenotypes to be common among COPD patients. The first phenotype consisted of young patients with severe respiratory disease, few cardiovascular co-morbidities, poor nutritional status and poor health status. A second common phenotype entailed older patients with moderate respiratory disease, obesity, cardiovascular and metabolic co-morbidities [60]. Han et al suggested that components of COPD phenotypes could be classified into several categories: clinical manifestations (age, sex, body composition, dyspnoea, depression), physiological manifestations (lung function, airway hyper responsiveness, hyperinflation), and radiologic manifestation (emphysema, airway wall thickening, bronchiectasis based on CT) [54]. Components like systemic inflammation, comorbidities and exacerbations frequency were considered to play central role in the phenotypes definition. Based on the review of 17 COPD cohorts, Miravitlles et al suggested more phenotypes based on recommended treatment [59]. This approach has led classification using three COPD phenotypes: mixed COPD-asthma phenotype; exacerbators; and emphysema-hyperinflation phenotype [59].

Recently, a Task Force of the European Respiratory Society conducted a comprehensive evaluation of the evidence on body composition considering fat loss, muscle loss, bone mineral density loss, adiposity, as well as nutrient deficiencies and potential for nutritional interventions in COPD [61]. This was a narrative literature review with agreed methodology but with no formal grading of the evidence (as opposed to a formal systematic review of current evidence) and only included the more recent randomised control trials and systematic reviews (in print between 2006 and 2013). Evidence from large epidemiological cohorts such as ECLIPSE [62] was not included. The ERS statement [61] has also highlighted the need to better understand the specific nutritional needs of the differing metabolic phenotypes with the development of targeted nutritional interventions.

The proposed metabolic phenotypes (Figure 2) reflect both ends of the spectrum of nutritional phenotypes (obese and malnourished as sarcopenic/cachectic) and were defined solely in relation to cardiovascular risk, impaired physical capacity and mortality risk (Table 2). Obesity was grouped into two categories, with impaired physical performance defining morbid obesity. Sarocpenia and sarcopenic obesity were based on the skeletal muscle index based on DXA Cachexia was separated into precachexia defined as unintentional weight loss independent of BMI or lean mass, while cachexia was determined based on unintentional weight loss and low lean mass. All phenotypes were determined focusing on increased mortality or cardiovascular risk, with no consideration of short-term outcomes such as the time to first exacerbation. The proposed cut-points of BMI, SMI, FFMi, weight loss used to define each phenotype category remain arbitrary but practically convenient.

Table 2 Metabolic phenotypes proposed by ERS Taskforce [61]

Metabolic phenotype	Definition	Clinical risk
Obesity	BMI 30-35 kg/m^2	Increased cardiovascular risk
Morbid obesity	BMI>35 kg/m^2	Increased cardiovascular risk Impaired physical performance
Sarcopenic obesity	BMI 30-35 kg/m^2 and SMI<2SD below mean of young M and F reference groups [63]	Increased cardiovascular risk Impaired physical performance
Sarcopenia	SMI<2SD below mean of young M and F reference groups	Increased mortality risk Impaired physical performance
Cachexia	Unintentional weight loss>5% in 6 months and FFMI<17 kg/m^2 (M) or <15 kg/m^2 (W)	Increased mortality risk Impaired physical performance
Precachexia	Unintentional weight loss>5% in 6 months	Increased mortality risk
SMI – appendicular skeletal muscle index; M – man; W – woman; FFMI – fat-free mass index		

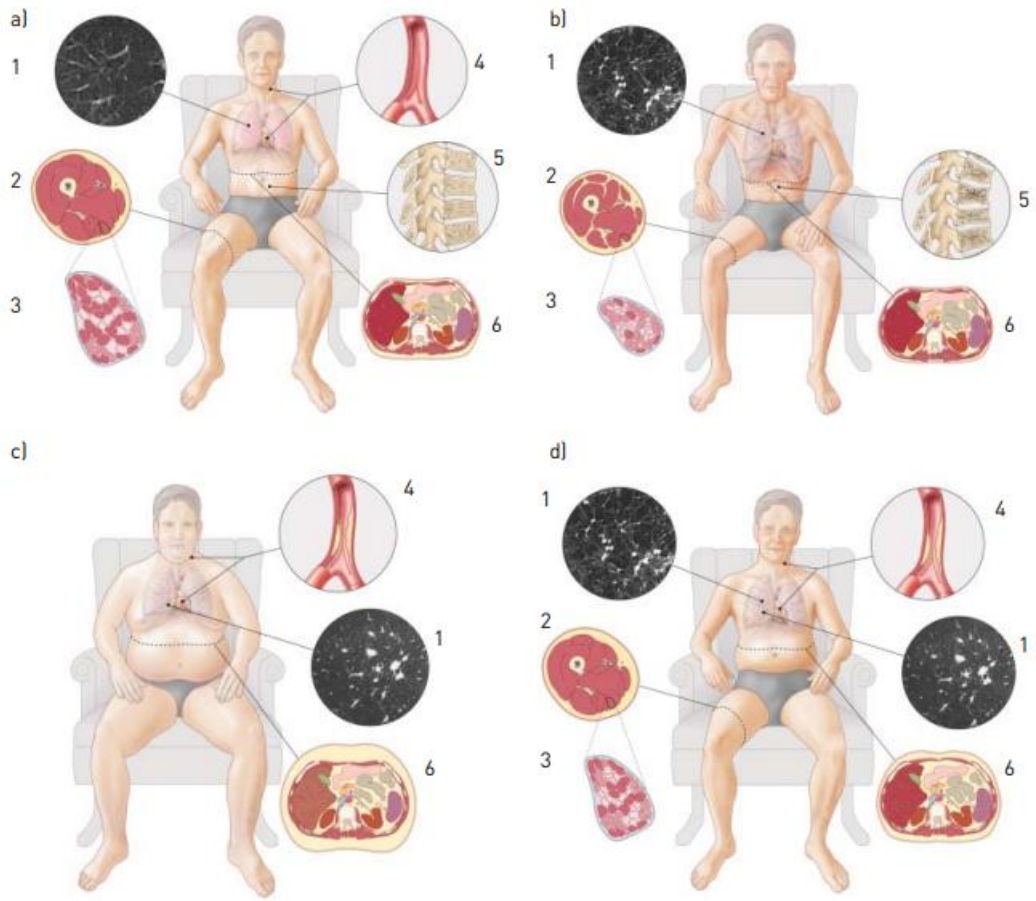


*Figure 2 Metabolic phenotypes in COPD according to ERS Task Force: a) healthy control, b) cachexia, c) obesity, d) sarcopenic obesity, with presentation of 1-lung CT, 2- quadriceps MRI, 3-muscle fibre, 4-arterial vessel, 5-bone tissue 6- abdomen MRI [61]] *figure c) is presented as in the original paper*

There is evidence suggesting that COPD patients who are malnourished have more co-morbidities [64], higher mortality [65, 66] or length of hospitalisation [67]. At the same time there are results suggesting that obese patients are more likely to be frequent exacerbators and therefore carry a higher risk of worse clinical outcomes [68]. Others have suggested that increased body weight has a protective effect against exacerbations [69]. Overall, this contradictory evidence strongly suggests that COPD population is very heterogeneous from a clinical and nutritional perspective, and potentially different disease management may be required for those with different disease phenotypes.

Independent of the approach taken to define COPD phenotypes, patients classified as ‘exacerbators’ or ‘frequent exacerbators’ appear to shape a separate COPD phenotype across different studies and reviews [38, 60, 68, 70]. The role of exacerbations in the disease progression has been shown in various COPD studies [38]. Patients with higher exacerbation frequency often had worse clinical outcomes and quality of life [28, 71]. However, most studies considering frequency of exacerbations did not consider the underlying cause for higher exacerbation

frequency. Frequent exacerbators could further comprise of two sub-phenotypes: those who respond rapidly to a minor exacerbation trigger (have low exacerbation resilience); or those who happen to have greater resilience, but persistent exposure to exacerbation triggers. Resilience to exacerbation would probably be aligned with other components of various COPD phenotypes, like inflammation or nutritional status measured with body composition. Depending on the phenotypes, the same trigger could or could not result in exacerbation and amongst those who respond with exacerbation, the same trigger could potentially cause exacerbations of different severity.

1.1.6 Systemic conditions, multicomponent markers and multidisciplinary approaches to treatment

The most effective treatment for COPD patients should address three key considerations: ameliorate disease progression and symptoms, prevent exacerbations and reduce the impact of the disease on the everyday life thus improving patients' quality of life. Current COPD management focuses on symptomatic relief by using short- and long-acting bronchodilators and corticosteroids [49]. Successful COPD management requires patient's full involvement, including lifestyle changes (smoking cessation is essential), pulmonary rehabilitation to improve patients' wellbeing, understanding of the disease and learning techniques of overcoming limitations caused by progressing symptoms [72]. Prevention of exacerbations is still limited and consists of regular vaccinations and smoking cessation. As the role of nutrition in COPD mechanism remains unclear, disease management does not include nutritional assessment, and nutritional education is limited to introducing the general importance of a healthy diet and the possible use of oral nutritional supplements to those who are losing weight.

Limitations of the disease management based on symptom relief alone have been recognised and different scores, merging respiratory and systemic markers, were introduced, in order to go beyond FEV_1. A BODE score (BMI, Obstruction, Dyspnoea, Exacerbation [73]) and its modifications (mBODE [74]; e-BODE [75]; BODEx [75]), estimate the likelihood of hospitalisation and death (higher with the higher score[73]). A DOSE index (Dyspnoea, Obstruction, Smoking, Exacerbation [76]) was designed to improve disease management, while SAFE index [77] is merging the quality of life score, airflow limitation and walk test, to define disease severity better. Recently, DECAF score has been validated and showed a great potential to predict the risk of mortality during an exacerbation. However, this is helpful to manage AE once occurred [78], not in advance. There is no consensus on the advantage of one index over another, especially that some of them fit different purposes, as well as there is no guidance on disease management in patients identified as being at high risk of poor outcomes. There is also limited translation of those markers into

clinical practice. Various scores are available (Table 3) but most of them are only used in research and having a high or low risk does not trigger any further action, it provides only a descriptive value.

Table 3 Comparison of components of various multidimensional COPD indices

Indices	Included measures					Interpretation	References
	BMI	FEV1	MRC	Other	Other		
BODE	✓	✓	✓*	6MWT	-	Mortality risk	[73]
e-BODE	✓	✓	✓*	6MWT	frequency of severe exacerbations (past 12 months)	Mortality risk	[75]
BODEx	✓	✓	✓*	x	frequency of severe exacerbations (past 12 months)	Mortality risk	[75]
mBODE%	✓	✓	✓*	x	oxygen uptake measured at peak exercise	Mortality risk	[74]
DOSE	x	✓	✓	smoking status	exacerbation rate (past 6 months)	Mortality risk	[76]
SAFE	x	✓	x	6MWT	St George's Respiratory Questionnaire	Stratification of severity in COPD	[77]
DECAF	x	x	✓***	Eosinopenia Consolidation	Acidaemia Atrial Fibrillation Score	Mortality risk during hospitalised exacerbation	[78]

6MWT – 6minute walking test, MRC - Medical Research Council (dyspnoea scale)
*mMRC, ** extended MRC

Non-pharmacologic therapy for COPD includes pulmonary rehabilitation (PR), which has been shown to relieve dyspnoea, fatigue and enhanced patients' control over the condition [79, 80]. Involvement in regular activities with other COPD patients also had a positive social impact, but not all the patients are willing to attend PR, irrespective of known benefits [81]. This group often comprises of patients with lowered self-worth and reduced help-seeking due to shame, guilt and fear of others evaluation [81]. For that reason, new platforms have been developed including home-based training and online groups [82, 83], which are dependent on patients' motivation and persistence

Recently, the Cochrane review of pulmonary rehabilitation effectiveness in COPD has been updated [80]. Based on 65 studies, the authors concluded that PR significantly improved both functional exercise and maximal exercise, when compared with usual care. Researchers reported an increase in maximal exercise capacity and the mean treatment effect for 6MWT was greater than the threshold of clinical significance [84]. However, in this review, PR was defined as at least four weeks of exercise training with or without educational and/or psychological support, where

supplementation was not an element of a single program. For those, who have attended pulmonary rehabilitation and found it helpful, there are maintenance rehabilitation programs but published results show a lack of further improvement in physical capacity due to maintenance rehabilitation. However, none of the studies considered the fact that maintenance rehabilitation can ameliorate rate of deterioration [85, 86]. The Cochrane systematic review of maintenance programs is still in the protocol phase.

The ERS statement [61] has highlighted the need for an understanding of the cross-talk between the shift in body composition in COPD and dietary management and in order to address needs of metabolic phenotypes with the development of targeted nutritional interventions. So far, it has been shown that COPD patients with higher levels of nutrients in blood have better lung function and improved clinical outcomes such as hospital readmission or mortality [87-90]. In general, supplementation has been seen as a potential therapy in many various conditions, and body of literature in supplementation has been systematically reviewed by Cochrane over 9280 times (18/02/2016). For last two decades, there has been a number of studies testing various supplementation patterns in COPD, including single vitamins, a combination of antioxidant nutrients or vitamins and amino acids. The literature is extensive, contradictory and many have aimed recently to review it systematically [91-94]. Selected most relevant reviews of nutritional supplementation in COPD from last 5 years are discussed below.

Cochrane reviews are considered amongst the highest standard in the evidence-based medicine, therefore it is of great importance that a review from 2012 [93] showed that more than two weeks of energy nutritional supplement (oral, enteral or parenteral) promotes weight gain among patients with COPD. It was more evident in malnourished patients, when compared with well nourished. This finding differed to that seen in the previous version of the review from 2005, where no significant effect on anthropometric measures, lung function or exercise capacity in stable COPD patients was found. Within the 17 studies reviewed in 2012, authors showed an increase in FFMi, FMi, MAMC, skinfold thickness and 6MWT suggesting a positive effect of supplementation on both lean, fat mass and to some extent on the physical capacity. The meta-analysis of the data was more rational in the current Cochrane review, compared with the previous version. A comparison of results within the group, pre- and post-supplementation, rather than between supplemented and placebo groups offered a better perspective on the size of the effect that supplementation had. Because results were focusing on energy intake added to a normal diet, therefore, increase in body weight was not a surprise. From a clinical perspective, a comparison of the proportion of the increase in lean and fat may have been more informative. The increase in fat mass potentially has a negative effect on inflammatory status, therefore,

supplementation would be more successful if was affecting lean mass in greater proportion, than the fat mass, which was not assessed in the review.

In 2012, another review of supplementation effects on COPD patients was published [92], with more attention paid to the differences in various supplementation types, durations, and effects, than in the Cochrane review. First, it was shown that nutritional supplementation had a positive effect on food intake, which answered the concerns of those who suggest that patients on supplements eat less. Next, Collins et al. showed that nutritional intervention, mainly in the form of ONS, improved body weight, muscle mass (MAMC), fat mass (skinfold thickness), and handgrip strength. Most of the studies in the review focused on malnourished patients (10 out of 13), therefore, results should not be generalised across the COPD population. A subsequent review by the same group [91] has also shown that significant increase in body weight of more than 2 kg was related to the improved respiratory and non-respiratory muscle function and strength. This was an important finding, as it could be implemented as a therapeutic target in malnourished COPD patients. However, what the review does not answer, is the type of supplementation that would be the most effective and resulting in an increase of lean mass greater than the increase in fat mass. This is probably due to the lack of sufficient information in the reviewed studies, which was also a reason for the lack of observations on the anti-inflammatory effect of the nutritional intervention.

There is no coherent, good quality systematic review focusing on when to consider vitamins supplementation in COPD patients. A meta-analysis from 2010 showed extreme heterogeneity of vitamins supplementation studies, due to a variety of vitamins that had been supplemented, the dosage and the duration of the supplementation, ranging from 4 weeks to 5 years. Moreover, different outcomes were measured including the effect on spirometry results, disease symptoms and exercise capacity [94].

In summary, energy supplementation has been shown to promote lean and fat gain, while vitamin supplements studies were contradictory and showed extreme heterogeneity [91-94]. Despite many studies, there was no conclusive evidence of any consistent benefit of micronutrient supplementation for COPD patients. There is no evidence to guide dose, composition or length of supplementation in order to achieve clinically most relevant improvement in COPD patients. There is no understanding of the specific micronutrient needs of COPD patients at different stages of the disease journey when stable or exacerbating. Several factors might limit the ability to demonstrate any value of supplementation. COPD severity is usually assessed by lung function, and nutritional supplementation may not be sufficient to improve it directly. Potentially, the ability to ameliorate the rate of lung function decrease would be useful as an alternative

outcome; however, this would require detailed information on individual lung function decline rate, which currently is challenging. Therefore, change in the lung function may not be the outcome of choice for the effectiveness of nutritional interventions, and other clinical outcomes should be explored. The same supplement could have a positive effect on a patient, but not another. Both 2012 systematic reviews [92, 93] have shown that the positive effect of supplementation was seen in malnourished COPD patients and not always in the well-nourished group. Therefore, different patients may require different supplements or only some patients require supplementation at all. This would support the view that understanding the differences in the underlying pathomechanism of the disease and grouping patients into phenotypes of similar characteristic could allow to identify, and then, target individuals who are likely to benefit from a given intervention. This would make it possible to better match interventions with the patient for the best effectiveness, a framework for personalised medicine based on different patient phenotypes. New tools and therapies can be developed only by finding patterns that repeat in different patients. This is why structured and systematic analysis of nutritional status in COPD patients is required.

Current COPD management and the approach to assessing disease severity are similar for most COPD patients, regardless of the disease phenotype. Considering the potential differences between emphysematous and bronchitic patients discussed earlier, it is plausible to assume that those two groups of patients would benefit from different, more individualised therapies. However, there are no simple and economic assessment techniques to predict clinical endotypes representing disease mechanisms and hence future treatment responses. Two patients could have similar BODE or DOSE score, while the underlying mechanism could be different, therefore they clinical outcome could be very different. When different COPD tools and management techniques are compared, their clinical value is assessed using their impact on hospitalisation or mortality (see Table 3). Unquestionably, mortality is the most objective clinical endpoint, but in chronic diseases like COPD, it is essential to improve the quality of life of the patient and their carers/families. Considering the nature of COPD, it may be best achieved through predicting the timeframe of the next exacerbation. This would facilitate the development of preventative mechanisms. Commonly used markers of lung function, whilst important and not sufficient in themselves to mark risk, therefore, there is a need for other markers that could predict time to next exacerbation and do this reliably in all COPD phenotypes.

1.1.7 Summary

Exacerbations have detrimental effect on disease progression and patients' life, and so there is a need to focus on exacerbations prevention. However, limitations that scientists face are not only lack of detailed understanding of triggers and pathomechanisms of the exacerbation, but also lack of understanding whether an individual has strong resilience or predisposition to exacerbations. Categorising patients into two groups – frequent or infrequent exacerbators, simplifies the picture. Assuming that patients categorised as infrequent exacerbators have less exacerbations in time, suggesting they may have better resilience against infections or their inflammatory consequences. Meanwhile, those considered frequent exacerbators, can present with two, three or seven exacerbations a year, yet all are considered together. Some of those patients can experience more exacerbations because of greater exposure to AE triggers, like seasonal exposure to bacterial and viral infections. However, it appears not to be the critical difference, as not all COPD patients experience AE during winter season. It appears that some COPD patients do not respond with acute exacerbation regardless of exposure to triggers, while others respond rapidly to any, even minor AE trigger. Because all COPD severity patients experience AE, it appears that disease severity does not carry a major impact on resilience to exacerbations, however disease activity may play important role. Disease activity, which relates to underlying pathomechanism, appear to be nutritionally sensitive. Inflammation, oxidative stress and apoptosis can be modified by change in nutritional status, as well as macrophage responses that can be altered by change in provision of nutrients. Therefore, it appears reasonable to assume that nutritional wellbeing could influence resilience to exacerbations. Previous research exploring the role of body weight or body composition in determining different COPD phenotypes support the relationship between nutrition and COPD activity and resilience to exacerbation.

1.2 Role of nutritional status in COPD

There is a body of evidence on the relationship between poor nutritional status and health [95, 96]. The robust and sound evidence was obtained in gastroenterology [97-99], cancer [99, 100] and kidney diseases [101], but other chronic conditions, including COPD still require exploration and better understanding. Poor nutritional status was related to increased rate of complications (17% among well-nourished and 43% among severely malnourished patients [102]), 30% longer length of hospital stay than well-nourished counterparts [102, 103]), and increased mortality risk [102-104]. Malnutrition also increases a cost of healthcare, as the cost of disease-related malnutrition in the United Kingdom in 2009 was estimated to account for up to 10% of the total expenditure on health [105].

In this thesis, nutritional status marks the extent to which the body's demand for energy and nutrients is met by that available from the diet and endogenous metabolism. Any mismatch between demand and supply is expressed in terms of the structure and function of the body. Changes in function can occur rapidly and independently of changes in structure, and generally reflect a loss of metabolic or physiological control at the cellular, tissue or whole body level. Changes in structure, particularly in terms of weight and body composition, are much slower to occur and hence, at the whole body level can be seen as a summative (longer-term) measure of the less obvious changes in individual tissues and organs.

A comprehensive nutritional status assessment should represent what you eat, what you are and what you can do. However, this should be understood as far more than just food intake, body weight and exercise capacity. What you eat is mostly represented by diet, and there are various approaches to food intake assessment (e.g. food recall, food frequency questionnaire) and the choice of method depends on the aim (to compare to reference ranges on a population level, to explore the change in time for an individual, to compare between patients with similar condition)[106]. Nevertheless, what you eat is also determined by the appetite and level of absorption, which cannot be assessed by dietary tools.

There is no one pathway, by which malnutrition influences disease processes and consequences of malnutrition can be observed in most organs and systems [107]. The evidence suggests that acute malnutrition leads to abnormalities in innate immune function, e.g. demonstrated by reduced response to skin test with tuberculin or candida antigens [108]. This malfunction of immunological response could be detrimental in diseases, where both inflammation and malnutrition are present, like in COPD. Disproportionate intake of nutrients can lead to multiple metabolic changes including a decrease in muscle mass and function, tissue regeneration, and

wound healing (e.g. vitamin A, C, glucosamine, arginine) [109, 110]. On a cellular level, the immune response can be affected by malnutrition due to altered lymphocyte proliferation, reduced lymphocyte responsiveness, inefficient oxidative burst or change in cytokines production (e.g. folic acid, pyridoxine, zinc, iron, eicosanoids, taurine) [111-114]

Weight loss, changes in body composition, especially loss of skeletal muscle mass and abnormal muscle structure as a shift between fibre types and decrease of muscle fibre cross-sectional area [4, 115, 116] with fatigue and deterioration of physical abilities [117], occur in many COPD patients. The so-called 'J-shaped' relationship between weight as BMI and mortality with both low BMI and high BMI being associated with increased morbidity and mortality has now been unpacked to reveal that the increased morbidity and mortality at the two extremes of BMI are attributable to two differing relationships that together contribute to the J-shaped effect [118-120]. There is an inverse relationship between BMI and mortality evident at normal through low BMI and is largely attributable to mortality associated with weight-losing diseases such as emphysematous lung disease and lung cancer. At the same time, there is also a positive relationship evident from normal through to high BMI in conditions where excess weight is gained and mortality is largely attributable to cardiovascular disease patients [119, 120]. Not all patients with chronic lung disease are underweight and there are many patients with COPD patients who are overweight or obese [119] who do not have the same morbidity and mortality as those with COPD who are underweight – this paradoxical effect is often referred to the obesity paradox [69] and largely reflects the differing presentations of COPD and their underlying pathophysiology. Therefore, there is a need to explore to what extent nutritionally sensitive mechanisms relate to disease processes in COPD.

1.2.1 Immunonutrition and COPD

The inflammatory response to respiratory infection, although an essential part of innate immune response, carries the risk of severe tissue depletion and immunosuppression. The balance between inflammatory response sufficient to resolve infection and anti-inflammatory activity to subsequently dampen the effect is essential for effective and safe infection resolution [121]. Various factors can influence this process and there is emerging evidence suggesting important role of nutrition.

The initial inflammatory stimulus triggers a metabolic response including a cascade of pro-inflammatory cytokines – interleukins (IL), interferons, tumour necrosis factors (TNF), and transforming growth factors (TGF) (Figure 3) [122]. Also, in response to an infectious agent a broad range of leukocytes are attracted to the site, including phagocytes (neutrophils, basophils,

eosinophils, monocytes and macrophages), and lymphocytes. The host immune response to bacterial or viral infection (or burn or wound) triggers chain of metabolic changes in the whole body [123].

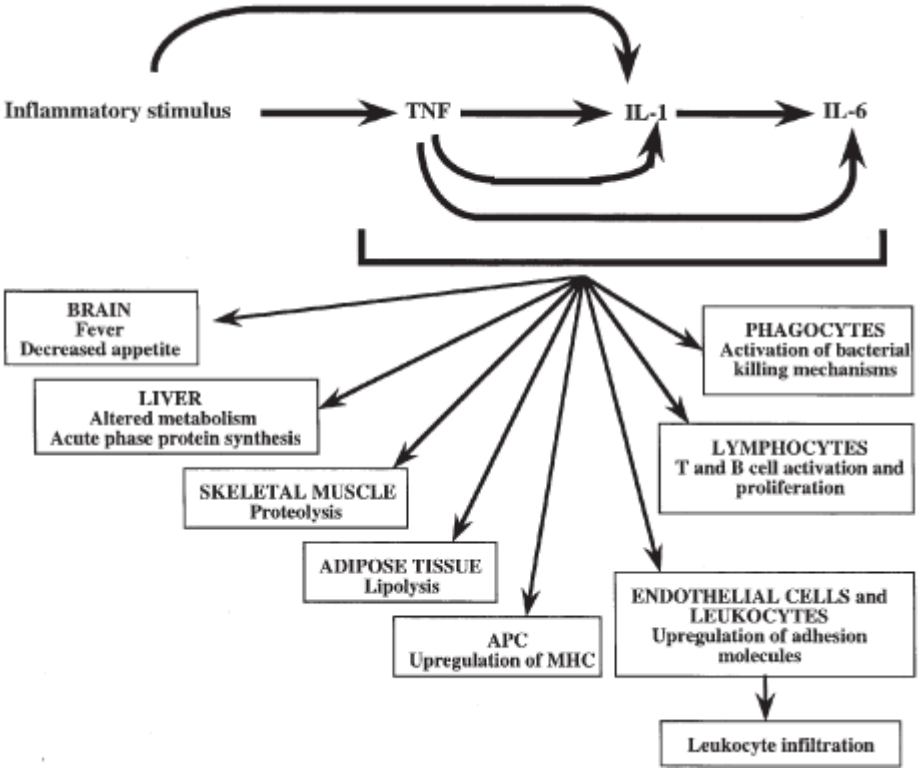


Figure 3 Key roles of proinflammatory cytokines in mediating the host immune response. APC-antigen-presenting cells; MHC-major histocompatibility complex [reproduced from [123]]

The response will differ between individuals depending on the stimulus, age, function of systems and organs, but is also subject to nutritional status of the individual. This has been proposed from epidemiological studies showing higher infection rates amongst malnourished patients[124], but also on a cellular level showing role of vitamins on lymphocyte maturation and function [125]. Malnourished patients, who have a reduced ability for cytokine production, had poor prognosis in recovery from infection and trauma [122].

Therefore, a new concept of a two-way relationship between nutritional status and immune response has been proposed. Immunonutrition was defined as 'modulation of the activities of the immune system, and the consequences on the patient of immune activation, by nutrients or specific food items fed in amounts above those normally encountered in the diet' [126]. Various nutrients have been shown to influence or even modulate inflammatory response. Lipids can alter membrane phospholipids and result in change in cytokine and lipid-derived mediator production;

antioxidants supress oxidant effects and alter cytokine production; while amino acids can influence acute-phase response [126].

The main cytokines produced by monocytes, (TNF, IL-1, and IL-6) activate neutrophils, monocytes, and macrophages to initiate bacterial and tumour cell killing, but also mediate the systemic effects of inflammation such as fever, loss of appetite, and wasting of peripheral tissues [122, 123]. Loss of appetite causes limited food intake, prompting tissue wasting and results in loss of tissue lipid, protein, and micronutrients, to supply the delivery of nutrients such as glutamine, amino acids, glucose, fatty acids, and micronutrients[122]. Released nutrients are delivered to the immune system and facilitate a number of other processes (tissues repair, cytokine production, healthy tissue protection from the effects of oxidants) [122].

Cellular studies have also suggested that the nutritional status of an individual not only determines availability of nutrients from tissues, but also determines function and survival of lymphocytes and macrophages during temporary starvation, as would be seen in severe COPD exacerbation. The peripheral blood lymphocyte was considered an ideal system for metabolic studies (easily obtainable, can be activated to proliferate), but most importantly, reflects both the genetic makeup and biochemical environmental history of the individual at the time the cells were formed [127]. By growing lymphocytes in a serum-free media and subsequently, adding various nutrients in different amounts and combinations, the authors were able to define components essential for lymphocyte response. Shive et al provided in vitro evidence for immunonutrition – absence of riboflavin from the medium led to significant decrease in lymphocyte response, while lack of amino acids like serine and glycine did not change the response [127, 128].

Immunonutrition could play a role not only during acute inflammation event, through facilitating response to the infectious stimuli, but could potentially modulate chronic inflammation. Lipid raft microdomains within the plasma membrane of endothelial cells, called caveolae, have been implicated as mediators of vascular inflammation. Layne et all suggested that caveolae promote endothelial activation and increase macrophage recruitment [129]. Omega-3 polyunsaturated fatty acids and some polyphenolic compounds have shown to modify the lipid raft microenvironment and interrupt caveolae-mediated signalling resulting in reduced inflammatory response [129].

Another immunonutrition aspect relates to autophagy, the catabolic pathway involving the engulfment and degradation of a cell's own components through the lysosomal machinery to enable energy conservation [130]. In vitro studies suggest that amino acid or glucose deprivation selectively stimulates the phagocytosis of heat-inactivated Gram-positive and Gram-negative

bacteria [130]. This suggest potential for nutritional strategies for the treatment of patients who are infected with multi-resistant bacteria [130].

There are various mechanisms and pathways by which nutrition can modulate the immune response. Facilitating acute response to infection through assurance of lymphocytes and macrophages function or modulating response during chronic inflammation are only few of potential mechanisms. COPD patients often present with persistent low-grade inflammation during stable condition [115], and acute inflammation during exacerbations [45]. In both scenarios, immunonutrition and nutritional modulation could play and important role. There is growing body of evidence showing relationship between nutritional status and COPD progression, focusing not only on a cellular level nutrition, but even more on a whole body level.

1.2.2 Markers of nutritional status in COPD

There is a general interest in the role of nutrition in COPD but its application in clinical practice is limited. Recently, a dedicated Task Force within ERS set to summarise evidence in the role of nutrition in COPD and how nutritional considerations might be incorporated into clinical practice [61]. In other reviews and studies four different metabolic phenotypes of COPD patients were identified that reflect differences in body composition - cachectic, obese and sarcopenic and sarcopenic obese [61, 66, 131-141]. Body composition expressed as fat-free mass index (FFMi where Fat Free Mass is expressed per unit height in metres) was used both by ERS and previous researchers, as a marker of malnutrition and lean depletion. Based on the supposition that malnutrition, weight loss and FFMi decrease are largely attributable to an inadequate intake, an insight to change in appetite in COPD patients is indicated. Furthermore, considering that poor nutritional status and respiratory condition would affect the ability to perform muscular work, some have proposed physical capacity as functional markers of body composition and COPD. Nutritional status, however closely related to food intake, can be assessed and monitored without direct focus on food intake. Therefore, this next section aims to explore the value of nutritional status in COPD measured with body composition (especially FFMi), appetite and physical capacity.

1.2.2.1 Body size and shape for nutritional screening

The human body can be described on many levels, but the simplest approach considers body size, shape and proportion. Height and weight are the most commonly used anthropometric measurements due to both simplicity and clinical utility in predicting risk of ill-health. Over the life course of individual, these measurements allow monitoring changes in body size and shape, a predictive factor of health and wellbeing in adults. Increased body weight (excessive fat tissue) can be distributed evenly or within a particular area. Truncal adiposity has been identified to be a

risk factor for coronary heart disease or metabolic syndrome, while fat deposition at thighs and hips does seem to have lesser effect [142-144].

Both height and weight, along with BMI, are used in clinical practice because they are simple, quick and carry only a small cost. Obtaining accurate and precise measures of height and weight are essential not only for understanding patients' nutritional status but also for clinical purposes, like chemotherapy dosage (dose calculation based on body surfaced area from height and weight) or dietary restrictions [145]. Regardless of the apparent simplicity of both measurements, certain mistakes are common, including: keeping the shoes on, not positioning the head in Frankfort plane, not instructing the patient to take a deep breath in just before the measurement. An experiment performed in a local hospital has shown that the same patient measured on the same day on multiple hospital wards and clinics had height recorded within over 10cm range and weight with an almost 7kg difference between the lowest and the highest reading [personal communication K. Durkin]. Quality control of anthropometric measurements was reviewed and showed that 'The measurements appear simple and straightforward and are made so frequently that for many there is insufficient regard to quality, thereby compromising the confidence that can be placed in their reliability in assessing response to therapy, growth-monitoring, surveys, or trials' [146]. The discrete problem exists in GP practices, where due to shortage of time, doctors rely on patients recall, rather than performing measurements themselves. This often leads to a failure to recognise a true change in weight until the difference is visually obvious or until the patient raises concerns.

There are some physiological circumstances, which emphasise limitations of BMI. There are circumstances where BMI remains in the normal range, but lean depletion is masked by excessive fat mass (sarcopenia). Professional athletes and fit individuals with high muscle mass could be categorised as overweight. Increased proportion of lean mass, at the expense of fat mass, leads to increasing body weight (one cubical unit of muscle tissue is heavier than one cubical unit of fat tissue due to the difference in tissue density). Regardless of the limitations, BMI is still the simplest, quickest and cheapest screening tool for nutritional status and has been incorporated into many other screening instruments (e.g. Malnutrition Universal Screening Tool or MUST [147]) and when used with care can aid clinical decision making.

Malnutrition represented simply by low BMI, has been related to worse clinical outcomes in various disease, including COPD [61, 66]. Consequently, those with BMI values representing normal weight were assumed to have better clinical outcomes. Moreover, there is evidence in COPD that the higher the BMI, even above the normal range of 25kg/m^2, the lower the risk of hospitalisation or mortality [118, 148, 149]. Patients with severe COPD, who were overweight or

obese according to BMI, had greater muscle mass, muscle strength and exercise capacity [139]. This observation called the “obesity paradox”, concurs with the observations of a similar paradox in cardiovascular diseases [149], where underweight patients seem to have a greater risk of poor clinical outcomes than overweight counterparts [118]. Recent studies show that the positive effect of higher BMI may be related to the higher lean mass, rather than higher body weight in total [66, 150]. However, BMI seems to be insufficient to assess nutritional risk in COPD when taking into account prevalence of sarcopenia and limitation when considering different body build.

The initial studies focusing on nutritional status in COPD appear to be selectively biased against high BMI. In 2016, five publications focusing on nutritional status in COPD were identified (excluding physical capacity studies) [151-155] (PubMed only, 05/10/2016). Among these, four publications included patients with the average BMI below 24kg/m^2. This suggest that current research focuses on selected group of COPD patients (preferably normal-weight), while the worldwide average BMI of COPD patients was above 25kg/m^2 [156], suggesting overweight to be more common than normal-weight. Moreover, the average proportion of underweight patients (BMI<21kg/m^2) was estimated to be 13.3% worldwide and 8.4% in the UK [156]. Meanwhile`, over 20% of COPD patients in studies published in 2016 were underweight [152, 157] and some studies restricted cohort to underweight patients only [158]. This probably reflected nutritional concerns in clinical practice where only obviously malnourished patients are offered nutritional review and advice. Consequently, cohorts with a higher BMI, who are believed to have a lower risk of poor clinical outcomes, even believed to be protected by the ‘obesity paradox’, have been gradually of lesser interest to the researchers. Lung function markers were worse in the lowest BMI group (≤21kg/m^2) and improved with increasing BMI. However, better lung function in obese may be a selection bias in that fatter people have more symptoms and therefore present at earlier stage of the diseases processes. Quality of life and physical capacity were worse in both extremes of the BMI spectrum, compared to patients in the normal range of the BMI [159]. This suggests that the mechanisms that underlie poor lung function and worse quality of life at extremes of BMI are likely to be different.

Recent study of large COPD cohorts included in a genome association study suggests that majority of COPD patients have high-normal weight or are overweight [160], suggesting that the risk of malnutrition is low if screening tools like MUST are used [147]. However, when comparing BMI and body composition, nutritional status appears to be of some concern. In the ECLIPSE cohort, the average BMI was 26.5 ± 5.7kg/m^2 [62], and decreased with the increasing COPD severity. Similarly, the fat-free mass index also decreased with increasing disease severity. In the patients with moderate COPD (the highest BMI in the cohort), the mean FFMi was 18.4 kg/m^2 in men and

16.2 kg/m^2 in women [62] which is lower than the 50th centile of a healthy population (19.4 kg/m2 and 16.2kg/m^2 men and women respectively), and mean value in men was equivalent to below 25th centile [161]. This suggests that overweight and increased BMI, even if protective for some patients, may mask lean depletion and poor nutritional status in others. Although not reported in the paper, this low lean and high fat mass could have been related to the varied frequency of exacerbations in the cohort. Although the average exacerbation rate in the ECLIPSE was 0.9 events in the year prior to enrolment the range varied from zero to seven exacerbations per year, suggesting that the patients in this cohort were largely infrequent exacerbators but that the cohort would also include some frequent exacerbators. As the analysis was for the whole cohort, no subgroup analysis by BMI or FFMi was reported.

Anthropometry and BMI could be considered a nutritional assessment of a choice, due to its relative simplicity and low cost, yet should be interpreted with care. Low risk of malnutrition based on BMI should not be assumed to confirm good nutritional status. Higher BMI was suggested to have a protective effect against long-term outcomes but there is a need to determine what effect it has on short-term clinical outcomes such as time to next exacerbation.

1.2.2.2 Body composition

Human body compartments can be defined on a functional level (skeletal muscle, adipose tissue, blood, bone and others) or a molecular level (water, fat, protein and minerals) (Figure 4) [162].

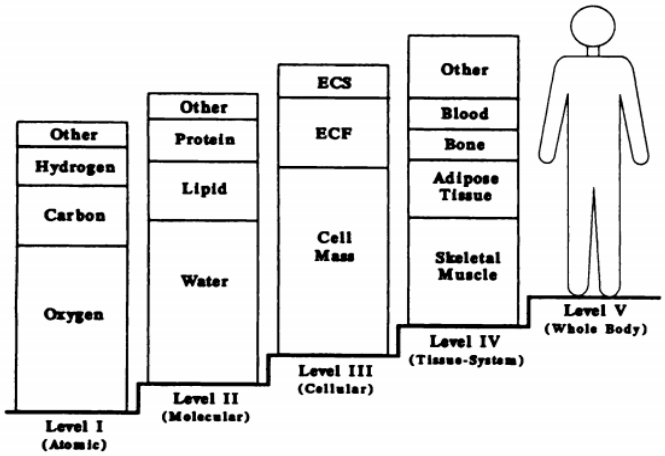


Figure 4 Levels of body composition according to Wang [162]

The simplest approach is to consider the body in a two-compartment model consisting of two chemically (and densitometry) distinct compartments, fat mass and fat-free mass [163-165]. Because the proportion of water, protein and mineral changes with age [166], the three-compartment model including fat mass, total body water and fat-free dry mass was proposed [167]. The three-compartment model does not consider variation in bone mass and assumes a constant ratio of protein to mineral, therefore, a four-compartment model emerged [168]

including direct measurement of bone mineral content. Currently, most studies reporting body composition measurements are restricted to two compartments - fat-free mass (also called lean mass) and fat mass although there some also report body water volume (sometimes with information on intracellular and extracellular water volume).

Many different tools and methods with varying precision can be used to assess body composition [169], but from a clinical perspective attention has been focussed largely on muscle mass and fat mass. As the gold standard for body composition analysis would be chemical analysis of the cadaver, all in vivo techniques can only be thought of as surrogate markers. Body composition might be estimated by a number of methods, of which the simplest involve only arm circumference and skinfold thickness [170]. Highly specialised methods, like dual energy X-ray absorptiometry (DXA), body impedance analysis (BIA) or deuterium dilution using deuterium oxide (D_2O), give more precise information on body composition, but they require specialist equipment and trained staff, as well as cautious interpretation [171].

The BIA technique is one of the most commonly used, due to its relatively low cost, good quality (reproducibility and repeatability) of results and portability. The technique is based on the assumption that lean tissue contains 73% water and electrolytes, so can act as an electrical conductor. By measuring physical properties, resistance and reactance of human body, a volume of extra- and intra-cellular water can be calculated [172]. This enables calculation of lean mass, and by using total body weight, provides fat mass.

The DXA technique is based on the concept that photon attenuation is a function of tissue composition [173] and assumption that human body consist of three compartments with different photon attenuation (fat, bone mineral and lean soft tissue). As DXA is a dual-energy technique, it measures the differential absorption of x-rays of two different energies [174]. Some consider DXA to be the most precise technique for assessing body composition, however as every surrogate technique, it has limitations. The results should be interpreted with caution, especially when used in elderly with metal implants or very obese (method precision is drastically decreasing if the anterior-posterior thickness exceeds 23cm [175]).

Deuterium dilution has been agreed to be a gold standard for assessment of total body water and is often used as a reference for other techniques. Deuterium dilution uses the principle that the dose of non-radioactive isotopic tracer (deuterium oxide 99.9 atom %) is evenly distributed in a pool of water and concentration of tracer in small volume represents concentration in the total volume, can be easily calculated. The D_2O has the appearance and taste of tap water and is not a hazardous substance, therefore, can be used in any age group or medical condition [171].

Introduction

The variety of techniques available to measure nutritional status requires a careful selection of a method adequate for the purpose, especially that differences in results depend on the technique [176]. In **Appendix I**, there is a comparison of advantages and limitations of the most commonly used methods to assess body size and composition in COPD. From all the methods assessing body composition, BIA has obvious advantages in terms of clinical practicality and has been widely used in COPD research. Regardless its popularity and common use [65, 66, 139, 150, 177-180], there are no guidelines or standardised procedures for assessment or interpretation of the results.

Lean depletion (low FFMi) was often highlighted as a clinical presentation in COPD. In 2014, a review of the variability of FFMi in COPD patients (based only on the Web of Science database) was presented at a conference [see **Appendix M**, [181]]. 94 articles were identified to report fat free mass index in COPD cohorts between 1990 and 2014. Out of those, 14 studies provided data on the mean and standard deviation (SD) for FFMi in men and of these, 11 also provided results for female COPD patients. The mean FFMi for men ranged from 16.8 kg/m^2 to 19.8 kg/m^2. Similar variability was found in the female results, with the mean FFMi varying from 14.7 kg/m^2 to 18.2 kg/m^2. This variability was even more prominent, when lean depletion prevalence was estimated using various lean depletion cut-offs. In studies included in this review three different lean depletion cut-offs were identified for men (16 kg/m^2, 17 kg/m^2 and 17.4 kg/m^2) as well as for women (14 kg/m^2, 14.6 kg/m^2 and 15 kg/m^2). Further analysis showed that when using an FFMi cut-off of 16 kg/m^2, the percentage of male COPD patients identified as lean deplete varied from 4.6% to 36.9% in reviewed cohorts. This increased to a range of 19.6% to 65.5% when an FFMi cut-off of 17.4 kg/m^2 was used. A similar spread of data was seen in the female results, emphasising the complexity of the lean depletion diagnosis in the COPD population.

The amount of lean was usually related to the level of lung obstruction [67, 182-184], however, some studies showed no difference in lung function between lean depleted and non-depleted patients [178]. Consistently, lean depletion was related to reduced physical capacity [67, 182, 184-186]. Most studies investigating body composition and clinical outcomes reported higher mortality and longer hospitalisation in COPD patients with lean depletion [67, 133, 150, 187], however, one study showed no difference in outcomes between non-depleted and lean depleted patients [136].

Understanding of the body composition concerns not only the amount of each body compartment but also the relative proportions of lean and fat. Obesity is often understood as excessive amount of fat, but ‘sarcopenia’ (age-related changes in muscle mass and strength), ‘cachexia’ (muscle wasting and weakening related to disease) and ‘sarcopenic obesity’ (lean mass loss concealed by excessive fat mass) [188, 189] were recognised as common features of COPD. These terms

describe different conditions, but there appears to be some confusion about those expressions still as they are often used interchangeably, and often are assigned based on impression, rather than established criteria. Four international groups aimed to gain consensus on definition and standards for sarcopenia [63, 189-191], but each group published separate criteria for sarcopenia. However, all new definitions included the same three domains– walking test, grip strength, and lean marker. Definitions varied in the cut-off points for each measurement and classes of sarcopenia severity. Valuable for research, proposed criteria for sarcopenia are not immediately or necessarily applicable to clinical settings (e.g. appendicular lean mass using DXA scan, which is not widely available).

As different criteria for sarcopenia have been published and applied, it is not surprising that the apparent prevalence of sarcopenia in COPD ranges from <1% to 50 % [192]. The observation that the mean BMI in sarcopenic groups was normal [131, 178, 193], emphasises the importance of nutritional assessment going beyond body mass. Moreover, there is a lack of agreement whether sarcopenia should be defined using structural or structural and functional markers, as some of the muscle function factors cannot be captured by a change in mass or cross-sectional area .Dam et al. [192]] reported that prevalence of sarcopenia shifted from the range of 7 - 50% when based on lean mass alone to the range of 0.5 – 5.3% when included muscle function as well.

Inflammation was of a great interest when to consider body composition, as increased inflammation was hypothesised to be the cause of muscle catabolism and cachexia [194]. Results, however, were inconsistent. Studies have shown no difference in serum CRP [195], IL-6 [195] and TNFα levels [195, 196] between lean depleted and non-depleted COPD patients, as well as same or no difference in airway inflammation between the two groups [183]. CRP levels were shown to be FFM-related, but in one study the inflammation level was higher in a lean non-depleted group [197], while in another CRP was higher in the cachectic patients [196]. Some have concluded that inconsistency of inflammation results was related to change in sensitivity of laboratory techniques over the years [194, 197], however, those reviews included studies with underweight/normal weight patients or lean depleted/non-depleted patients, which represent two different comparisons. Studies reporting differences between lean depleted and non-depleted groups used various different definitions for lean depletion, as indicated in the mini-systematic review of body composition in COPD presented earlier. Therefore, the relationship between inflammation and body composition in COPD remains unclear and possibly should be considered in continuous, rather than categorised fashion, to avoid the effect of arbitrarily selected criteria for lean depletion.

The balance between muscle degradation and tissue regeneration in different conditions is no longer maintained, which has been extensively described [64, 66, 178], but no sufficient explanation of the cause or mechanism of this process has been provided. A sophisticated system of protein turnover gives skeletal muscle impressive capacity to self-regenerate, but it is very sensitive to factors such as inflammation, malnutrition or oxidative stress [198]. However, the evidence is not sufficient to define mechanisms driving lean loss and consequences of long-term imbalance in muscle synthesis and degradation. Plausible mechanisms underlying loss of lean tissue in COPD are discussed further. There is a need to assess true prevalence of lean depletion and its implications in COPD, when analysed in a standardised way focused on clinical outcomes.

Evidence up to date suggests great potential for the use of body composition in COPD, especially FFMi, which may aid clinical decision making and be used to direct care. Published results show a similar tendency, however, the range of available techniques requires standardisation and unification, to provide comparable results. Simple and cheaper measurements than suggested DXA are needed in order to facilitate diagnose of body composition abnormalities on an everyday basis. Body composition, especially FFMi was measured in many COPD cohorts, however its potential value in predicting exacerbation risk or time of next exacerbation was not explored in details.

1.2.2.3 Appetite loss as a gateway to malnutrition

Considering limitations and lack of consensus on methodology and interpretation of body composition in COPD, there is a need for an alternative marker of nutritional status. Malnutrition can occur as a consequence of an inadequate energy and nutrients intake and through increased losses or needs. It has not been established to what extent malnutrition occurring in COPD is due to inadequate intake or change in demands, while among patients attending the COPD clinics it appears to be a combination of both mechanisms.

We are what we eat, and food intake can be limited by food accessibility, availability, food habits and by a decrease in appetite. Anecdotally, the majority of COPD patients experience loss of appetite around exacerbation, while only some patients experience a long-term decrease in appetite. There is no evidence comparing nutritional status among patients with acute loss of appetite and patients with a chronic decrease in appetite. Clinical experience suggests that overweight COPD patients experience change in appetite to a similar extent as those who present malnourished, however, the effect of appetite loss on nutritional status in those two groups is not clear and requires investigation.

Appetite and nutritional status are closely related, as poor appetite limits the food intake and increases the risk of not meeting the needs for energy and nutrients. Feeding behaviour is regulated by complex mechanisms, a balance of stimulating and inhibiting forces in the central nervous system, in particular, the hypothalamus. The majority of signals have an anorexic effect [199-206], and to counteract it, a gut-derived ghrelin is the only example of a peripheral hormone with orexigenic actions [205]. Leptin and ghrelin – two opposite appetite signals, may play an important role in COPD patients [207, 208]. Understanding the interrelation and possible imbalance between the two signals could aid understanding of malnutrition in COPD.

The assumption that leptin and ghrelin levels change as appetite deteriorates in AE, is a sound hypothesis. However, the level of appetite hormones is not the only regulator; non-hormonal drivers of appetite play an important role. Sensory factors [209], socioeconomic factors [210], but also changes in both central and peripheral appetite mechanisms, by some called 'anorexia of ageing', could determine food intake as well. There are a number of questionnaires that have been developed to assess appetite in elderly, including SNAQ and CNAQ [211, 212]. The original questionnaire was developed by The Council for Nutritional Strategies in Long-Term Care, which was an expert panel formed in May 1998 to identify evidence-based recommendations for the management of undernutrition in long-term care. Hanisah et al. have shown that regardless of causes of change in food intake, SNAQ (simplified CNAQ) was most valid and reliable appetite questionnaire to monitor malnutrition in elderly [213]. Therefore, the question is whether appetite can be influencing COPD progression, or whether it is the disease that modifies processes that regulate appetite. More importantly, now there is a need to understand when and how much appetite changes in stable COPD and at exacerbation and whether differences in appetite mark clinical outcomes in COPD.

1.2.2.4 Physical activity – marker of well-being and nutritional status

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) has recommended regular physical activity for all COPD patients as an element of disease management [28], yet evidence of this regular exercise on disease progression is not clear. Rehabilitation and Chronic Care Task force set up by ERS published a focused literature review on prevalence, determinants, consequences, measurement and potential treatment relevant to physical activity in COPD [214]. Physical activity was defined as 'a complex behaviour that can be characterised by type, intensity, duration, patterns and symptom experience' while exercise was considered as a subset of physical activity, which is planned, regular and purposeful [214]. Physical activity could also be considered when assessing leisure-time, daily living, and occupational activities, which emphasises the complexity of accurate assessment of physical activity. A wide range of tests used to estimate the

amount and the effect of physical activity were identified. Similarly to body composition assessments, requires thorough validation and international standardisation in order to systematically review and meta-analyse the data in the future. Regardless the diversity of approaches to measuring physical activity, the evidence suggest a negative effect of low levels of physical activity on lung function decline, increased risk of hospitalisation and predicts higher all-cause mortality [214].

The negative effect of decreased physical activity has been demonstrated [215-217], which emphasises the value of pulmonary rehabilitation for COPD patients. It has been shown that PR relieves dyspnoea and fatigue and enhances patients' control over their condition [79], however, there is emerging evidence that in some circumstances increase of physical activity could have a detrimental effect on health [218, 219]. Early rehabilitation program (6 weeks long) introduced within 48 hours from hospital admission due to COPD exacerbation did not reduce readmission rates in 12 months and in fact was associated with an increased mortality rate [218]. Also, a high-intensity rehabilitation course (at 95% of estimated maximal oxygen uptake VO_2 max, for as long as possible) was related to higher mortality rate when compared with rehabilitation course tailored at usual walking exercise intensity [219]. This suggests that increased physical activity during acute illness or very high-intensity training could have a negative effect on the disease processes in COPD.

Various physical capacity tests were developed to estimate physical activity in clinical setting. Physical capacity tests can be considered as surrogate measures of lean depletion and can be used to monitor the extent of structural and functional changes over time. The most commonly used physical capacity tests are walking tests (6 minutes, 12 minutes, incremental shuttle walk, limited distance test) [220, 221] or muscle strength and endurance tests (hand grip, knee) [222]. Muscle tests could be voluntary (simpler performance) [222] or stimulated (more objective) [223]. There are also other, less commonly used physical capacity tests, like sit to stand (number of repeats) [224], timed up and go (3 meters) [225] or short physical performance battery (3 different exercises) [226].

Physical capacity tests are commonly used as they represent the effect of chronic condition on the everyday life. Short walking distance, weak grip or struggle with the sit and stand test, represents patients' struggle in normal life, not only good or poor tests score. The poor walking score could be an indicator of difficulties on everyday basis e.g. shopping, meeting with friends and family. Poor grip affects lifting things like shopping, cups with drink, grandkids. A low sit to stand score could indicate a high risk of limited activity and increase the risk of a sedentary lifestyle. Altogether, physical capability tests give a glimpse of challenges that patients experience in day-

to-day life and could be used to understand not only physical limitations but also social and personal impairments.

Physical activity plays an important role in maintaining health, however, it is often decreased in COPD patients. Different functional capacity tests have been used to measure the level of physical activity, and now there is a need to identify the simplest and most affordable test that would help to determine patients at high risk of exacerbation in the near future. Complex and time-consuming assessments are unlikely to be incorporated into clinical practice, therefore the focus should be on those techniques that have low equipment cost and short procedure, like grip strength or walk test. There is a need to explore if those tests have any potential to identify patients at risk of exacerbation in the near future and whether potential patients benefit would overcome clinical effort and engagement.

Combining markers to better understand the nutritional status of COPD patients seems like an approach that should be taken forward and explored in detail. Focusing on one domain at a time is insufficient and has led to inconsistent and contradictory statements regarding nutritional status in COPD. The inconclusiveness of previous results, to some extent, could have been caused by different nutritional phenotypes of patients within each COPD cohort. The ERS metabolic phenotypes [61] suggest the direction of future research, highlighting the issue around sarcopenia and sarcopenic obesity, which were often overlooked, as some studies have suggested [140, 227]. However, there is more evidence needed in clinically most relevant nutritional phenotypes and clinically (not arbitrary) based criteria for phenotypes. Emerging evidence of specific co-morbidities in different disease phenotypes (osteoporosis in emphysema phenotype, idiopathic arterial hypertension in airway phenotype)[228] emphasises the difference in underlying pathomechanism and the need for considering COPD as a combination of different patients sub-groups (phenotypes) and not one condition. Once nutritional phenotypes are well identified, this could help in developing targeted interventions, including supplementation that reflects needs of different nutritional phenotypes.

Ultimately, disease phenotypes should reflect the underlying disease mechanisms, however, the lack of understanding of the pathomechanism in COPD limits such an approach. For now, defining phenotypes in COPD should be focused on identifying patients at the greatest risk of poor clinical outcome, especially short-term clinical outcome (e.g. time to first exacerbation) so that disease management could be improved. In order to be introduced into the clinical practice, phenotypes should be based on as few measurements and tests as possible, using simple and the most reproducible techniques available for the purpose.

1.2.3 Muscle – source of a limiting nutrient or energy?

In the human body, muscles are the biggest store of protein; therefore, muscle tissue can be used as a source of amino acids, but also of micronutrients and trace elements built into muscle tissue, such as potassium or calcium. Therefore basic assumptions for lean depletion in COPD and effects on lung health include: a) the lungs requires some nutrients to facilitate increased rate of remodelling and metabolism caused by lung tissue damage (smoking, infections), or b) COPD with its inflammation and recurrent infections has significantly altered and increased requirements, which are difficult to meet with normal diet, let alone, with decreased appetite that often occurs in those patients, therefore all nutritional stores are activated.

The first assumption could be considered on several levels. Lungs may be sending out a signal 'requesting' a single or multiple component(s). This could be mediated by general or specific paths. A general signal could be sent as an inflammation overspill. The mediator is transported via the blood stream to all systems and organs, consequently mobilising the component(s) from wherever it is possible. The specific path would involve lungs excreting molecules that can be picked up only by selected receptors located in the muscle tissue. In both scenarios, the muscle could be mobilised to provide the lungs with the missing component(s) as a priority over the normal muscle metabolism. Another possibility is that increased metabolism and remodelling in the lungs requires a higher level of a component and the component delivery to the lungs is prioritised by all systems and tissues. Therefore, the general pool of this component is minimalized and may not be sufficient to maintain muscle turnover in balance. All of the above considerations are highly speculative, because not many nutrients have been investigated and identified to play a role in lungs metabolism, let alone knowing whether or how much a nutrient requirement increase during the chronic or acute state.

It is difficult to define whether the decrease in muscle mass is due to a decrease in protein synthesis, an increase of degradation or both. The synthesis could be limited due to lack of an amino acid required for synthesis of actin or myosin, low level of a cofactor or low activity of protein-based enzymes. Those changes can be a consequence of increased requirements, low intake, and malabsorption or utilisation alterations. However, it may be that synthesis is not decreased, but protein loss is exaggerated. This could be due to excessive activation of the Ubiquitin Proteasome Systems (UPS) [229, 230], which are responsible for muscle degradation. This process is described in more details further in this thesis. Importantly, some factors can increase the activity of UPS, it has been suggested that glucocorticoids and oxidative stress can additionally amplify the activity of UPS [229, 231] [232]. In addition, it may be that inhibitors of the degradation pathway are muted in their activity, or their concentration is decreased. It was

suggested that tocopherol and glutamine could be factors that, at lower concentrations, no longer have the ability to inhibit protein degradation pathways [233, 234].

The UPS is responsible for removal of damaged proteins after muscle activity and is a major mechanism of protein breakdown. The E3 Ubiquitin (Ub) ligases are selective towards sarcomeric proteins, and it has been shown that atrogin-1 (MAFbx) and RING finger protein 1 (MuRF1) are specific and rate limiting for muscle protein degradation [230, 235]. Once E3Ub ligases conjugate with substrate protein, the 26S-proteasome recognise and degrade the substrate.

MAFbx and MuRF1 lead to muscle degradation through different pathways. The MAFbx promotes degradation of muscle transcription factor (MyoD) and of an activator of protein synthesis (eIF3f) which in effect leads to decreased protein synthesis. MuRF1 controls half-life of structural elements of muscle motor proteins like troponin I, myosin heavy chains and light chains as well as myosin binding protein C [229, 230, 236]]. In cachectic patients levels of MAFbx protein and mRNA were increased, as well as MuRF1 mRNA level and total protein ubiquitination [236], suggesting the role of E3Ub ligases in muscle wasting.

It has been suggested that glucocorticoids, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and forkhead box O3 (FOXO3) regulates expression of MuRF1 [236] (Figure 5). The NF-κB is stimulated by oxidative stress, but also, there is a relationship between NF-κB and tumour necrosis factor (TNFα) which is an acute phase protein [231, 232]. Cytokines levels, including TNFα, go up in response to infection or inflammation, which additionally stimulates NFκB, leading to ubiquitin system activation. Rises in TNFα levels may also affect protein degradation through decreases in appetite and decreases in food intake [237]. On the other hand, vitamin E can decrease levels of TNFα [234]. Therefore, lack of appetite and smaller food intake may lead to lower levels of serum vitamin E, which in effect loses the ability to counteract TNFα.

In summary, the ubiquitin-proteasome system is a major pathway of muscle protein degradation and can be upregulated by oxidative stress and inflammation through activation of NFκB pathway but also stimulated by glucocorticoids and FOXO3 pathway (Figure 5). All three regulating factors (NF-κB, FOXO3, glucocorticoids) enhance the protein degradation system, but play a double role in muscle loss, as they also inhibit pathways of protein synthesis [229].

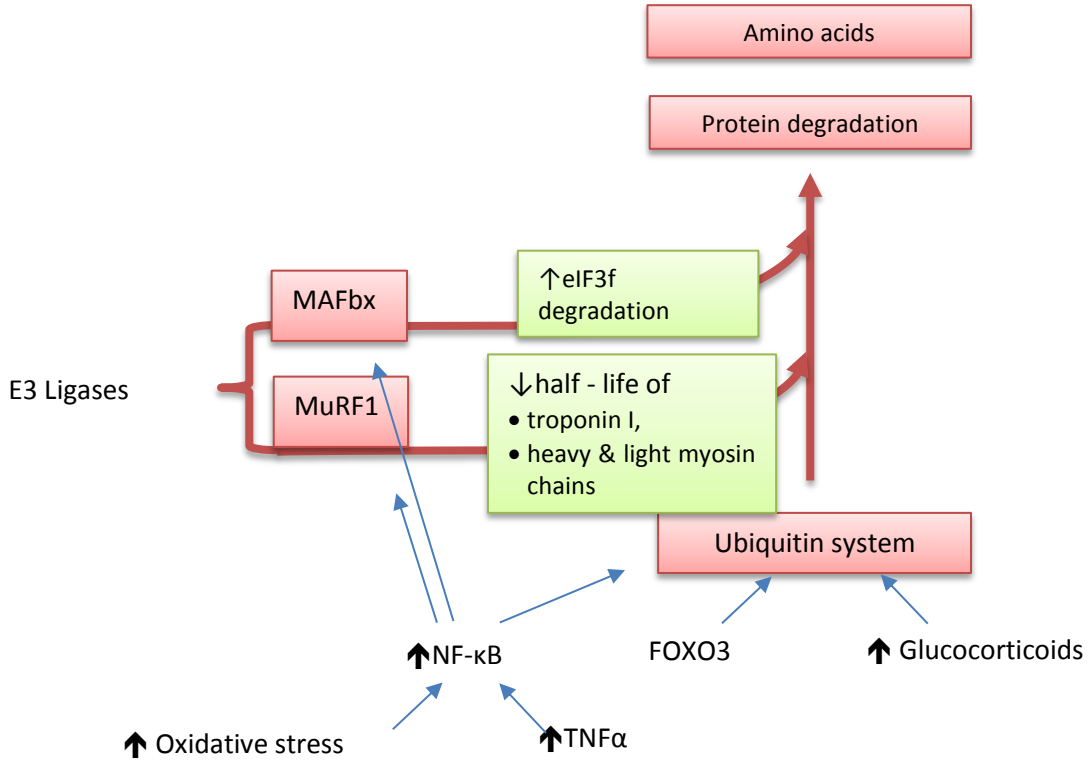


Figure 5 Mechanism of protein degradation through ubiquitin system

1.2.3.1 Muscle degradation in response to oxidative burst

The physiologic response to stressors increases the requirement for amino acids through the accelerated synthesis of acute-phase proteins in the liver, increased synthesis of proteins needed for immune function or synthesis of proteins associated with wound healing [109, 110]. Systemic inflammation is common in COPD patients [238], as the inflammatory response is essential for protection from infection. It is well established that both neutrophils and macrophages are attracted to the infection site in the lungs as well as elsewhere [239], and the role and potential mechanisms of immunonutrition in COPD were discussed earlier (see 1.2.1, page 46).

One of the mechanisms involved in inflammatory response involves phagocytic oxidative burst. This process is based on the oxidising potential of hypochlorous acid (HOCl) converted from chloride ion by superoxide anion. HOCl is a part of innate immunity, but overproduction or ineffective neutralisation by antioxidants can lead to oxidative stress and chronic inflammation [240]. To protect adjacent cells and tissues from HoCl, antioxidants are required to neutralise the effect of oxidative burst. One of the anti-oxidative mechanisms requires taurine (Tau) to generate taurine chloramine (TauCl) from HOCl. TauCl, which is more stable and less toxic than HOCl, decreases oxidative stress not only by limiting the amount of HOCl but also through suppressing respiratory burst (decreasing the activity of phagocytic cells) and by inducing expression of antioxidant enzymes (e.g.peroxyredoxin-1) [112, 113].

Respiratory burst has not only effect on phagocyted cells but also stimulates signal transduction, a process of activating signalling pathways by second messengers, kinases and phosphatases [241]. It has been demonstrated that nuclear factor κB (NF-κB) and mitogen-activated protein kinase (MAPK) signalling pathways were activated by the H2O2, a product of the respiratory burst [241, 242]. In turn, the NF-κB is involved in protein degradation. Considering the role of taurine in anti-inflammatory processes, and the fact that biosynthetic capacity to synthesise taurine (from methionine and cysteine in hepatocytes) declines with age and some pathological stages (e.g. sepsis) [111], it is possible that low taurine level is related to chronic inflammation and muscle wasting in COPD patients.

1.2.3.2 Secondary consequence of muscle wasting

The consequence of losing lean mass in regards to muscle mass could be twofold. Firstly, changes related to physically decreased muscle mass lead to limited physical activity. The evident role of the muscular system in physical activity explain the limitation of exercise when muscle mass is decreased. Functional impairment and increased risk of falls, furthermore declines physical activity. Consequently, the limited physical activity indirectly decreases muscle mass resulting in further decline in physical activity. Secondly, the smaller the size of the muscle compartment, the lower the activity of processes located in the tissue. Thirdly, small muscle mass cannot maintain its natural metabolism and starts to prioritise some processes over another which, at the time, appear less essential.

The decrease of muscle mass reduces the size of the metabolic pool with the ability of glucose uptake, branched chained amino acids (BCAA: leucine, isoleucine, and valine) degradation and metabolism [233] or efficiency of glycogenolysis. Decreased glucose uptake changes insulin metabolism and increases the risk of insulin resistance. If BCAA is not degraded in muscle, it may lead to decreases in their product levels (AcetylCoA and steroids synthesis) and possibly accumulation of amino acids up to toxic levels. Glucose synthesis in liver depends on alanine provided from the breakdown of muscle [243]. Potentially, a decrease of muscle mass reduces the level of those processes and decrease the efficiency of glycogenolysis.

There is no conclusive evidence on direction of relationship between nutrition status and COPD processes. Majority of presented mechanisms are based on the assumption that there is a direct link between the two elements. However, some may entertain the theory that deterioration of muscle structure and function has no direct effect on COPD outcomes and it happens independently, but in parallel with, other disease-related changes. There are several potential pathways linking nutrition and disease processes and outcomes, which are further discussed in the next section.

1.3 The role of nutrition in COPD - theoretical perspective

The diagnosis of COPD is usually made late in the disease development, often in the moderate or severe stage, once symptoms develop to the extent that limits patients everyday life [244, 245]. Lack of appropriate treatment, continuous smoking and potentially untreated exacerbation, cause a great burden to health and patients wellbeing in the pre-diagnostic period. However, inability to identify patients in the pre-disease or at an early stage of the disease, limits the ability to explore and understand mechanisms underpinning COPD development. For that reason, in this section a number of potential and hypothetical mechanisms and pathways is described, reserving the fact that some of the described mechanisms are based on limited evidence and are biologically and physiologically plausible, however, are only theoretical.

The ultimate aim of nutritional research in COPD would be to identify a pathway of changes and mechanisms of the disease, with detailed information and explanation of the role of different nutrients on the disease development and progression. Currently, available evidence and the complexity of COPD limits the understanding of the link between COPD and nutrition.

Relationship between nutritional status and disease severity, activity and outcomes could be through several different pathways: A) Poor nutritional status is instrumental for development and progression of COPD, therefore those with worse nutritional status have worse clinical outcomes; B) Poor nutritional status is a marker of the disease progression and severity, therefore poor clinical outcomes, driven by deterioration in the disease status, are indirectly related to poor nutritional status; C) Clinical outcomes are dependent on disease status, but for any given disease status those with worse nutritional status have worse clinical outcomes; D) Clinical outcomes are dependent on nutritional status, but for any nutritional status those with worse disease status have worse clinical outcomes (Figure 6).

Current evidence does not definitively support or refute any of the above hypothetical relationships, but for the purpose of this thesis, the assumption is that for any given disease status those with worse nutritional status have worse clinical outcomes (Figure 6C). Therefore, attention is now directed towards potential mechanisms by which difference in nutritional status could influence the clinical outcomes.

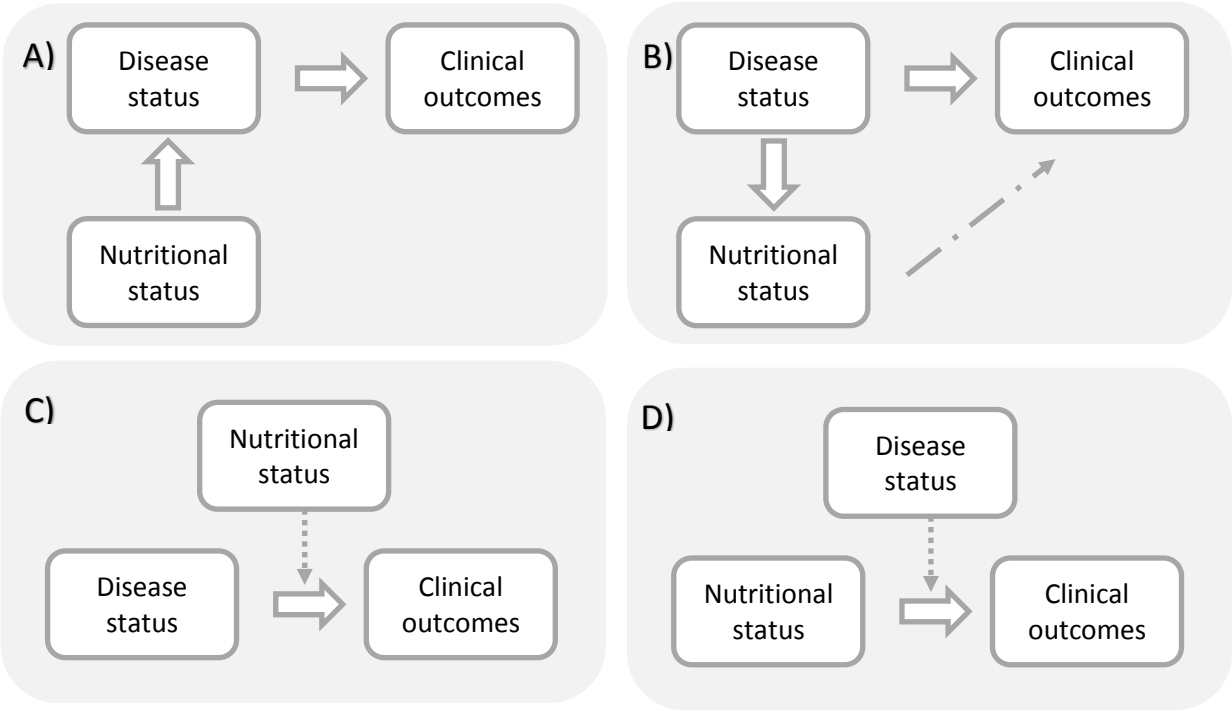


Figure 6 Potential relationships between nutritional status, disease status and clinical outcomes

Malnutrition in COPD, especially loss of FFMi, has been explored by many, and there is evidence suggesting that lean depletion is a prognostic indicator of poor outcomes in COPD [65-67], but the process and triggers of lean wasting and mechanism by which low amount of lean effects disease progression are still not fully understood and are not easy to determine or quantify. This complex process potentially results from changes in the control of metabolism, increased the cost of breathing, the imbalance between nutritional needs and intake or systemic inflammation and oxidative stress, but the mechanisms linking causes and loss of muscle mass are poorly understood [115, 246-248].

In this thesis, identified gaps in the evidence are explored using a large epidemiological study named Acute Exacerbation and Respiratory Infections in COPD study (AERIS). This was a unique longitudinal observational study of 127 COPD patients with high disease activity (record of exacerbations in the past year), followed up for 2 years with the first 12months analysed for this thesis, which allowed deep phenotyping of moderate to very severe COPD patients. The pre-set aims and objectives are detailed in the next chapter.

2 Research questions and aims

The central tenet being explored in this thesis is that differences in nutritional status in COPD patients are associated with differences in the development of the disease, progression of the disease and the response to treatment. A better understanding of how and why patients present with the differing nutritional state will aid clinical decision making, improve care and open new targets for interventions, resulting in improved patient outcomes.

To explore the central tenet, this thesis comprises of three core elements. First, a systematic review of literature was performed to explore the current evidence of nutritional status assessment in COPD patients. This systematic review focused on identifying the measurements of lean markers, how they were interpreted and their relationship to clinical outcomes.

Due to limitations identified in the systematic review for the methods used to assess nutritional status of COPD patients, an evaluation of these methods was deemed to be required. The second element of this thesis carried out this evaluation through a methods comparison study and quality control review. The aim of methods comparison study was to assess comparability of body composition assessment methods. The quality control review was performed in order to define inclusion and exclusion criteria for grip tests to be used in the local standard operating procedures.

Based on the results of the systematic review supporting the importance of the nutritional status in COPD patients, the third element of the thesis performed an assessment of nutritional status and its relationship to clinical outcomes. This was part of a longitudinal epidemiological study of a large COPD cohort, the AERIS study [249]. The nutritional component of the AERIS study encompassed baseline nutrition status characterization of COPD patients at enrolment and at multiple points over the next 12 months. During the study period, patients were provided with optimal medical care. COPD exacerbations were the primary clinical outcome; hence recruitment focused on patients with recurring exacerbations with at least one exacerbation in the 12 months prior to enrolment. During the study, patients' COPD status was assessed monthly, while nutritional markers were measured on a quarterly basis. Patients also attended the clinic at every COPD exacerbation. Measurements performed as a part of the study aimed to determine if the nutritional state was associated with patients' prior medical history, their disease severity, and activity, or their subsequent pattern and severity of exacerbations.

Research questions

Do patients with COPD who have a poor nutritional status have worse clinical outcomes (measured as time to first exacerbation (TTFE) and acute exacerbation rate (AER)) than COPD patients with good nutritional status and is this an effect that is independent of COPD severity and medical history?

Thesis aims

1. To explore to what extent poor nutritional status, measured with appetite, physical capability, and body composition, is associated with worse clinical outcomes in COPD in the 12 months follow-up.
2. To identify which of nutritional status markers best identify COPD patients at risk of worse, clinical outcomes as defined by time to first exacerbation and exacerbation rate over the 12 months.

Research questions

1. Does medical history, represented by the history of exacerbations in the past 12 months, influence nutritional status at baseline?
2. Does current disease status associate with current nutritional status (baseline)?
3. Does current nutritional status influence disease progression and clinical outcomes measured by the time to first exacerbation and acute exacerbation rate in the follow-up?

Outline of research questions is presented in the Figure 7.

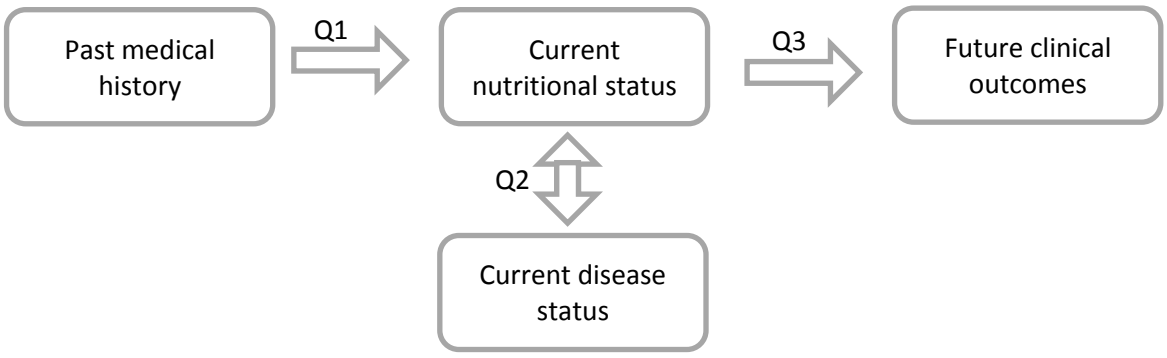


Figure 7 Outline of the research questions

Outline of thesis

To test the hypothesis and answer research questions, the results are divided into eight parts.

Systematic review of the role of nutrition status in COPD: Up to date number of publications explored nutritional status in COPD patients, therefore there was a need for a systematic review of current evidence. This chapter focused on exploring most commonly used methods of nutritional assessment (with focus on body composition and physical capacity markers). Assessment methods, reporting technique and relationship with the clinical outcomes in COPD were reviewed and reported.

Validation of selected methods used in nutrition status assessment for COPD patients – methods development: Based on the results of the systematic review a more detailed evaluation of body composition and physical capacity assessment techniques were required. A discrete study of body composition methods and evaluation of accuracy of grip tests were performed to explore comparability and quality of different methods.

Results: COPD severity and activity in the AERIS cohort: To understand the characteristics of patients in the cohort, their medical history, and severity of COPD, a description of clinical markers of COPD was presented. Characteristics of clinical outcomes of interest – time to first exacerbation and exacerbation rate, was analysed and compared with other respiratory markers. Further chapters focused on nutritional markers in the same COPD cohort.

Results: Exploration of body composition and its relevance in COPD: Previous studies did not explore the validity of body composition as a marker of TTFE in COPD, hence body composition was measured and in this chapter analysed to explore its relationship with clinical outcomes. This chapter aimed to identify which markers of body composition could aid in defining nutritional phenotypes, focused on clinical relevance. Because the methodology of body composition in the AERIS study was predefined, a validation study was set up and performed by the author to assess the difference in body composition results when using different techniques. The reliability and repeatability of data from the AERIS cohort were evaluated in comparison with four other methods.

Results: Exploration of appetite and its relevance in COPD: Taking the limitations of body composition assessment into consideration, appetite was chosen as a novel and simple nutritional marker that has potential to relate to clinical outcomes in COPD. This chapter analysed the utility of appetite score as an element of nutritional phenotyping in COPD. Appetite was measured at stable and exacerbation visits, therefore, results were discussed in contexts of stable COPD and during exacerbations.

Results: Exploration of physical capability and its relevance in COPD: Based on the supposition that the current nutritional status reflects what we eat, what we are and what we can do, this chapter focused on different physical capacity markers and their relationship with TTFE and AER outcomes in COPD. This chapter sought to explore which capacity tests have the greatest value in predicting clinical outcomes in COPD and could aid in creating a more complete nutritional picture of different disease phenotypes.

Multicomponent analysis of the exacerbation risk in COPD: This summative chapter sought to compare the clinical value of single nutritional when compare with the multicomponent approach, using nutritional markers identified in the previous chapters. Further exploration was then carried out to ascertain the simplest combination while maintaining its clinical accuracy at the identification of patients at high risk of poor clinical outcomes.

Summary, discussion, and future works: The value of obtained results and recommendation for future research were presented in this chapter.

3 Methods

3.1 Systematic review – methods

Detailed methods of the systematic review are described in the Chapter 4.1, page 89.

3.2 Comparison of selected methods used in nutritional status assessment for COPD patients – methods

Detailed methods of the comparison of body composition study and evaluation of a grip test quality assurance criteria are described in the Chapter 5.1, page 106.

3.3 AERIS study – methods

3.3.1 Overview of study design

To answer the key questions regarding the relationship between nutritional status and COPD, a longitudinal observational study, Acute Exacerbation and Respiratory InfectionS in COPD (AERIS) was used. Patients during study underwent a wide range of tests, but for the purpose of this thesis, only selected data were used including markers of nutritional status and COPD status. The nutritional assessment consisted of appetite score, physical capability, and body composition; COPD status was measured using lung function, inflammation markers, and various disease indices. All nutritional results were related to clinical outcomes focusing on acute exacerbations (AE) with a central focus on time to first exacerbation and exacerbation frequency.

3.3.2 Study description

AERIS study (ClinicalTrials.gov: NCT01360398) was a prospective, single centre, observational cohort study involving participants recruited from patient screening clinics, primary care, community services and outpatient departments. The study was conducted in Southampton General Hospital between June 2011 and June 2014 by a research team consisting of respiratory consultants, medical doctors, research nurses, laboratory technicians, scientists and the author was representing nutritionists. The AERIS study was a large investigator-led industry-sponsored project, with Dr Tom Wilkinson as a Principal Investigator. The author of this thesis had an opportunity to join the research team and explore the nutritional component in the AERIS cohort. However, the primary aims and the objectives of the AERIS project were focusing on the

microbiology of the exacerbations; nutritional component was included for better description of the cohort. This setup however, led to certain limitations in the design and results obtained for the purpose of the nutritional assessment. Regardless the limitations, the AERIS project has offered a unique opportunity to investigate nutritional status of COPD patients in a cross-sectional and longitudinal form. As a member of the multidisciplinary team delivering the AERIS study and as a nutritionist, the author has conducted nutritional assessments during the third of the study visits and supported the management of the nutritional data, including data entry, data cleaning and the creation of a data management plan.

After recruitment, 127 participants were seen monthly over 24 months follow-up, with additional visits within 72 hours of the onset of every acute exacerbation during the follow-up. The AERIS study was funded by GlaxoSmithKline, and a full description of the study procedures was published previously [249] (see Appendix N). The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and has been approved by the relevant institutional ethics and review board and the Southampton Ethics Board.

Standard medical care was provided by study clinicians during each visit, including medication review, smoking cessation advice and physical examination. Previous medical history, as well as patient-reported exacerbations, were checked by clinicians or respiratory nurse against the medical notes and GP records to reassure the accuracy of the past exacerbations frequency data.

Study subjects undertook a broad range of tests, measurements, and sample collection, but only selected results relevant to research questions in this thesis, were used in this analysis. Study measurements were performed with different frequency, and at different study visits, as detailed in the Appendix E. If a patient was found to be exacerbating on the arrival for a monthly visit, the regular visit was replaced with an exacerbation visit, and some tests were withdrawn for the patient's safety.

Considering the scale of the AERIS study, the uniqueness of its size, length, and structure the study addressed multiple aims and objectives. The overall AERIS aim was to estimate the incidence of all-cause acute exacerbations and of the acute exacerbations with sputum containing bacterial pathogens (overall and by species). In order to efficiently manage the study, each study team leader (microbiology, nutrition, imagining, and vaccinations) was responsible for delivery of specific aims. The author of this thesis, as a nutritionist, was focusing on the interactions between airway infection and systemic manifestations of COPD and nutritional status.

3.3.2.1 Inclusion and exclusion criteria

A number of inclusion and exclusion criteria were set to minimize heterogeneity in the AERIS cohort. The main inclusion criteria for the study participants were:

- confirmed diagnosis of COPD:
  - post-bronchodilator FEV_1 ≤80% of the predicted normal value;
  - FEV_1/FVC ratio ≤ 0.7;
- COPD stages from moderate to very severe (GOLD class 2 to 4);
- current or former smokers with smoking history ≥ 10 pack-years;
- minimum of one COPD exacerbation in the 12 months prior to enrolment, but recovered and ceased antibiotic and/or steroids treatment no less than 30 days prior to the first study visit.

The list of exclusion criteria included: confirmed diagnosis of asthma; α-1 antitrypsin deficiency; cystic fibrosis; tuberculosis; lung cancer; history of lung surgery and other conditions imposing pneumonia risk. Participants on long-term corticosteroid or antibiotic therapy at the time of enrolment were also excluded.

3.3.3 COPD markers and measures

COPD is diagnosed based on lung function obstruction and irreversibility of changes. COPD is recognized as a systemic disease with a greater range of symptoms than lung function impairment only, including persistent inflammation, potential eosinophilia, and common morbidities. The AERIS study has included both traditional measures of COPD (lung function) and systemic measurements: inflammation status, haematology profile, and full medical history. Data from all the participants enrolled to the study were included in the analysis, but only data relevant to the analysis plan and released by the study sponsor were included in the analysis.

3.3.3.1 Lung function

Lung function was measured as a primary indicator of disease severity in COPD. Forced Expiratory Volume in one second (FEV_1) and Forced Vital Capacity (FVC) were measured using MicroLab Spirometer at least 30 minutes post-bronchodilator, by a respiratory nurse.

Results from spirometry were used to categorise subjects according to the criteria from GOLD [28, 250]. According to these, COPD patients with FEV_1/FVC ratio of less than 70% can be stratified into 4 grades using a percentage of predicted FEV_1 (Table 4).

Table 4 Severity of airflow limitation categorised into COPD severity stages by GOLD

Stage		FEV_1
GOLD 1	Mild	≥80% predicted
GOLD 2	Moderate	50% – 80% predicted
GOLD 3	Severe	30% – 50% predicted
GOLD 4	Very severe	< 30% predicted

Each spirometry test was assessed for quality according to quality control criteria (Table 5). Only results with grade A or B were used in the analysis. Categorisation into category C was based on poor blows, which were caused by a slow start, short blow, coughing, extra breath or early stopping.

Table 5 Quality control criteria for spirometry in the AERIS study

Quality controls criteria	Quality categories
Repeatable (within 5% of highest FVC and FEV1) and all good quality	A
Either all repeatable (within 5% of highest FVC and FEV1) with some unacceptable blows, or all good blows and not repeatable (one of the two elements for quality criteria)	B
Poor blows and not repeatable (unacceptable)	C and below

In addition to the standard spirometry, a single breath diffusion test was performed to obtain transfer factor (TLCO; mmol/kPa/min) to measure the extent to which oxygen passes from the alveoli of the lungs into the blood. This test was performed by a respiratory technician.

3.3.3.2 Blood markers and inflammation

Blood samples were collected for the analysis of cell-mediated immune response, biomarkers, blood counts and haematology and RNA profiling, from all participants at study entry, quarterly, and at exacerbation. For the purpose of this project, only standard inflammation markers were used as no other markers were available at the time. Inflammation was identified by the presence of C-reactive protein (CRP using immuno-turbidimetric test), erythrocyte sedimentation rate by fibrinogen (using prothrombin-based derived fibrinogen assay) and interlukin-6 (IL-6 using high sensitivity ELISA). Local UHS laboratory reference range was 7.5mg/L CRP and 5g/L fibrinogen. As CRP values were recorded with no decimal places a level of 8mg/L was used as a cut-off point. For the fibrinogen levels two cut-offs were applied, 5g/L and 3.5g/L [251].

3.3.3.3 Exacerbation identification

In addition to routine visits, the research team conducted visits for exacerbating subjects within 72 hours of the onset of symptoms. An acute exacerbation of COPD (AE) was defined as a combination of at least two major symptoms (dyspnoea, sputum amount and sputum purulence)

or at least one major and one minor symptom (wheeze, sore throat, cold and cough) for two consecutive days [84, 252]. Exacerbations of COPD were captured via e-diary which participants were instructed to complete on a daily basis. The morning questionnaire consisted of a list of questions aimed at recording a worsening of symptoms within 24 hours i.e. “in the last 24 hours did you experience a worsening of breathlessness?”. When a combination of worsening symptoms persisted for at least 48 hours, a potential exacerbation of COPD alert was transmitted to all members of the research group. A responsible clinician contacted the patient to confirm exacerbation and arranged a visit within 72 hours of the onset of symptoms. Along with the exacerbation alert sent to the research team, the e-diary was also programmed to instruct the subject to contact research team as a precautionary measure. In few cases, exacerbation visits were missed when the research team was unable to see the patient within 72 hours. In such case, a second clinical opinion was obtained (phone call discussion with the patient) and if exacerbation was confirmed, the event was classified as a ‘missed exacerbation’. There was no additional post-exacerbation assessment. After the exacerbation visit participants returned to their regular visit schedule and attended regular monthly visits.

3.3.3.4 Medical history and medical review

At enrolment, every patient underwent detailed medical history review and physical examination including: body temperature; systolic/diastolic blood pressure; oxygen saturations; heart rate and respiratory rate after at least 10 minutes of rest. Smoking history was recorded at enrolment and smoking status was verified at every study visit.

3.3.3.5 COPD scores and indices

At enrolment, each patient was requested to fill a COPD assessment test (CAT), which is a validated self-administered instrument designed to provide a straightforward and reliable measure of health status in COPD patients [253]. The CAT comprises of eight items and has a scoring range of 0-40 points, with higher scores indicating a greater impact of the disease on patients’ life. Full questionnaire is presented in Appendix H.

Another COPD marker, a BODE score was used. It comprises of BMI, the degree of airflow Obstruction (FEV1%), Level of functional Dyspnoea (by modified medical research council dyspnoea scale) and exercise capacity by 6-minute walk test. The detailed scoring system is presented in the Appendix H. BODE is a simple multidimensional score for mortality risk, with the highest mortality risk among those with the highest BODE score [73]. The score was categorised into low risk group (BODE ≤4) and high risk group (BODE>4).

3.3.3.6 Clinical outcomes

For the purpose of this thesis, clinical outcomes were defined as measures of exacerbations – time to first exacerbation and exacerbation rate. These were chosen because currently exacerbation is recognised as an event that has the greatest influence on the COPD progression and patient health. Commonly used outcome measures, mortality, hospitalisation and length of hospital stay, were not appropriate for the analysis due to a small number of events in the follow-up.

3.3.3.6.1 Time to first exacerbation

Time to first exacerbation (TTFE) was defined as the number of days between the enrolment visit, and the first day of the first exacerbation. Using TTFE as a measure of clinical outcome allows the identification of which tests have the most value in identifying patients at high risk of future exacerbations in the near future. This could enable identifying which measures should be performed on a regular basis, to allow the most efficient monitoring of the disease activity.

3.3.3.6.2 Exacerbation rate

Exacerbation rate (AER) was calculated as the total number of exacerbations within the first year (before the 12^{th} month visit) divided by a number of days between the 12^{th} month visit and enrolment visit (expressed as one if the number of days was equal to 365). For patients who failed to complete full 12 months on the study, AER was calculated for the time the patient was active in the study. AER was estimated for the full 12 months assuming the same frequency of exacerbation for the remaining part of the year e.g. if a patient who had 2 exacerbations withdrawn after 6 months, his annual AER was 4.

For some analysis, patients are often categorised into infrequent and frequent exacerbators. The term ‘frequent exacerbators’ was first used in 1998 and was based on a median number of exacerbations per year (3 AE/y), which was applied as a cut-off point [71]. Acknowledging the limitations of such arbitrary chosen cut-point, exacerbations frequency was classified into two categories into frequent exacerbators (>2AE/year) and infrequent exacerbators (≤2AE/year), as previously used [71]. Considering that some patients experienced exacerbations every two months or even more often, an additional category within frequent exacerbators was defined as very frequent exacerbators with more than 4AE/year.

3.3.3.6.3 Severity of exacerbations

The severity of exacerbations was classified by clinicians according to patient information and prescribed treatment (Table 6).

Table 6 Exacerbation severity – criteria in the AERIS study

Exacerbation severity	Criteria
Mild	Worsening symptoms of COPD that are self-managed by the patient
Moderate	Worsening symptoms of COPD that require treatment with oral corticosteroids and/or antibiotics
Severe	Worsening symptoms of COPD that require treatment with in-patient hospitalisation or home care intervention

The analysis of the severity of an AE was complex, as patient experienced multiple exacerbations, often of different severity. As there was no agreed index of severity for multiple exacerbations, analysis of severity in this thesis was focused on the first exacerbation only.

3.3.4 Nutritional status markers

Nutritional status was assessed using anthropometric measurements with appetite score, physical capability tests, and body composition assessment. Measurements were performed according to local standard operating protocols (SOP), of which full list is in Appendix BAppendix E (Table 79).

3.3.4.1 Anthropometry

Anthropometric measurements included height, weight, mid-upper arm circumference, triceps skinfold thickness, and waist circumference. All measurements were performed in standing position in three repetitions with the mean of the three values used for analysis or otherwise specified.

Height was measured using a Leicester Height Measure stadiometer. The patient's head was positioned in Frankfort Plane and the height measurement was made at the point of maximal inhalation. The Frankfort Plane is believed to be the natural anatomical position of the human skull. It can be best thought of as a plane passing through the inferior margin of the left orbit (the point called the left orbitale) and the upper margin of the ear canal (the point called the porion). This methodology helped to obtain three accurate, precise and repeatable results, which were required to be within 2mm of one another. Weight was measured using Seca class III medical grade scales (model number 861, precision up to 0.05g) in light clothing.

Height and weight were used to calculate BMI. The BMI, together with proportion of unplanned weight loss and acute disease effect, was further used in a screening tool for nutritional risk, a 'Malnutrition Universal Screening Tool' (MUST) [147]. Detailed instruction of the score is in the Appendix H. A detailed description of other anthropometry measurements is presented in the Chapter 6.1, page 122.

3.3.4.2 Body composition

Body composition was assessed by Bioelectrical Impedance Analysis (BIA) [172, 254] using the Bodystat QuadScan 4000 with Bodystat BIA analysis software version 4.08. Tests were performed in a supine position, at least two hours after a meal. A tetrapolar surface electrode arrangement was used with self-adhesive disposable electrodes (Bodystat long electrodes) attached to the right hand (electrodes behind the knuckles and on the wrist next to the ulna head) and right foot (behind the toes and on the ankle at the level of and between the medial and lateral malleoli). The QuadScan was validated on a daily basis using a Bodystat-supplied resistor for which results must fit within the specified range of 500 ohms ± 4 ohms.

From the BIA tests a range of values were reported including raw data, as well as derived values e.g. fat-free mass or total body water. All results were recorded as provided by the QuadScan software. At the time of analysis only fat-free mass, fat mass and impedance at 50kHz were available (access to remaining raw data was limited by the sponsor), therefore, the analysis was limited to those variables, and calculation of fat free mass using any other than proprietary equations was not possible.

As previously suggested, fat mass and fat-free mass were adjusted for height as in the example below [255] and presented as fat mass index and fat-free mass index.

$$Fat\ Free\ Mass\ Index = \frac{Fat\ free\ mass\ [kg]}{Height\ [m]^2}$$

3.3.4.3 Appetite

To collect basic information on appetite and food habits the Council on Nutrition Appetite Questionnaire (CNAQ) was used. This is a patient administered, multiple choice, 8-items questionnaire with five answers given to each question (Figure 8). Each answer is given points (a=1, b=2, c=3, d=4, e=5) with the maximum score of 40 points. The CNAQ questionnaire was validated in the elderly population in the US showing that a score of ≤28 points was indicative of a significant risk of at least 5% weight loss within the next six months [211]. Previously validated criteria were used to interpret results with the aim to establish the most relevant cut-off point for COPD population that would relate to the risk of exacerbation.

There are three major advantages of CNAQ: it is simple and quick to fill by patients; the cost of implementing and using it in everyday practice would be very low; questions cover both appetite and food intake limitations (satiety, nausea), which to some extent overcomes the limitations of a single appetite question.

1) My appetite is:
   a) Very poor
   b) Poor
   c) Average
   d) Good
   e) Very good

2) When I eat
   a) I feel full after eating only a few mouthfuls
   b) I feel full after eating about a third of a meal
   c) I feel full after eating over half a meal
   d) I feel full after eating most of the meal
   e) I hardly ever feel full

3) I feel hungry
   a) Rarely
   b) Occasionally
   c) Some of the time
   d) Most of the time
   e) All of the time

4) Food tastes
   a) Very bad
   b) Bad
   c) Average
   d) Good
   e) Very good

5) Compared to when I was younger, food tastes
   a) Much worse
   b) Worse
   c) Just as good
   d) Better
   e) Much better

6) Normally I eat
   a) Less than one meal a day
   b) One meal a day
   c) Two meals a day
   d) There meals a day
   e) More than three meals a day

7) I feel sick or nauseated when I eat
   a) Most times
   b) Often
   c) Sometimes
   d) Rarely
   e) Never

8) Most of the time my mood is
   a) Very sad
   b) Sad
   c) Neither sad nor happy
   d) Happy
   e) Very happy

Figure 8 The CNAQ questionnaire

3.3.4.4 Physical capability

The physical capability was assessed using grip tests and walking distance.

Grip testing consisted of a strength test and an endurance test (both voluntary contractions) using the MIE Pinch/Grip Digital Analyser and the Clinical Analysis System (CAS) Software version 1.40. One piece of equipment was used in the study. The device was regularly tested, to check accuracy and, if necessary, recalibrated annually according to the SCBR Quality Assurance strategy.

Grip strength test was performed on the non-dominant side in three repetitions. The reported value was a mean of all high-quality repetitions (Newton), poor quality tests were rejected and not included in the calculation of the mean. If both strength and endurance tests were performed, grip strength test was conducted before the endurance test to avoid excessive muscle tiredness and reassure obtaining maximal grip strength. Grip endurance test was performed once only on the non-dominant side. The target value was established individually as 50 ± 5% of maximal voluntary contraction (MVC) as per local SOP. The threshold of 50% MVC was chosen as the blood flow through the muscles in the forearm is occluded when MVC reaches 60% [256]. Also, for isometric contractions tension of less than 15% MVC can be held for a very long time, while with increase to 50% MVC endurance time would be expected around 60 seconds [256].

Once the grip endurance test was started, participant was instructed to grip the handles for as long as possible whilst keeping the line displaying the force of their grip, within the green area, representing target value. Criteria for rejection of the test were discussed and detailed process was described in Appendix G.

Walking distance was measured with a six-minute walk test (6MWT) according to the ATS guidelines [220, 221]. Subjects were asked to walk at their own pace (which reflects the submaximal level of functional capability) for 6 minutes, and the total distance was measured based on 30m-long laps. Heart rate and oxygen saturation were measured during the test using fingertip pulse 'MIR Oxi' Pulse Oximeter. The subject was allowed to stop and rest if they experienced any pain or breathlessness, which was recorded. There was no practise or warm up performed, as per ATS criteria [220, 221].

3.3.5 Data analysis and quality assurance

High-quality data have been collected that has been shown to be repeatable and reproducible with low inter- and intra-observer variability. For each nutritional measurement, there was a Standard Operating Procedure prepared by a quality assurance manager in accordance with current international standards. For measurements which have no international standards (e.g. grip endurance), an internal quality process was initiated, and quality control criteria were defined. All measurements were performed by trained and competent measurers, who were re-evaluated on an annual basis. The quality of performed measurements was also monitored on a regular basis by a quality assurance manager.

All nutritional data were assessed after first 30 participants completed six months follow-up and performance standards were evaluated. Identified flaws were analysed, discussed and correcting procedures were implemented, including staff re-training and re-evaluation.

The quality of results depends not only on the quality of measurements but also correct coding and data entry. Therefore, the second step of the quality check was performed after the study completion, during the data cleaning process. To reassure high quality of information in the dataset, each variable entered into the database was compared against the original clinical research files for each patient.

To reassure that results obtained during each nutritional assessment were handled appropriately, a data management plan was prepared (Appendix O). The document included instruction and guidance on data entry, data coding and data cleaning, as well as storage and archiving plan. The document was prepared according to the guidance from the Quality Assurance Manager in Southampton SCBR and reviewed by a senior BRC researcher.

3.3.6 Statistical analysis

Data analysis was performed according to statistical analysis plan (Appendix P), which was developed with the guidance and quality assurance of the study statistician (Dr Ngaire Coombs). The analysis was performed using statistical software package SPSS 20.0, while GraphPad Prism 6 was used to prepare selected graphs.

Analysis of data was performed using a stepwise approach. First, simple descriptive statistics were used, e.g. what is the average, minimal and maximal age of COPD patients in this cohort. Results were presented as mean ± standard deviation (SD) for normally distributed variables and median and interquartile range (IQ) otherwise. Distribution was assessed using distribution graphs (histograms). Next, when applicable, difference of the same measure between two groups was tested e.g. does men and women have different lung function. Student's t-test and the Mann-Whitney U-test were used to compare normally and non-normally distributed data between two or more groups respectively, while Kruskal-Wallis test was used for comparison between multiple groups of non-normally distributed data. P values for the differences between groups and linear trends across the groups were analysed, taking $p<0.05$ as a threshold of significance. Further, if two variables were expected to present linear relationship, a Pearson (presented as 'r') and Spearman (presented as 'Rho') correlation analyses were undertaken to examine associations between normally and non-normally distributed variables, respectively.

After exploring variables within each domain, a relationship between measured variables and markers of clinical outcome were tested. To explore the relationship between various markers and TTFE (days to first event), several different analysis were performed. Kaplan-Maier graphs were used to plot TTFE for each individual with grouping individuals into two or more predefined (chapter specific) sub-groups e.g. do frequent exacerbators have shorter TTFE than infrequent

exacerbators. Differences between the sub-groups in TTFE were assessed using log-rank test. TTFE for each sub-group was reported using median expected TTFE, 95% confidence interval and p-value.

To explore the effect of more than one variable on TTFE, a Cox regression model was used. Tested variables were first inserted individually, to confirm or reject its relevance in tested model. Next, all variables that were identified to be relevant on an individual basis were used and entered together. If needed, interaction terms were explored. The relevance of tested models was assessed using hazard ratio (HR) and p-value, with the presentation of data using 95% confidence intervals.

A receiver operating characteristic (ROC curve) analysis was used to test how well tested variable can predict the occurrence of the event (exacerbation). The analysis was performed for 30, 100 and 365 days, which were arbitrarily chosen timeframes. The 30 days threshold (representing one month) was selected as a clinically relevant threshold. Information that a patient is highly likely to exacerbate in next 30 days gives clinician time to take steps to prevent the exacerbation. In addition, the introduction of potential intervention a month before the exacerbation leaves time for the intervention to have an effect, while e.g. 14 days window could be potential too short. The 100 days criteria (representing three months) was chosen as a sufficient warning about potential exacerbation to be identified by a patient himself, with time to seek medical help. A 365 days timeframe was used to understand the relevance of tested variable in a larger cohort, considering that majority of patients in the cohort have exacerbated by the end of year one.

The interrelation between variables, excluding clinical outcomes, was explored when appropriate. Venn diagrams were used to test the prevalence of co-occurrence of two or three selected markers, e.g. how many patients with low grip strength have also stopped during walking test.

This was an exploratory analysis of novel data, which resulted in a large number of comparisons. All the analysis were driven by plausible biological mechanisms and aimed to explore potential relationships. This exploratory approach was chosen to understand and describe features of nutritional phenotypes in COPD. This study was constrained by the study design of the primary study AERIS in that the number of subjects studied was not determined to directly address the nutritional questions. Therefore, risk of bias was high with type I and II errors being possible. Considering this was smaller sample than would be expected to answer research question in this thesis, obtained negative results are likely to be correct, while positive findings are especially at risk of type I error.

3.4 AERIS - Methods discussion

All research studies have restrictions related to techniques, populations or results interpretation. There is also a list of caveats related to study design, as the single project can only be tailored to address the main research question, while secondary aims face design imperfections. Limitations are inevitable in research projects, however, by considering them and taking into account during the data analysis and results interpretation, prevents from overstatements and generalisation beyond the actual research capabilities. In this thesis, the caveats were related to study design, methods used in the assessment of nutritional and COPD markers, as well as in the selection of clinical outcomes. The study design and available outcomes were beyond control, but techniques used for nutritional assessment were tested for accuracy and precision. Limitations related to exploration around body composition, appetite, and physical capacity are discussed in details in relevant chapters.

3.4.1 Study limitations

The AERIS study was designed to answer the main study objective (incidence rate of acute exacerbations of COPD), while nutritional explorations were the tertiary aim. Therefore, some of the nutritional status data were obtained with not optimal frequency (e.g. appetite score), while other markers might not have been powered to accurately answer nutritionally focused research questions.

Whatever the study design, it is chosen outcomes that determine quality and usefulness of generated results. In this cohort, there was a number of limitations related to selected clinical outcomes. The most robust clinical outcome in chronic diseases is mortality rate, but in this cohort, less than 10% patients died during the follow-up, so it was not applicable for the main clinical outcome. Also, considering that focus was on identifying ways to improve patients' quality of life, focusing on mortality would not be relevant. Therefore, alternative outcomes were chosen – TTFE and AER. To capture each exacerbation during the follow-up, an exacerbation monitoring process was required. Questionnaires filled by patients twice a day (morning and evening), as well as peak flow measured daily, were used to monitor changes in symptoms severity. Based on the daily results, potential exacerbations were identified and the patient was contacted to confirm changes in symptoms. If clinically confirmed, the patient was invited to the hospital and reviewed within 72 hours from the exacerbation onset. This type of setup required great determination, dedication, and motivation of patients, as well as flexibility to come to the hospital with short notice. Intensity and responsibilities of the patients were one of the reasons for initial dropouts,

however the majority of patients have proven great determination to continue with the study for 24 months with daily duties.

Identifying exacerbations soon after onset was proven to be as challenging, as estimating the duration of each exacerbation. Long-lasting exacerbations might have been considered as two exacerbations, because the duration of exacerbation was estimated based on patient-reported symptoms, rather than measures of e.g. inflammation. Hence, patient's attitude and coping mechanisms were potentially influencing prevalence and duration of exacerbations. Patients with a higher threshold for coping with symptoms fluctuation could have potentially missed mild exacerbation. Similarly, those with lower threshold were at risk of overestimating exacerbations and indicating exacerbation when natural progression and day-to-day variation was more prominent.

Time to first exacerbation was considered as a marker of high clinical relevance and therefore was used to test the predictive value of nutritional markers. AER was regarded as an informative and descriptive marker rather than influencing disease management on an everyday basis, like TTFE could potentially be. Those two outcomes are closely related. Those who have more exacerbations during the year are most likely to have shorter TTFE at any point, compared to those who experience fewer exacerbations overall. A patient who experiences one exacerbation per year, depending on the date of the last exacerbation, could have an event anytime between 1 to 365 days. For two exacerbations a year, it would be more likely to be between 1 and 180 days, while for four exacerbations or more, it suggests an exacerbation every 90 days or less. In real life, this is not as clear and proportional. However, represents a concept that for those with more than four exacerbations a year, AER would be more accurate and informative, than for those with less than two exacerbations a year.

TTFE and AER were used for different purposes. Testing markers against TTFE can aid in everyday practice and help identify patients with increased risk of an exacerbation within defined timeframe. This opens the possibility to individualised and targeted interventions to prevent exacerbation, to go beyond the treatment of developed infections. Those with more active disease and more frequent exacerbations would be expected to have shorter TTFE, simply because they have more exacerbations with shorter exacerbation-free periods. However, it is not only disease activity that would influence TTFE, but seasonality could also have an effect. There is evidence that COPD patients have more exacerbations and more of COPD patients experience exacerbations in cold seasons, with a peak in late autumn and late winter [47, 257]. This suggests that at any point in the 'cold' season the TTFE is likely to be shorter, independent of disease severity and activity, than if measured during the 'warm' season.

AER can be used to measure the overall impact of exacerbations on the disease progression and patients' everyday life. Testing markers against AER provide information about overall resistance to exacerbations, with an indication of which markers have the ability to identify patients with the more active disease.

Both TTFE and AER have drawbacks. Using TTFE alone carries a risk of misrepresenting patients who experience only one or two exacerbations a year, but the first exacerbation happened soon after the baseline assessment. In addition, in some patients disease activity fluctuates during the year with higher exacerbation frequency in winter, compared to summer. Therefore, if TTFE was assessed in summer, when the exacerbation-free period is extended, this would underestimate overall disease activity. AER is an overall statement of the observed 12 months. For patients who experience exacerbation less often than once a year, annual AER could overestimate disease activity if measured during the 'event' year, or underestimate if the last exacerbation happened shortly before the enrolment. AER is a summative statement and irrespective of relationship with tested markers, this would not enable the introduction of interventions to prevent exacerbation in the 'high risk' pre-exacerbation period.

During the study follow-up, some of the monthly visits were replaced with exacerbation visits. When that happened, the list of assessments and tests performed was limited to a minimum, in order to reassure patients' comfort during the chest infection. In such cases, the nutritional assessment was restricted to appetite score, while body composition and physical capability tests were excluded. This approach was necessary to maintain participants in the study, however it limited the opportunity to understand changes in nutritional status at the time of exacerbation.

AERIS study was a commercially sponsored project by GSK Biologicals SA. Consequently, some of the data were not available for the analysis, due to limitations introduced by the Sponsor. Limitation in data access was related to data cleaning process on the Sponsor's side (second-year data), as well as due to an ongoing review of selected nutritional data. In order to complete this thesis within the timelines, an arbitrary decision was made to use only data currently approved by the sponsor. Therefore, the data on body composition were limited to lean mass and fat mass with one raw impedance value, which limited ability to explore the relationship between phase angle or other impedance indices and disease status. In addition, data used in this thesis were limited to the first 12 months of the follow-up. There are various limitations when the follow-up is restricted to one year. Exploration of long-term changes in nutritional status in COPD, including full body composition dataset, will be assessed in the future, once whole study dataset is available.

3.4.2 Statistical analysis caveats

The cohort size was determined in accordance with the primary AERIS study objective, which focused on bacteria presence and exacerbations. Therefore, the cohort size was not determined to address nutritional research questions. Considering size and frequency of tests and measurements patients underwent during every study, some measurements had to be performed less frequently, in order to avoid large dropout. The pragmatic approach led to some restrictions in nutritional assessment, which to some extent limited specificity of nutritional research questions. A cohort of 127 participants with two sexes and three disease severities lacked the power to run comparisons between sub-groups of different sex and severity, due to small sub-groups sizes. Questions related to the change in appetite score around exacerbation would benefit from more frequent assessments.

One of the challenges in statistical analysis was the vastness of the data. The whole study generated over 180 million data points, therefore statistical analysis that would incorporate all aspects was impossible. In addition, many statistical tests assume a linear relationship between variables. The majority of nutritional and biological markers represent non-linear associations e.g. BMI and mortality risk is the highest at the lowest and highest BMI values representing a u-shape relationship, while the use of inhaler can only increase lung capacity until it reaches a plateau and regardless further inhaler dose increase lung capacity will not increase representing a peak-and-plateau type association. Therefore, results of statistical analysis assuming linear association (e.g. chi-square test) of nutritional data, which are likely to have non-linear association, required careful and thoughtful interpretation.

3.4.3 Other aspects

To allow for any data collected to be of the highest quality, the author received training from a certified trainer, in the methodologies and skills used to perform the measurements. Using skills and expertise, the author has performed comparison of used methods (grip and body composition) and evaluated the quality of nutritional measurements obtained by the research nurses. During the study course, the author was a point of contact for any queries related to nutritional assessment in the study. The author was actively involved in the data entry, data cleaning, and data quality check. The author performed all of the statistical analysis presented in this thesis, except for one analysis of the longitudinal data (random effect model).

4 Systematic review of the role of nutrition status in COPD

The first paper on nutrition status in COPD was published in the 1960s [258]. Over the last 50 years 1062 further papers were published in PubMed (search term 'nutrition COPD'; 03/02/2016). Thousands of COPD patients have their nutritional status measured in various ways, but there is no conclusion as to how the nutritional status interacts with the disease or what is the best way to improve someone's nutritional status.

Public interest in COPD and role of nutrition was growing exponentially over the years, with over 2.77 million results in Google (search term 'nutrition COPD'; 03/02/2016). Regrettably, only a small proportion of the nutritional information provided to the public is based on scientific evidence. Instead, many inaccurate or misleading opinions are expressed, such as: milk and dairy products increase mucus production and should be avoided by COPD patients [259], while evidence does not show any link between dairy products consumption and mucus production [260, 261].

A high interest in nutritional status of COPD patients has led to over a thousand scientific papers on nutritional status in COPD, but this research activity has not led to significant improvement of disease management or nutritional guidance for those patients. A significant change in nutritional status in COPD patients compared to healthy individuals suggests a relationship between the disease and nutrition. Various age, medical history and comorbidities, lifestyle and nutritional status at the onset of the disease and during the disease development – all play a role in the way organism adapts to this chronic condition. Also, previous research has focused on all COPD patients or only on those malnourished. This might have led to omission or misinterpretation of some crucial findings, considering that even the first historical COPD phenotypes, pink puffers and blue bloaters, often presents the opposite extremes of nutritional status (respectively visibly malnourished vs. high body weight). Therefore, understanding the role of nutritional wellbeing is challenging and at this stage, can only be hypothesised and disputed in general terms.

It could be suggested that for any given disease those with poorest nutritional status will have a worse prognosis. However, there is no clear definition what poor nutritional status is and how many nutritional markers need to be changed to have a serious effect on disease processes. Measuring weight does not give sufficient information because individuals with the same total body weight (70kg) could have balanced body composition (approximately 50kg lean & 20 kg fat) or excess of fat tissue masking lean depletion (e.g. 40kg lean & 30kg fat). It has been

demonstrated that as health deteriorates and body composition worsens, the ability to recover from acute illness also deteriorates and risk of mortality rises [66, 133].

Therefore, a multidimensional assessment is required to obtain information about nutritional wellbeing. Many available markers of nutritional status are surrogate in their nature and have some limitations, but each method has its use, importance, and role. Clinical use is dictated by time and cost-efficiency, whereas the research setting requires tools that provide most accurate and detailed measure to answer the research question. The set of nutritional markers used routinely in clinical setting, may be simpler, but less specific or sensitive, than those used in a research setting.

Assessment of nutritional status can reflect what we eat, what we are or what we can do. The choice of assessment technique depends on the questions that are asked and anticipated answers. There is no standardisation to what measurements should be used beyond BMI, no guidance to what nutritional domains could be most affected by a disease, which makes the choice of nutritional assessment technique very subjective. To understand what nutritional assessments are used most commonly in COPD, and what is the relationship between those markers and COPD, a systematic review of the literature was performed.

4.1 Methods

Many recent publications on nutritional status in COPD focus on malnutrition and wasting [64, 180, 184, 262-264], with great interest in body composition, lean depletion or sarcopenia [133, 136, 178, 265]. Therefore, a systematic review of methods used to assess nutritional status in COPD studies, with a focus on lean markers, was needed to understand what measurements were used, how they were interpreted and relationships to clinical outcomes that were considered. The review strategy was based on a PICO analysis (population, indicators, comparison and outcome analysis). The inclusion criteria were: the population of patients with any stage of COPD, stable or exacerbating, over 40 years old with no serious comorbidities. The question for the systematic review was: ' Are lean markers (structural and functional) able to predict clinical outcomes in COPD patients?'. Lean markers related to function (grip strength or grip endurance) or structure (anthropometry measures or lean mass) were selected as indicators, with outcomes established as frequency of exacerbations, time to next exacerbation, frequency of hospitalisation or mortality. Studies were included if they reported a single assessment or a period of follow-up. No limitations of study length or follow-up period were set to enable an inclusive assessment of current knowledge on lean markers and their relationship with clinical outcomes. Therefore, any observational studies related to the topic of this review were included, except for interventional studies. The only exemption was when an intervention was preceded by baseline assessment. In such cases results from baseline were included, but not from the follow-up.

Using MEDLINE (1946 to July 2013), EMBASE (1974 to Week 28 2013), EBSCO, Cochrane Library and Web of knowledge a systematic review was performed to identify potentially relevant studies published between 1993 and July 2013. To identify the largest number of relevant articles a broad search strategy was used with limitation to English-language publications only. The search terms and MeSH terms included: COPD, chronic obstructive pulmonary disease, emphysema, bronchitis, the length of stay, exacerbation, mortality, survival rate, lean mass, fat-free mass, FFM, FFMi, lean body mass (detailed list of search terms presented in the Appendix B). The electronic search was supported by scanning the reference list of included articles.

Exclusion criteria were grouped into three categories 1) interventions, 2) population differences, 3) insufficient data. Interventions were considered if patients were undergoing pulmonary or multidisciplinary rehabilitation, studies after lung transplant, studies using supplementation or nutritional support, drug effectiveness studies and pre- and postoperative studies. Population differences were considered when patients had an additional diagnosis of cancer, AIDS or HIV, diabetes, kidney diseases or pulmonary infections (pneumonia, influenza). Studies with irrelevant information or insufficient information were excluded when: i) BMI or BODE scores were used as

the only nutritional markers, ii) lean markers were not related to clinical outcomes or iii) body composition results did not include lean or muscle mass.

Studies were included if results of body composition or physical capability were compared to each other or/and to clinical outcomes measured by frequency of exacerbations, frequency of hospitalisation and length of stay or mortality rate.

Studies were initially screened by reading title and abstract and full texts were reviewed if studies were not screened out. The review was performed by a single researcher, but strict adherence to inclusion and exclusion criteria and clearly defined aims were implemented to limit bias. Any uncertainties were resolved through discussion amongst the author and supervisory panel.

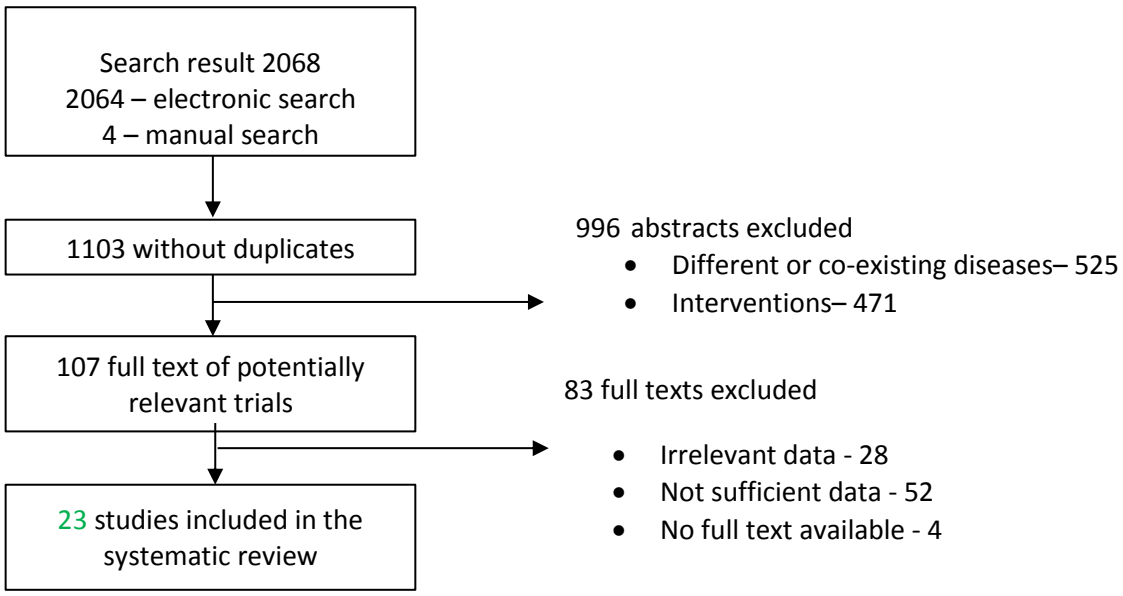


Figure 9 Flow chart of papers selection proces in the systematic review

The above consort diagram (Figure 9) describes search strategy with 2068 potentially relevant articles initially identified, reduced to 1103 after duplicates were removed. Title and abstract review excluded a further 996 articles because they did not meet inclusion criteria. Of 107 remaining studies 28 studies were excluded due to irrelevant data (different physical capability tests, lack of lean mass assessment), 52 trials did not present sufficient data (missing values for FFM or FFMi, lack of relationship with the clinical outcome) and four articles could not be retrieved in full text. A total number of articles included in this review is 23 of which four were identified by the manual search. Ten trials presented follow-up data between 6 months up to 7 years, and remaining 13 studies performed assessment on a single occasion. Nineteen studies performed an assessment in stable COPD and three assessed patients during an exacerbation. Stable COPD was defined as no exacerbations or medication change in previous two months (4 studies), six weeks (2 studies), three months (2 studies), or 6 months (single study) prior to the

assessment. In 6 studies the definition for "stable COPD" was not described and another four studies did not specify if subjects were screened due to their medical stability.

Clinical outcome was reported in 19 studies with information on hospitalisation (6 studies), exacerbations (3 studies) and mortality (7 studies). Basic information on study participants is summarised in Table 76, Appendix A, page 245.

For included studies the following data were extracted: sample size, age range, men percentage, follow-up period, use of oxygen (long-term oxygen therapy (LTOT) or non-invasive positive pressure ventilation (NPPV)), functional data ($FEV_{1,}$ hand grip, walking tests), body composition data (FFM, FFMi BMI), anthropometric data (MUAC, TSF, mid-arm muscle area (MAMA)), clinical outcome data (hospitalisation frequency, length of stay (LOS), exacerbations frequency, all-cause mortality). When data were not reported in the text but presented graphically, it was used as the source of data. In one study values of FEV_1 data recorded in percentage were considered improbable and assumed to be in millilitres. Assessment of papers quality, according to CASP checklist [266], was performed and indicated overall low and medium quality of studies (Table 7).

Table 7 Assessment of the quality of papers included in the systematic review using CASP checklist [266] for cohort studies

Reference	Q1	Q2	Q3	Q4	Q5	Q6	Q9	Q10	Q11	Overall quality
Ansari 2012 [265]	y	y	y	ct	n	n	y	ct	y	low
Benedik 2011 [136]	y	y	y	y	n	n	y	ct	y	medium
Budweiser 2008 [184]	y	y	y	y	n	n	y	ct	y	medium
Coleta 2008 [267]	y	y	y	ct	n	n	y	ct	y	medium
Cortopassi 2011 [186]	y	y	y	y	n	n	y	ct	y	medium
Eisner 2007 [132]	y	ct	y	y	n	n	y	ct	y	medium
Faganello, 2010 [268]	y	y	y	ct	n	ct	y	ct	ct	low
Gelamo Pelegrino 2009 [264]	y	y	y	ct	n	n	y	ct	y	medium
Giron 2009. [67]	y	y	y	n	n	n	y	ct	y	medium
Hallin 2011 [180]	y	y	y	y	n	n	y	ct	y	good
Heijdra 2003 [137]	n	ct	y	ct	n	n	y	ct	y	low
Hitzl 2010 [65]	n	ct	n	y	n	n	y	ct	y	low
Marino 2010[269]	y	ct	n	y	n	n	y	ct	y	medium
Kurpad 2006 [270]	y	y	n	y	n	n	y	ct	y	low
Mehrotra 2010 [271]	y	y	y	n	n	n	y	ct	y	medium

Reference	Q1	Q2	Q3	Q4	Q5	Q6	Q9	Q10	Q11	Overall quality
Sabino 2010 [139]	y	y	n	n	n	n	y	ct	y	low
Schols 2005 [66]	y	y	y	y	n	n	y	ct	y	good
Soler-Cataluña 2005 [272].	y	y	y	y	n	n	y	y	y	medium
van Wetering, 2008 [273]	y	y	n	n	n	n	y	y	y	low
Vermeeren 2006 [178]	y	ct	n	n	n	n	y	ct	y	low
Vestbo 2006 [150]	y	y	y	n	n	n	y	ct	y	medium
Vilaro 2010 [179]	n	y	n	y	n	y	y	n	y	low
Waschki 2011 [274]	y	y	y	n	n	n	y	ct	y	medium

Y – yes; n – no; ct – can't tell
Q1 Did the study address a clearly focused issue?
Q2. Was the cohort recruited in an acceptable way?
Q3. Was the exposure accurately measured to minimise bias?
Q4. Was the outcome accurately measured to minimise bias?
Q5.Have the authors identified all important confounding factors?
Q6-Was the follow up of subjects complete and long enough?
Q9. Do you believe the results?
Q10. Can the results be applied to the local population?
Q11. Do the results of this study fit with other available evidence?

After the extraction of data, it was not appropriate or feasible to perform a meta-analysis or even summary statistics due to extreme heterogeneity of results, reporting formats and grouping strategies, as described in the results section.

4.2 Results

4.2.1 Systematic review of body composition assessment methods

Nutritional status was measured in many COPD studies, with recent publications focusing on the function of lean mass in COPD patients. Considering that lean mass can be measured in various way, this systematic review aimed to review the most commonly used lean markers, both structural and functional. The need for such review was identified after multiple lean depletion cut-offs were found to be used interchangeably. With a selection of the most appropriate (informative) FFMi cut-off in mind, the focus was on the methodology of measurements– techniques and equipment used to measure lean mass, the format in which data were reported and the criteria against which data were interpreted, as well as its relationship with mortality, hospitalisation, and exacerbations.

Out of 23 included studies, 19 focused on lean mass measured with bioelectrical impedance analysis (BIA) [65-67, 132, 136, 137, 139, 150, 178-180, 184, 186, 264, 267-269, 273, 274], while two studies used Durnin & Womersley body composition tables [170] to estimate lean mass [270, 272]. None of the studies that met the inclusion criteria were using DXA, while many of the rejected studies did. Body composition in reviewed papers was assessed using 13 different BIA machines. 12 different equations were applied to estimate FFMi (Table 8) and results were interpreted in various ways with 5 different FFMi cut-off points for lean depletion which were derived using various methods and cohorts (Table 9). COPD studies most commonly used FFMi cut-offs of FFMi <16kg/m^2 for men and FFMi<15kg/m^2 for women. These cut-points were developed using the study by Schols [275], and they were determined by the linear association between FFM and body weight in normal-weight to underweight patients. Based on the assumption that FFM adjusted for ideal body weight represents 63% total body weight for women and 67% for men, FFM values were estimated and then adjusted for body height. There was inconsistency in the use of cut-offs, as some of the studies were inclusive of the cut-off value (≤) and some were not (<). Another FFMI interpretation was based on sex-specific values of the lowest 10^{th} centile of the total population sample [150]. The FFMi cut-off was established at the level of <17.05 kg/m^2 for men and <14.62 kg/m^2 for women. Another alternative cut-off of 17.4 kg/m^2 for men and 15.0 kg/m^2 for women was also used, although methodology of its development was not clear [276].

Table 8 Equipment and equations used to measure body composition in studies included in the systematic review

study	BIA Equipment	Equation for calculation of fat-free mass
Hallin 2011 [180]	Hydra EFC/ICFXitron 4200, Xitron Tech, USA	Body density estimated; Body fat = (2.127/body density)-1.4 FM=body fat *(W-TBW); FFM=W-FM
Giron 2009 [67]	BIA 101 RJL Akern, Italy	NA, "manufacturer software"(BiaVectorBodygram)
Marino 2010 [269]	Ironman, Tanita, UK	NA, "following manufacturer recommendations"
Ansari 2012 [265]	BIA NUTRIGUARD-M, Data Imput GmbH, Germany	-11.81+0.245*W+0.298*H^2/I+0.148*H+5.284*sex (M=1, W=0)
Gelamo Pelegrino 2009 [264]	BIA 101, RJL Systems, USA	-6.06+H*0.283+W*0.207-R*0.024+sex*4.036 (M=1, W=0)
Sabino 2010 [139]	Biodynamics 310, Biodynamics corporation, USA	
Faganello 2010 [268]	BIA 101A RJL Systems, USA	
Coleta 2008 [267]		NA
Schols 2005 [66]	Xitron Technologies, USA	NA
Van Wetering 2008 [273]	Bodystat 1500, Bodystat Ltd, Britain	NA
Vermeeren 2006 [178]		M: 8.383+0.465*H^2/R+0.213*W W:7.610+0.474*H^2/R+0.184*W
Cortopassi 2011 [186]		NA, probably manufacturer software
Vilaro 2010 [179]		NA
Eisner 2007 [132]	BIA RJL systems quantum II , USA	M: 3.587 + 0.326*(H^2/R) +0.304*(W) + 0.136*× Xc W: 5.161 + 0.439*(H^2/R) +0.152*(W) + 0.053 * Xc
Benedik 2010 [136]		N/A, probably manufacturer software
Hitzl 2010 [65], Budweiser 2008 [184]	STA/BIA 101 Medical Inc, Germany	-4.104+0.518*H/R+0.231*W+0.130*Xc+4.229*sex (M=1, W=0)
Vestbo 2006 [150]	BIA-103 RJL Systems, USA	M: 6.493+0.4936*(H^2/R)+0.332*W W: 5.091+0.6483*(H^2/R)+0.1699*W
Heijdra 2003 [137]	BIA RJL systems quantum , USA	Body density estimated; %Body fat =100[(4.570/body density)-4.142] FM=%body fat *(W)/100; FFM=W-FM

H – height, W – weight, R – resistance, Xc – reactance, I – impedance, M-men, W-women, TBW – total body water, FM- fat mass, FFM – fat-free mass

Table 9 Characteristics of the studies based on which FFMi cut-offs for lean depletion were derived

	Population			FFMI Cut-off [kg/m^2]		Cut-off methodology	Age specific	Weight adjusted	Measurement technique	Equipment
	Condition	Age [y]	N (%M)	M	W					
Schols 1993 [275]	COPD, moderate to severe; stable	65±8 M 62±9 W	255 (80)	16	15	Metropolitan Insurance Life Tables + Assumption Lean=63(67)% body weight	No	No	FFM patients specific regression equation generated in 32 normal to underweight patients with COPD	BIA 101, RJL systems, Detroit, MI, supine, right side.
Kyle 2001 [276]	Healthy	22 - 94	343 (59)	17.4	15	Not obvious	Possibly	No	% IBW from 1983 Metropolitan LIT (midpoints for medium frame for each height), mean for all ages > 100% of IBW	5kHz Xitron 4000B generator (Xitron technologies San Diego, Ca, USA)
Kyle 2003 [277]	Healthy	18-98y,	5629 (53)	16.7	14.6	FFMI values for BMI=18.5 defined as a cut-off. *	No	Yes	Standard BIA	Bio-Z2, Spengler, Paris, France used as a generator, 50 kHz
Vestbo 2006 [150]	COPD - poor definition		1898 (≈50)	17.05	14.6	The lower 10th percentile derived from the total CCHS population sample (not only COPD), determined for men and women separately.	No	No	Standard BIA	BIA-103 RJL (RJL Systems, Clinton TWP., MI) with a 30kHz microamper device
Coin 2008 [278]	healthy		1866 (23)	17.8	14.6	10th percentile for 60-69 y men and women in the study population	Yes	No	retrospective, whole body scan dual-energy X-ray fan beam densitometer with dedicated software	DXA (Hologic QDR 4500W)

4.2.2 Systematic review of body composition results and reporting format

Extracted data were reported as FFM in kilograms, FFM in percentage of total body weight or FFMi. Out of 10 studies that reported FFM [67, 132, 136, 137, 186, 267-270, 272], only two provided results by sex [137, 270] (Table 10), while out of 13 studies reporting FFMi [65, 66, 137, 139, 150, 178, 184, 264, 267-269, 273, 274], only 6 provided sex-specific results [65, 66, 137, 150, 184, 273] (Table 11). Average FFMi among included studies was in the range of 15.9 ± 3.1 kg/m^2 up to 17.2 ± 2.2 kg/m^2, with range of 16.8 ± 2.4 kg/m^2 to 19.8 ± 2.8 kg/m^2 for men and 15.0 ± 1.9 kg/m^2 to 18.2 ± 2.7 kg/m^2 for women. Prevalence of nutritional depletion or lean depletion varied between the studies and was based on a range of criteria (Table 12).

Table 10. Body composition (fat-free mass) in COPD cohorts included in the systematic review (mean±SD)

	Fat free mass				
	Total [kg]	Total [%]	Grouped [kg]	Men	Women
Marino 2010 [269]	44.2 ± 7.7	x	x	x	x
Eisner 2007 [132]	49.3 ± 12.6	x	x	x	x
Soler-Cataluna 2005 [272]	52.5 ± 12.2	x	x	x	x
Giron 2009 [67]	47.8 ± 7.8	68.2 ± 9.1	x	x	x
Benedik 2011 [136]	40.2 ± 18.9	50.0 ± 13.0	x	x	x
Faganello 2010 [268]	42.6 ± 7.1	x	41.8 ± 7.8 EX 43.5 ± 6.1 NONEX	x	x
Coleta 2008 [267]	x	68.6 ± 9.3	68.5 ± 9.6% S 69.2 ± 7.8% NS	x	x
Cortopassi 2011 [186]	62 ± 10.5	x	x	x	x
Kurpad 2006 [270]	41.2 ± 5.6	x	x	41.2 ± 5.6 kg	x
Heijdra 2003 [137]	x	x	x	61.2 ± 9.3 kg 83 ± 7%	48.3 ± 8.3 kg 69 ± 8%

FFM – fat-free mass, S – survivals , NS – non-survivals, EX – exacerbators, NONEX – non-exacerbators,

Table 11. Body composition (fat-free mass index) in COPD cohorts included in the systematic review (mean±SD)

	Fat-free mass index [kg/m^2]			
	Total	Grouped	Men	Women
Vermeeren 2006 [178]	17.2 ± 2.2	x	x	x
Sabino 2010 [139]	x	13.7 ± 0.5 BMI<18.5 17.3 ± 0.3 BMI>25 15.3 ± 0.3 BMI 18.5-24.9	x	x
Faganello 2010 [268]	16.2 ± 2.0	16.0 ± 2.2 EX 16.4 ± 1.8 NONEX	x	x
Marino 2010 [269]	16.6 ± 2.3	x	x	x
Coleta 2008 [267]	15.9 ± 3.1	16.1 ± 3.2 S 15.1 ± 2.4 NS	x	x
Waschki 2011 [274]	x	18.9 ±2.6 S 17.6 ±2.4 NS	x	x
Gelamo Pelegrino 2009 [264]	16.1 ± 2.1	x	x	x
Vestbo 2006 [150]	x	17.2 G1 17.3 G2 17.1 G3 16.1 G4	18.7	16.0
Budweiser 2008 [184]	x	x	18.2 ± 2.0	15.0 ± 1.9
Hitzl 2010 [65]	x	x	18.2 ± 2.0	15.0 ± 1.9
Heijdra 2003 [137]	x	x	19.8 ± 2.8	18.2 ± 2.7
Schols 2005 [66]	x	x	16.8 ± 2.4	16.1 ± 2.5
Van Wetering 2008 [273]	x	x	17.8 ± 1.7 G2 17.0 ± 1.5 G3	16.9 ± 2.2 G2 16.3 ± 2.2 G3

S – survivals , NS – non-survivals, EX – exacerbators, NONEX – non-exacerbators, G1, G2, G3, G4 – GOLD stages

Table 12 Prevalence of weight and lean depletion with criteria of depletion in COPD cohorts included in the systematic review

STUDY	Depletion defined by BMI			Depletion defined by BMI & FFMI	Depletion defined by FFMI		
	% (no.)						
	T	W	M		T	W	M
Sabino 2010 [139]	22 (7)	x	x	x	x	x	x
Gelamo-Pelegrino 2009 [264]	x	x	x	x	52.9	55	47
Hallin 2011 [180]	55	66	29	x	x	x	x
Vestbo 2006 [150]	x	x	x	13.1: normal /↑BMI, ↓FFMI 0.7: ↓BMI, FFMI >p10 2.4: ↓BMI, ↓FFMI 50: normal BMI, ↓FFMI	x	24.6 15.8	2.9 15.1
Marino 2010 [269]	3.8 (1)	x	x	x	x	30	31.3
Giron 2009 [67]	x	x	x	38 T	x	x	x
Van Wetering 2008 [273]	x	x	x	16 (20): G2 23 (12): G3	15 (19) G 2 21(11) G 3		
Vermeeren 2006 [178]	x	18	10	15: (25W, 11M) NW&↓FFMI 1: ↓BMI & normal FFMI 11: ↓BMI & ↓FFMI	x	40	20
Schols 2005 [66]	x	x	x	29M, 27W: Cachexia 6 M, 5W: Semi-starvation 10M, 10W: Muscle atrophy 56M, 59W: No impairment	x	x	x
Heijdra 2003 [137]	x	x	x	x	3	5.3	x
Soler-Cataluna 2005 [272]	x	x	18.8 (18)	x	17.7 (17)	x	x
Waschki 2011 [274]	x	x	x	x	9.5 (13) S 26.9 (7) NS		
Budweiser 2008*[184]	16.1 (15)	x	x	x	35.4 (33)	x	x
Hitzl 2010*[65]	16.1	x	x	x	35.4	x	x
Kurpad 2006 [270]	62.5 (15)	x	x	x	x	x	x

T – total, M-men, W-women, TSF-triceps skinfold, MAC-mid arm circumference, FMI- fat mass index, NW-normal weight, OW-overweight, MAMA-mid arm muscle area, S-survivors, NS – non-survivors,

Functional markers of lean mass reported in reviewed papers included a hand grip strength test in 9 studies [137, 178, 180, 186, 265, 267, 269, 271, 273] but none looked at grip endurance. Hand grip tests were performed using 5 different dynamometers and combination of different operating procedures: in sitting or standing position, with straightened or bent arm, on various sides (left, right, dominant, non-dominant) and a number of repeats (one, three, four). Studies

were reporting mean value of all repeats, the sum of results from both hands, a single maximal score either from all performed tests (on both left and right hand) or from one selected side (Table 13). Interpretation of study results was also inconsistent, with reporting of raw values without interpretation or with results presented as a percentage of predicted value based on reference ranges for healthy adults.

Table 13 Handgrip test results in COPD cohorts included in the systematic review (mean±SD)

STUDY	Hand grip strength			
	total	men	women	% predicted
	[kg]			
Vermeeren 2006 [178]	31 ± 9 D 38 ± 11 ND	x	x	x
Hallin 2011 [180]	20.4 ± 6.5 D 27.7 ± 9.8 ND	x	x	x
Marino 2010 [269]	30.5 ± 8.0	x	x	x
Coleta 2008 [267]	26.8 ± 10.1 T 27.5 ± 10.2 S 23.0 ± 9.3 NS	x	x	x
Cortopassi [186]	37.8 ± 7.5		37.8 ± 7.5	
Van Wetering 2008 [273]	x	x	x	77.7 ± 18.8 G2 81.5 ± 14.7 G3
Mehrotra 2010 [271]	x	39.5 ± 8.2 S 37.7 ± 9.0 NS	22.7 ± 5.3 S 21.2 ± 4.0 NS	x
Heijdra 2003 [137]	x	38.9 ± 9	21 ± 9	97 ± 32
Ansari 2012 [265]	x	55.46 ± 17.17 T 24.71 ± 8.8 L 30.75 ± 10.19 R	35.86 ± 9.24 T 16.0 ± 5.05 L 19.8 ± 5.01 R	71.96 ± 21.75 M 81.05 ± 18.26 W

S – survivals , NS – non-survivals, T – total, EX – exacerbators, NONEX – non-exacerbators, G2, G3, – GOLD stages, D- depleted, ND – non-depleted, M-men, W-women, L-left, R-right

4.2.3 Systematic review of the relationships between body composition and clinical outcomes in COPD

There is emerging evidence that lean markers have potential value as a predictive marker of clinical outcomes among COPD patients (Table 14). The majority of studies that evaluated relationship between lean markers (structural and functional) suggested a potential value of lean measures to be marker of mortality [2, 65, 66, 150, 267, 271, 274] or hospitalisation [67, 136, 270, 273], but this requires further investigation.

Handgrip was found to be a valuable predictor of hospitalisation [273] and exacerbation [265] but not of mortality [267, 271], although results might be skewed because of a broad range of test performance techniques. No studies have considered the potential value of any of reviewed markers as a predictor of time to next exacerbations.

Table 14 Predictive values of structural (fat-free mass index (FFMi)) and functional lean markers (handgrip strength (HG), 6-minute walk test (6MWT) and clinical outcomes in COPD patients – summary of a systematic review

	Study	HG	FFMI
Hospitalisations	Van Wetering 2008 [273]	++	
	Kurpad 2006[270]		+
	Benedik 2011 [136]		-
	Giron 2009 [67]		+++
Exacerbations frequency	Ansari 2012 [265]	+++	
	Faganello 2010 [268]		
Mortality	Vestbo 2006 [150]		+
	Hitzl 2010 [65]		+++
	Soler-Cataluna 2005 [272]		+
	Schols 2005 [66]		+++
	Waschki 2011 [274]		-
	Coleta 2008 [267]	-	-
	Mehrotra 2010 [271]	-	

+ positive relationship $p>0.05$, ++ $p<0.05$, +++$p<0.01$
- lack of relationship, empty cell – no results available

4.3 Discussion

There is a growing body of evidence exploring the relationship between poor nutrition and COPD patient outcomes. The nutritional challenges in COPD relate to both malnutrition and obesity (including sarcopenic obesity), chronic and/or acute changes in appetite, as well as limitations in functional capability. Deterioration of some or all of those markers relates to deterioration in the patients' quality of life and increase in risk of poor clinical outcomes. Previous results suggest worse clinical outcomes in those with lower FFMi, therefore, the aim of this review was to assess in a structured and systematic way if structural or functional markers of low lean mass can be used as a predictor of clinical outcomes like exacerbations, hospitalisation and mortality.

This review has led to three main findings. First, lean depletion was reported within a 10-fold range (from less than 5% to over 50%) depending on the applied methodology; and 6 out of 9 studies have shown relationship between low lean mass (FFMi) and exacerbations or mortality. Secondly, out of 9 studies measuring grip strength, only in 4 studies did the results allowed comparison of grip strength with clinical outcomes, 2 of which showed relationship with hospitalisations and exacerbation frequency, but none have shown relationship with mortality. Thirdly, no results for grip endurance were available and none of the reviewed studies used TTFE as a clinical outcome.

The evidence to date is not consistent with showing that COPD patients with low FFMi have a higher risk of hospitalisations and mortality. In studies included in this systematic review, body composition was most commonly assessed using BIA. Results exposed significant variation in the methodology of the measurements and inconsistency in data presentation with combination of various categories and sub-groups (whole cohort, sex specific, BMI-specific, average for lean depleted or non-depleted, for groups divided by exacerbation history). It now becomes a feasible hypothesis, that the lack of consensus on the role of body composition in COPD, especially FFMi, could be largely determined by methodological heterogeneity.

To clarify the confusion about terms relating to lean depletion, various international groups have been working on a consensus for a definition and diagnostic criteria for lean depletion including: European Working Group on Sarcopenia in Older People (EWGSOP) [63], ESPEN Special Interest Group [190] and International Working Group On Sarcopenia (IWGS) [279] and Foundation for the National Institutes of Health Sarcopenia Project (FNIHSP) [280]. The summary of sarcopenia definitions from those four groups is shown in Appendix D, Table 77. Despite the efforts to gain consensus, there are still four different criteria to identify sarcopenia, none of which have been implemented into clinical practice in the UK. Diagnostic criteria, are not entirely applicable to the

clinical settings. Also, lack of evidence of the relevance of chosen cut-offs with clinical outcomes in different conditions needs addressing. Lean mass measured as appendicular lean mass (ALM) using DXA, is the most challenging aspect from a clinical perspective – it is feasible in research, but not in everyday practice. Simpler and cheaper measurements need to be chosen to assess lean mass in order to diagnose sarcopenia on an everyday basis.

Considering that BIA was the method of choice of most research groups, there is a need for standardised operating procedures for BIA measurement. Body composition assessed with BIA requires standardisation in terms of the choice of equipment, approach to measurement of resistance and reactance, algorithm to derive estimate of TBW and FFM and interpretation of results, including an international agreement for lean depletion cut-offs. There is also an urgent need to standardise data reporting including sex-specific result. Reporting body composition as an average for men and women jointly is fundamentally flawed, as demonstrated significant difference in lean mass between men and women. Otherwise, average lean mass is determined by the proportion of men (higher lean) and women (higher fat) in the cohort and does not reflect potential body composition abnormalities related to the disease.

Functional markers, specifically grip strength, has demonstrated similar heterogeneity of methods and reporting as shown for FFMi. No two studies assessing grip strength have followed the same protocol, with differences in every aspect of measurements – number of repeats, hand used or even derivation of the final value (single highest value, mean or sum). There is a need to standardise this to allow collection and comparison of results in the future.

In summary, lean mass and grip strength, but not grip endurance, were measured in multiple COPD studies but only some of the reviewed studies showed relationship between lean markers and clinical outcomes. There was a great variability in the measurement methodology and variability of data presentation methods; hence, results did not qualify for meta-analysis. There is a need to bring standardisation and unification into COPD research when assessing and interpreting body composition results, with focus on carefully selected methods and interpretation criteria defined based on relationship with the most important clinical outcomes, including risk of mortality but also time to next exacerbation.

The systematic review has exposed a gap in the evidence of the relationship between nutritional status and clinical outcomes, especially TTFE in COPD patients, showing the need for such explorations. What remains unclear is what is the direction of causality between low lean and clinical outcomes. Additionally, there is insufficient evidence to determine whether COPD patients with worse nutritional status are more likely to exacerbate in the future. Based on the supposition

that low lean mass and poor appetite can cause physical weakness, appetite and physical capacity appeared to be of interest.

5 Comparison of selected methods used in nutrition status assessment for COPD patients

The systematic review of the nutrition status assessment techniques in COPD patients has shown great variability of methods used for body composition measurement. Use of BIA was shown to be most common; however DXA was also used frequently. BIA and bioelectrical spectroscopy (BIS) are based on the assumption that lean tissue contains 73% water and electrolytes, so can act as an electrical conductor [172]. By measuring the physical properties, resistance and reactance of the human body, the volume of extra- and intra-cellular water can be calculated using regression equations derived from large cohort studies. This enables calculation of lean mass, and by using total body weight, it provides quantification of fat mass. Fat tissue is the main resistor in the human body hence impedance will increase with fat content, but results also depend on hydration status (lean mass underestimated in dehydration) and body temperature (lean mass overestimated in a higher temperature). In contrast, DXA relates to differences in tissue photon attenuation and it measures differential absorption of x-rays of two different energies [174]. All three methods are surrogate markers of body composition and require accurate and carefully developed equations to translate physical properties into lean mass. To determine the extent to which body composition results by BIA are comparable to other methods, the comparison described in this chapter was proposed.

A number of validation studies have looked at reliability of body composition measurements and some focused on COPD patients. Based on a validation against age- and BMI-matched controls, Balasio et al concluded that multi frequency BIA can be useful for assessing body composition in COPD patients [281]. However contradictory to this, in 2013 a cross-validation between BIA, BIS and DXA in stable COPD patients suggested that BIA underestimated FFM, and BIS showed FFM difference of more than 9 kg when compared to DXA [282]. This comparison which was performed in an overweight cohort, and another study focusing on severely obese patients demonstrated differences between BIA and DXA so significant, that authors concluded that BIA was unreliable [283].

Due to the contradictory evidence of BIA validity, a need to evaluate reliability of the BIA equipment used in the design of a large, longitudinal epidemiological study (AERIS) was generated. Previously, DXA was most commonly used as a reference method, but it has been shown that DXA measurements in obese individuals with anterior-posterior thickness over 23cm are less reliable [175]. Therefore, D2O method, which is an agreed gold standard for measurement of total body water, was used as an additional point of reference [171].

5.1 Study design and methods

The comparison of multiple body composition assessment techniques was designed, developed and delivered by the author, who obtained all the relevant approvals (Appendix J). Participants were asked to attend a University Hospital Southampton NHS Foundation Trust Research Centre on a single occasion if they met the inclusion criteria (Table 15).

Using the Southampton COPD register for recruiting volunteers, with assistance from the research nurse team, the author has identified participants and arranged the study visits and carried out all the study visits, except for medical review (support from the Southampton COPD group clinicians) and spirometry (support from the Southampton COPD group research nurses). Samples collected during the study visit (saliva) were subsequently delivered to Southampton BRC nutritional laboratory, where members of the nutrition laboratory processed and analysed samples.

All measurements were conducted in the same order, to ensure consistency, between September and December 2015. First, a medical review was performed, to establish disease status and identify possible medications or conditions that could affect any of the body composition assessment techniques. Lung function was reviewed based on spirometry results from last six months, if available. Otherwise, respiratory nurse performed the spirometry testing. When patients were confirmed to be clinically eligible for the study, further screening was performed by the author. Due to time restrictions and population characteristics, COPD patients with small metal implants were enrolled, but detailed information about the implant size and location were obtained. Once patients were enrolled, further measurements were performed on the day of enrolment. Anthropometric measurements and body composition measurements were performed (D2O, BIA, BIS, and DXA) in the same order for each patient.

Table 15 Inclusion and exclusion criteria for the body composition comparison study

Inclusion Criteria	Exclusion Criteria
• Written informed consent • Men of women (postmenopausal or sterile) 40 - 85 years of age • Confirmed diagnosis of COPD • Ability to comply with study requirements	• Subjects taking long-term oral corticosteroids or other systemic immunosuppressive medication • Subjects with severe oedema • Subjects with co-morbidities which in the opinion of the investigator would confound study outcomes including an active cancer diagnosis, diabetes mellitus, uncontrolled cardiac conditions including arrhythmias, cardiac failure and angina, kidney malfunction, TB, dementia • Subjects with confirmed history of asthma, up to the discretion of an investigator • Any subject deemed in the opinion of the investigator not clinically suitable to participate in the study • Use of pacemaker • Pregnancy

Recorded data included demographic data, lung function and disease severity based on the GOLD criteria (for details see 3.3.3.1, page 73). Anthropometric measurement included height, weight and waist circumference (for details see3.3.4.1 page 77). Body composition was measured using:

- Bioelectrical Impedance Analysis BIA (Bodystat QuadScan 4000 (BIA))
- Bioelectrical Impedance Spectroscopy BIS (Seca mBCA with Seca MBCA 1.0 software)
- Dual-energy absorptiometry (DXA) - Total body scan using DXA Horizon Hologic, measurement in the supine position on a flat and open X-ray table
- Deuterium dilution (D2O) - A standard dose of 5g D2O was used. Pre-dose and two post-dose samples of saliva (approx. 2mL each) were collected at 3 and 4 hours to measure deuterium enrichment in saliva as a representative sample of body water. Deuterium abundance was determined by isotope ratio mass spectrometry (IRMS). The IRMS analysis was performed by the external lab and obtained results were then recalculated from D2O fractional abundance into total body water, and next into fat-free mass, following the protocol from the International Atomic Energy Agency [171] (Table 16).

To reassure adherence to SOPs for each technique, participants were asked to: refrained from eating 2 to 3 hours before they were measurements; emptied bladders approximately 30 minutes prior to the BIA/BIS measurement; refrained from physical exercise for 12 hours prior to the test and did not drink caffeinated drinks or alcohol for 24 hours before the test; remove all metal elements (e.g. jewellery) and made note of any metal implants.

The BIA was chosen as a point of reference. As BIA is based on stepwise calculations (impedance used to calculate total body water, which is in turn used to estimate fat-free mass and fat mass), data analysis was performed looking at results of each step. Therefore, analysis focused on the following variables: total body water (TBW), fat-free mass (FFM), fat mass (FM) and fat-free mass index (FFMi). Total body water was recorded from D2O, BIA and BIS, but not from DXA (not available in standard DXA report), while FFM, FM and FFMi were recorded for all methods (Table 16).

Table 16 Source of data or calculations used to obtain total body water (TBW), fat mass (FM), fat-free mass (FFM) and far-free mass index (FFMi)

	BIA	BIS	DXA	D2O
TBW (l)	Recorded directly from machine report (proprietary equations used)			[Dose D2O (mg)/ ^{2}H in saliva (mg/kg)]/1.041
FM kg)				body weight (kg) – FFM (kg).
FFM (kg)				TBW (kg) / 0.732.
FFMi (kg/m^2)				FFM / height^2

^{2}H-deuterium enrichment

The procedure to measure total body water by deuterium dilution using saliva was not previously used by the nutrition research team in Southampton BRC, therefore, the author, in collaboration with local quality assurance manager (Dr Kesta Durkin), has developed a study specific standard

operating procedure for the sample collection and analysis. Results of the laboratory analysis, along with the results obtained during the study visits were inputed and analysed by the author.

Fat-free mass can be estimated using various calculations based on impedance measurements from BIA. The simplest assumption is that lean mass is proportionate to the relationship between the resistance of a body and its height, called resistance index ($FFM=Ht^2/R$). First, resistance index was compared with FFM estimated by D2O to investigate whether additional parameters (sex, weight.) play an important role in FFM calculation.

Next, calculations including additional factors like age, sex, weight and reactance were compared using raw data from BIA (resistance). To estimate the role of resistance and proportion of change in resistance and its influence on the change in body composition, the simulation was performed using raw data from BIA (resistance) and different equations for FFM (Table 17). By using average age, height, weight (sex specific) and reactance for the study cohort, and by changing resistance value, it was estimated to what extent change in reactance is represented by a change in FFM.

Table 17 Equations to calculate FFM in COPD patients

Equation	Original source	Validated against
$4.104+(0.518*H^2/R)+(0.231*W+0.130*Xc+4.229*sex$ (M=1, F=0)	Kyle 2003 [277]	RJL, Xitron
$2.38+(0.58*H^2/R)+0.23*W$	Schols 1990 [284]	D2O
M: $0.00132*Ht^2-0.04394*R+0.30520*W-0.1676*age+22.66827$ F: $0.00108*H^2-0.02090*R+0.23199*W-0.06777*age+14.59453$	Segal 1988 [285]	Densitometry (4 labs data)
M: $8.383+0.465*H^2/R+0.213*W$ F: $7.610+0.474*H^2/R+0.184*W$	Steiner 2002 [176]	D2O

H-height, W-weight, Xc-reactance, M – men, W - women

An accurate measurement and relevant cut-offs are essential to identify those who are depleted, otherwise, the risk of miscategorisation can be high (both false negative and false positive). For the purpose of this analysis, FFMi cut-offs 15 kg/m^2 for women and 16 kg/m^2 for men were used, and prevalence of lean depletion was compared between the methods. Selection of these cut-off was due to their use most commonly in COPD cohorts.

Results were presented as a mean with standard deviation (distribution was assessed and was shown to be normal) and compared between the methods using Bland and Altman plots. Difference in the mean for each measurement and technique, separate for men and women, was presented using error bar. Measurement by BIA were used as points of reference for the analysis. Rank order of results for each method was compared. Comparison of the results between the

methods was also performed using ANOVA testing. Considering the small sample size (n=9 for each gender) statistical analysis was limited due to high risk of type I or type II errors.

5.2 Data quality check

After study completion, data entry was followed by quality check. Results of only one test (one participant) raised concerns. The results of this individual were carefully analysed. Total body water of this individual measured by D2O was found to be extremely low – over 30L below estimations by all other techniques. The difference between 3 and 4 hours sample readings was verified to confirm tracer equilibrium. The difference between the two time points in this individual was 16.6ppm, which was the highest in the cohort (average difference of 1.9ppm). To test to what extent this difference could influence the TBW readings, calculations were performed for each value individually, and results differed by 3.6L in TBW and 5kg in FFM. Therefore, the level of discrepancies between D2O and BIA cannot be attributed to not reaching the tracer equilibrium. Detailed consideration of other possible errors was required, however this individual was excluded from further analysis of data.

The subject with these abnormal results was a male patient, obese (115kg, the highest body weight in the group, but not the highest BMI), with no obvious reason for abnormal readings. The subject did not have any metal implants, relevant comorbidities or medications that could influence readings in either technique. Because TBW estimated by D2O technique seemed to be below physiological levels, all the steps of the D2O method were reviewed for potential errors or inaccuracies. Stepwise consideration of possible errors and their likelihood is presented in Table 18.

In summary, the abnormal readings were most likely related to a combination of multiple methodological issues. The most probable reason for the extremely low level of the label in the saliva sample (which effected in the low readings) was related to a combination of a number of small errors including sample size, sample dilution and sampling not at equilibrium. The last could have been related to aberrations in sample absorption, but in general, the abnormal result was considered to be methodological and not biological, as others have discussed [146].

Table 18 Potential errors and likelihood of it for the excluded results of D2O analysis

Possible errors			Likelihood	Reasoning
Was the dose correct?	Sample preparation	Dose different from others	Very low	Horizontal audit during sample preparation process
	Sample storage	Container not hermetic – leak, evaporation	Low	Horizontal audit during sample preparation process
How much of the dose was consumed?	The act of swallowing	Spillage, spitting	Low	No incidences recorded during the visit
	The act of measuring remaining dose	Loss of remaining sample before weighing - spillage Weighing process inaccuracy	Low Medium	Additional protocol in place, human error possible Scale calibrated regularly, human error possible
Were the sampling and analysis performed accurately?	Sampling at correct time	Sampling earlier than protocol indicates	Low	Timer was used at the visit to monitor sampling time
	Equilibrium reached	Absorption disturbed Increased losses	Medium Medium	Difficult to assess and monitor for Difficult to assess and monitor for
	Sample secured from dilution	Dilution by patient (e.g. drink just before sampling) Dilution at storage Dilution in laboratory	Medium Low Medium	Patient with the researcher most of the visit Protocol in place Protocol in place, human error possible
	Correct sample	Sample mislabelling	Medium	Labelling prepared prior to visit No other results concerning, human error possible
	Correct calculations	Typographical error	Low	Horizontal audit of the calculations

5.3 Results

The cohort consisted of mostly moderate COPD patients (average FEV1 58.3%) without obvious wasting (all patients BMI >20). In general, women were older than men (70.1 ± 6.6y vs. 67.6 ± 7.8y) and had on average lower BMI (28.4 ± 7.0 kg/m^2 vs. 29.7 ± 3.8 kg/m^2). The results of body composition by all techniques are presented in Table 19.

Table 19 The difference in body composition markers measured by BIA, BIS, DXA and D2O (n=18; ANOVA)

	BIA	BIS	DXA	D2O	p-value
MEN (n=9)					
TBW (l)	48.3 ±7.3	42.7 ±7.8	X	43.4±6.1	0.114
FFM (kg)	62.8 ± 10.2	57.8 ± 10.6	60.8 ± 9.7	59.3 ± 8.3	0.512
FFMi (kg/m^2)	21.2 ± 2.5	19.5 ± 2.6	20.5 ± 2.4	20.0 ± 2.0	0.286
FM (kg)	25.2 ± 6.2	30.3 ± 6.3	26.9 ± 5.8	28.7 ± 7.6	0.371
FMi (kg/m^2)	8.6 ± 2.2	10.3 ± 2.4	9.1 ± 2.0	9.7 ± 2.5	0.339
WOMEN (n=9)					
TBW (l)	33.1 ± 4.3	29.4 ± 5.8	X	30.4 ± 4.0	0.243
FFM (kg)	40.3 ± 5.8	38.4 ± 7.2	41.56 ± 5.4	42.2 ± 6.5	0.599
FFMi (kg/m^2)	15.8 ± 2.0	15.1 ± 2.7	16.6 ± 3.0	16.4 ± 2.3	0.574
FM (kg)	31.3 ± 12.2	33.1 ± 11.0	29.5 ± 9.6	30.1 ± 11.5	0.905
FMi (kg/m^2)	12.5 ± 5.6	13.2 ± 5.1	11.7 ± 4.3	12.0 ± 5.1	0.924

Results of this study showed no differences between techniques, and variation in results of BIA was similar to the variation of results in all other tested techniques. On average, BIA provided higher readings of TBW than other techniques, both in men and women (Figure 10). This led to lower average FM, (Figure 11), and higher FFM (Figure 12) when compared with other techniques, which was more evident in men. The FFMi from BIS in men was on average higher than estimated by other techniques, but was lower in women (Figure 13). The BIA and BIS provided most similar readings, with smallest 95 CI in all the above comparisons. Results for all methods and variables presented the same rank order (data not shown).

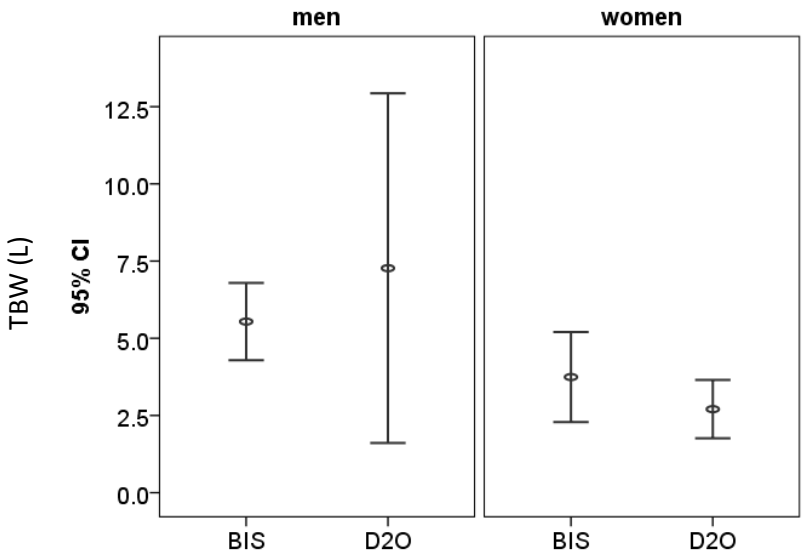


Figure 10 Differences in total body water (TBW) between bioelectrical impedance analysis (BIA as reference value) and bioelectrical spectroscopy (BIS) and deuterium dilution (D2O), (mean, 95%CI)

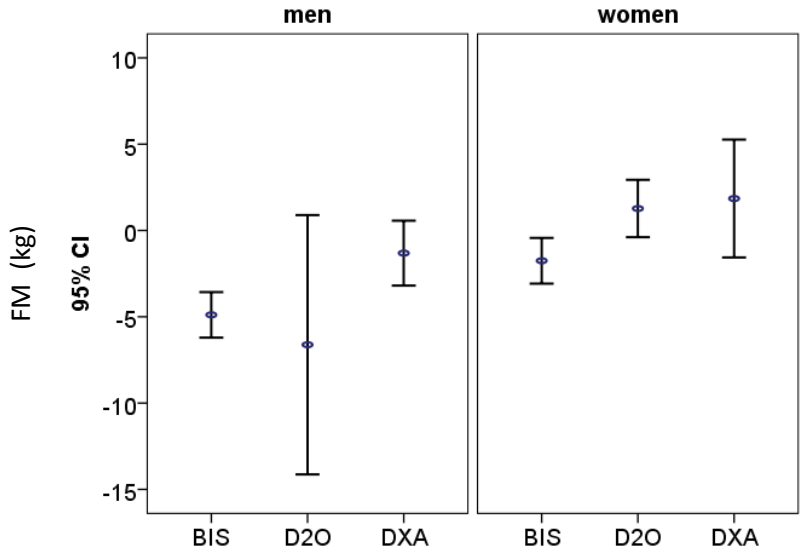


Figure 11 Differences in fat mass (FM) between bioelectrical impedance analysis (BIA as reference value) and other methods: bioelectrical spectroscopy (BIS), deuterium dilution (D2O), dual energy absorptiometry (DXA)

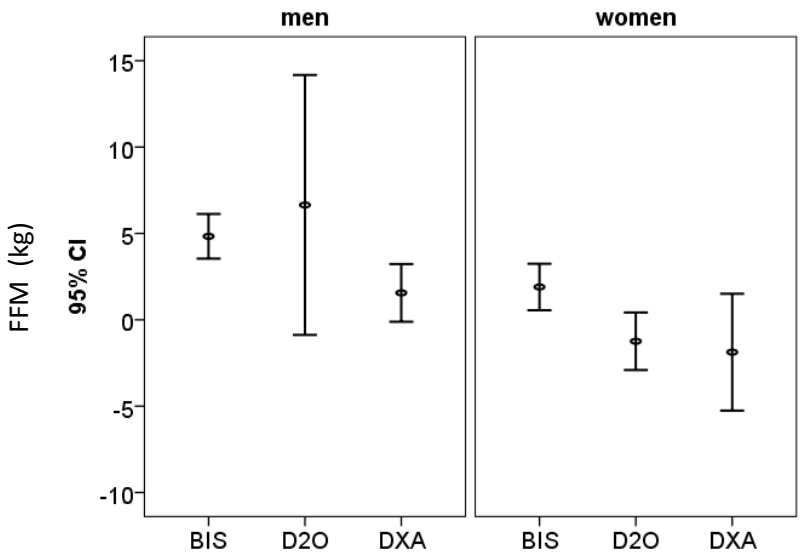


*Figure 12 Differences in fat-free mass (FFM) and fat-free mass index (FFMi) between bioelectrical impedance analysis (BIA as reference value) and other methods: deuterium dilution (D2O), bioelectrical spectroscopy (BIS), dual energy absorptiometry (DXA) (paired sample T-test, *p<0.05)*

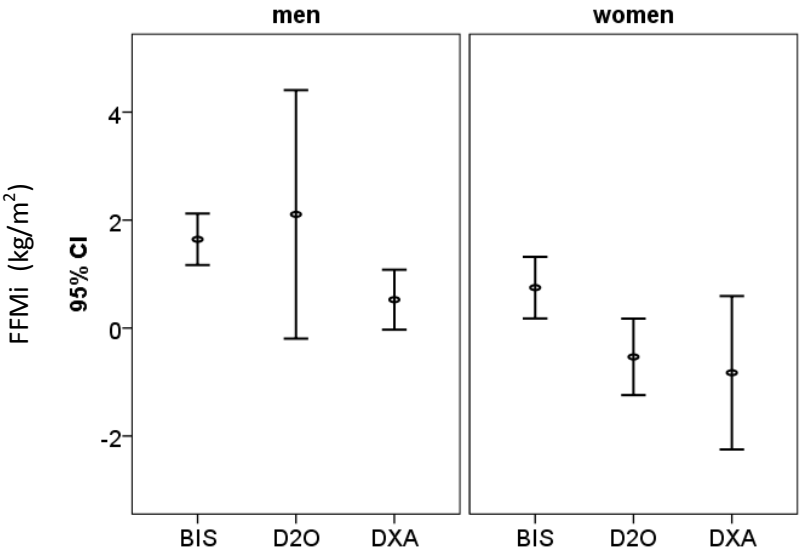


Figure 13 Differences in fat-free mass index (FFMi) between bioelectrical impedance analysis (BIA as reference value) and other methods: bioelectrical spectroscopy (BIS), deuterium dilution (D2O), dual energy absorptiometry (DXA)

5.3.1 Raw impedance data analysis

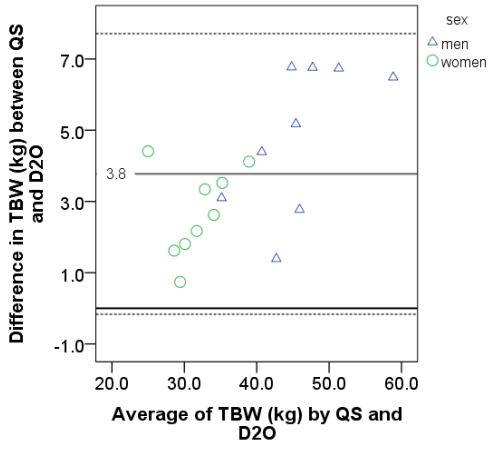
Impedance results were only obtained by BIA and BIS, however, BIA presents results for total body, while BIS provides individual body parts (left leg, right arm). Also, machines use a different range of frequencies, therefore a direct comparison of the raw data was not possible. In data simulation of the effect of impedance on FFM, an increase in resistance by 10 ohms resulted in FFM decline by 0.5-1.0kg in both men and women, which on average represents 1.5 to 2.5% decrease in FFM depending on weight and equation used.

When BIA raw data were used in four different FFMi equations, results on an individual level varied between equations within a range of 1.9kg to 11.3kg. Two equations were consistently providing lower results than others [176] [285], while Schols equation [284] resulted in the highest FFM in 15 out of 18 cases.

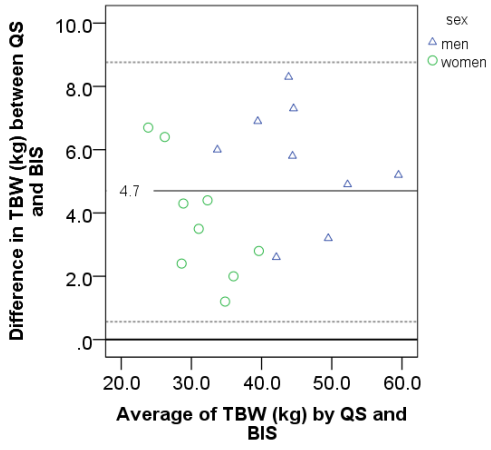
5.3.2 Total body water

Results obtained by BIA were consistently higher than D2O, with increasing difference with TBW rise. The difference between TBW by BIA and D2O was ranging between 0.7kg and 6.8kg, with the mean difference of 3.8kg (Figure 14a). All results were within 2SD of the mean. In general, the difference between the methods was rising with the increase in TBW. For men, it was on average 10% of the TBW. There was an evident grouping of results for each sex, which is biologically feasible, as women on average have smaller TBW than men, both due to lower total body mass and smaller proportion of lean mass. The average difference between the two methods was over 2kg higher for men than for women (4.84 kg vs. 2.7 kg respectively).

The average difference between BIA and BIS was 4.7kg, with higher readings from BIA (Figure 14b). Sex groupings were similar to those discussed previously. The difference between the methods was the smaller, the higher the TBW, which was more evident for women than men.



A)



B)

Figure 14 Bland and Altman plot for TBW comparing BIA with A) D2O, B) BIS

5.3.3 Fat-free mass and fat mass

First, resistance index was compared with FFM estimated by D2O (Figure 15). FFM based on resistance index was on average 3.8kg lower for women and 9.8kg lower for men compared with

D2O results. Next, proprietary equations were used, and the difference in FFM between BIA, D2O, BIS and DXA were analysed. The FFM estimated by BIA and D2O was ranging between -5.6 kg and +8.5kg, with the mean difference of 1.1kg higher readings from BIA (Figure 16). The mean average for women was -1.2kg, while for men it was +3.5kg. The higher the FFM, the higher difference between the methods. For lower FFM values, results from BIA were below the results from D2O, which changed over FFM of 50kg.

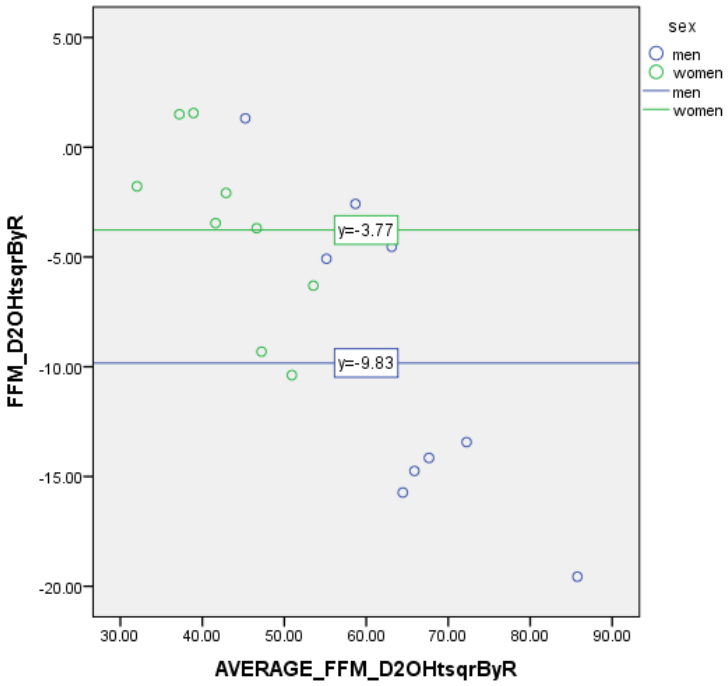


Figure 15 Bland and Altman plot comparing FFM (kg) by D2O and resistance index (Ht^2/R)

The difference between BIA and BIS was higher for men (+5.0kg) than women (+1.9kg) and was decreasing with the increase in FFM (more evident in women, Figure 17). For all techniques, the majority of the results were within ±2SD. One case was consistently borderline or outside the 2SD range. After further investigation, this individual was found to have FFM consistently lower by BIA comparing with every other technique (-2.3kg BIS, -3.1kg D2O, -9.4kg DXA), however, it was DXA that was more different to D2O, than BIA (Figure 18). This patient had extremely high body weight (BMI of 45.2) which would be in line with the theory that precision of DXA total body scan decreases with very high weight and BMI. The machine weight precision limit is 160kg, however, high BMI indicates high girths and suggests a risk of anterior-posterior thickness over 23cm that could lead to imprecise readings.

The FM was presenting the opposite pattern compared with FFM for each method. Women had a higher mean difference in FM between the compared methods, with evenly distributed cases.

Similarly, to FFM, most results were within 2SD for each method, with outliers representing same cases as FFM outliers. From all the three methods, BIA was obtaining consistently lower results than BIS for all but one case. The difference between BIA and D2O in 6 cases was lesser or equal to 1kg, which was more than compare DXA with D2O (4 cases) or BIS with D2O (3 cases), with no case repeating in all three comparisons (Figure 16, Figure 17, Figure 18).

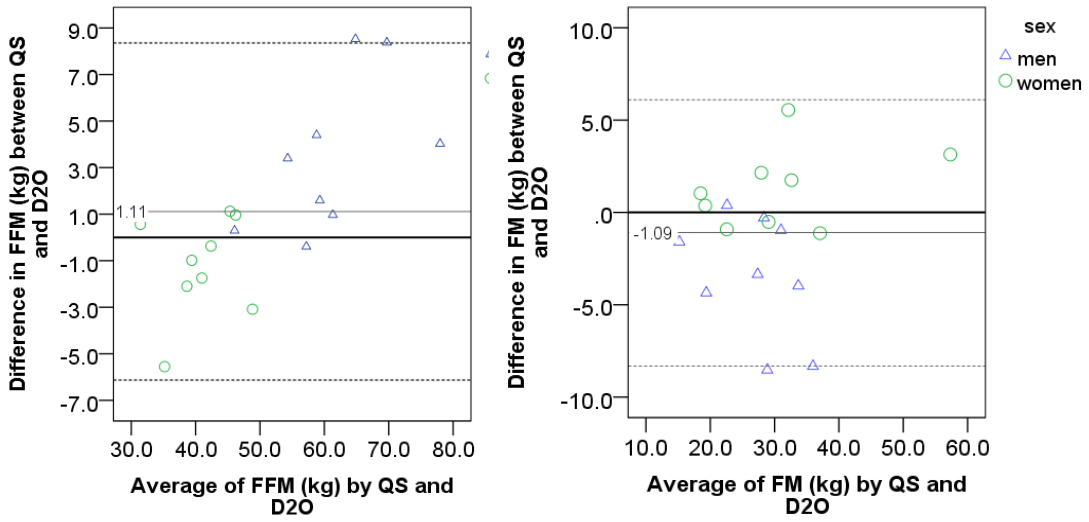


Figure 16 Bland and Altman plots for FFM and FM comparing BIA with D2O

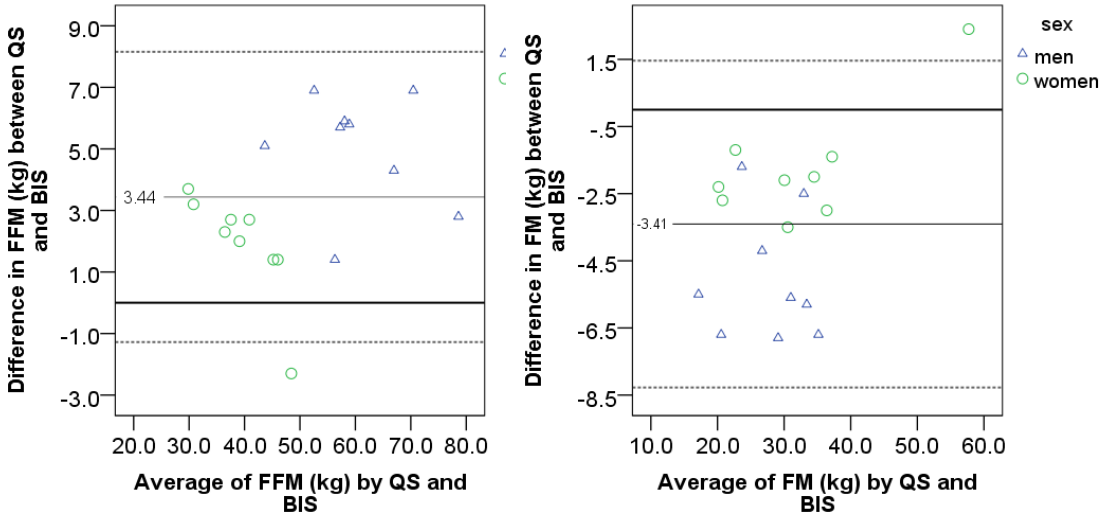


Figure 17 Bland and Altman plots for FFM and FM comparing BIA with BIS

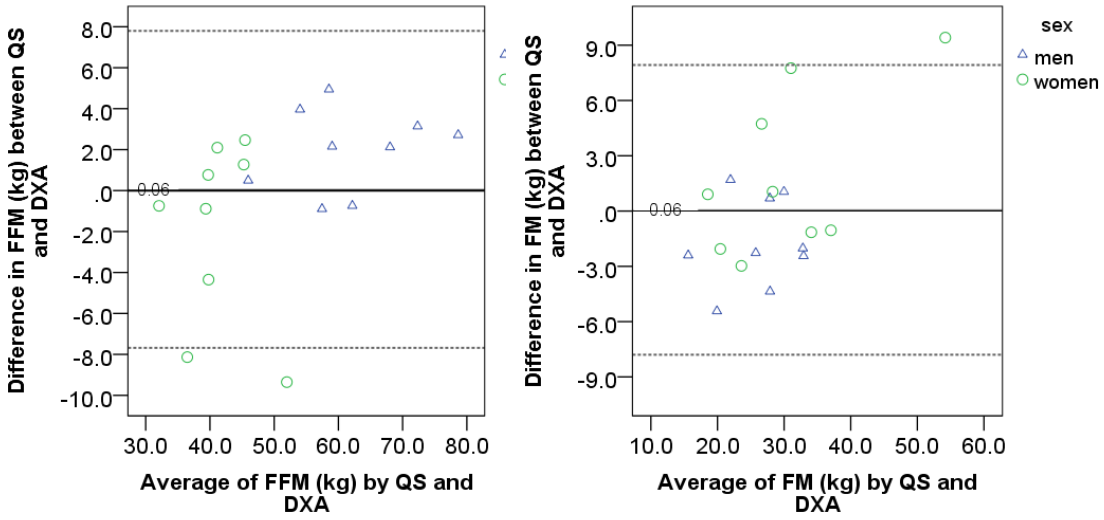


Figure 18 Bland and Altman plots for FFM and FM comparing BIA with DXA

5.3.4 Fat-free mass index

On an individual basis, the difference between the FFMi readings from different techniques varied within a wide range – from 0.5kg/m^2 to 5kg/m^2 in women and 0.8kg/m^2 to 2.7kg/m^2 in men (Table 20). Two participants were identified as lean depleted based on the DXA results, four based on the BIA and five using BIS readings. The two participants identified by DXA were also categorised as lean depleted by the other two methods.

Table 20 The difference in FFMi on individual and population level between BIA and BIS, D2O, DXA

The average difference between FFMi by BIA and…		Individual level		Population level
		Minimum	Maximum	Mean
men	BIS	0.46	2.51	1.71 ± 0.67
	D2O	-0.13	2.71	1.14 ± 1.04
	DXA	-0.29	1.64	0.66 ± 0.68
women	BIS	-0.98	1.58	0.75 ± 0.74
	D2O	-2.44	0.39	-0.53 ± 0.92
	DXA	-3.98	0.89	-0.83 ± 1.85

5.4 Summary

This study has demonstrated that different methods used for estimation of body composition provide different results, however the rank order of the results was the same for each technique. This suggest that standard interpretation criteria based on a predefined cut-off point may carry a risk of misclassification of some results. Potentially, use of centiles, tertile or quartiles approach (reflecting rank order) could help to avoid the issue of misclassification, as all used techniques provided the same rank order of individual results.

The TBW in this cohort was higher than in previous studies of COPD patients. On average it was over 10 kg higher than results from 2013 [286] and almost 20 kg higher than results from 1997 [287]. In both compared cohorts TBW was measured by D2O in stable COPD patients, however, both cohorts included more severe and younger participants, which may account for the difference between the cohorts.

The different results obtained by each method (BIA, BIS, D2O, DXA), suggest that the interpretation of body composition could be significantly variable by simply changing technique used to assess body composition. Results from all techniques are based on measurement of a surrogate marker and number of calculations to estimate lean mass. Calculations are based on equations derived from regression models in various populations. Although this is the only approach currently available, it carries a high risk of error on an individual level. Big variation of FFMi results on an individual level in this cohort suggests that by simply changing the measurement technique an individual can be considered to have lost or gain up to 5kg/m^2 of lean, which could result in a misrepresentation of body composition and shifting between non-depleted and lean depleted categories. The range of FFMi values obtained by various techniques in this study suggest that any individual who has FFMi within two units from the FFMi cut-off could potentially be at risk of false positive categorisation. Change in BIA readings (resistance) by 10ohms was shown to results in FFM misrepresentation by up to 3%, further emphasising the need to accurately follow the standard operating protocols for the measurement. Therefore, this study further supports that estimation of body composition on a population level could be reliable, but requires careful interpretation on an individual level.

Currently, there is no evidence if results from different BIA analysers are comparable or interchangeable. There is a wide range of machines offered on the UK market from eight major providers (SECA; Tanita; Maltron International Ltd; Impedimed; Biodynamics Corporation; RJL Systems; InBody CO.,LTD; Bodystat), each offering several different BIA analysers and each using their own proprietary equations to estimate water volume, which could lead to different FFM results. That represents another challenge with regards to body composition measurements.

In summary, body composition estimated using BIA, BIS, D2O and DXA differed between the techniques, but the rank order of the results was the same for each technique. Use of tertiles or quartiles of body composition to identify individuals with low lean mass in the cohort, could minimise the risk of misclassification of body composition on an individual level.

6 Results - Exploration of body composition and its relevance in COPD – AERIS study

Low body weight and low amount of lean tissue are systemic manifestations of COPD, which have been reported by many authors [66, 178, 182]. Patients who are underweight and/or with lean depletion show poor functional capability [132, 139, 185], longer hospitalisation due to exacerbations [67], higher risk of re-exacerbation [67, 187], and higher mortality risk [66, 133, 150, 288]. The body of evidence suggests that low amount of lean and low body weight have a negative effect on outcomes in COPD, especially in more severe COPD, but some studies have shown no relevance between low body weight and clinical outcomes [267, 273]. The inconsistency in the results could be related to the prevalence of sarcopenic obesity (loss of lean masked by increased fat mass). Overall, there is a strong body of evidence suggesting that low amount of lean and low body weight have negative effect on outcomes in COPD, but all previous studies have focused on mortality, hospitalisations and overall exacerbation risk, while there is no evidence of relationship between body composition and TTFE. Considering the variability of previously used study methods, a structured and systematic approach to body composition assessment in COPD was introduced to explore the predictive value of body composition markers for TTFE and AER.

Body composition in its simplest form can be presented as lean and fat. There is not sufficient evidence to say what is ‘normal’ lean mass and ‘normal’ fat mass in patients with COPD, nor what body composition has the most detrimental effect on health status, and why. To answer that question several potential mechanisms should be considered. One is that low amount of lean represents malnourishment, which is a risk factor for poor outcomes. Therefore, patients with a high amount of lean would not be considered malnourished, and would be expected to have better outcomes. Similarly, low-fat mass in chronically ill patients could suggest wasting processes and result in poor outcomes, while high amount of fat would be considered to have a protective effect because overweight patients are not malnourished. Some have even suggested a protective effect of overweight (obesity paradox [69]). However, high amount of fat would also be considered as a risk factor for a worse outcome, as fat tissue secretes pro-inflammatory mediators [289]. Therefore, there are some uncertainties regarding the proportion of lean and fat tissue that would be most advantageous for COPD patients, but those with a low amount of lean and low amount of fat tissue would be expected to have the most unfavourable body composition.

Body composition

Lean mass can be measured by several techniques including bioelectrical impedance analysis (BIA), dual energy x-ray absorptiometry (DXA) or estimated based on anthropometric measurements of skinfolds and body composition tables. The BIA technique is the most commonly used, due to its relatively low cost, good quality of results (reproducibility and repeatability) and portability. The challenge related to use of BIA is selection of regression models for translation of impedance data into lean and fat mass. A number of equations have been published and used (see Table 8, page 94) leading to results for an individual varying between 2kg to 11kg depending on the equation used (see5.3.1, page 113).

From a clinical perspective, identifying those with lean depletion is important, but current results show the prevalence of lean depletion varying from 3% to 53% [137, 264]. This broad range could be a result of multiple factors including different methodology of measurement (equipment, calculations) various interpretation criteria, different age in compared populations or various underlying conditions in COPD.

In 1990, to standardise lean mass presentation in different populations and enable comparison between various cohorts, an index of fat-free mass (FFMi, kg/m^2) was introduced. FFMi among COPD studies included in a review published in 2014 (see Appendix M2) was in the range of 16.8 ± 2.4 kg/m^2 to 19.8 ± 2.8 kg/m^2 for men and 15.0 ± 1.9 kg/m^2 to 18.2 ± 2.7 kg/m^2 for women. There was a visible difference in FFMi ranges between healthy and COPD patients, as demonstrated by data modelling (Appendix C), which also indicated that severe COPD patients had lower lean mass than moderate COPD patients. The attempt to standardise data presentation requires further efforts, as demonstrated in the systematic review (see chapter 4, page 87).

Variation in the prevalence of lean depletion could be caused by a range of available interpretation criteria [150, 275, 276, 278]. It would be expected that higher cut-off value (e.g. 17.4kg/m^2 vs. 17.0 kg/m^2) would identify a greater proportion of patients as lean depleted. The combined effect of the variability in FFMi across different COPD populations, together with various cut-offs, makes it extremely difficult to know with certainty, what the true prevalence of lean depletion in COPD is. There is a need for a unified approach, to assess prevalence of lean depletion in consistent way, which is relevant for short-term and/or long-term clinical outcomes. As discussed in systematic review, none of the FFMi cut-offs used so far have been determined using their ability to determine different clinical outcomes. Relationship with mortality was extensively explored [221, 290] , however relationship with TTFE has not been explored.

COPD cohorts often consist of a different proportions of emphysemic and bronchitic patients, with the suggestion that they have different body composition [262], with wasting being more prevalent in emphysematous patients [291]. Research of body composition and its role in COPD

has focused on patients with visible malnutrition, cachectic type patients, that are potentially dominated by patients with emphysema. Patients with predominant bronchitis often present with normal weight or overweight, which caused lesser interest in the nutritional wellbeing of those patients. This would suggest that cohorts with an average low BMI (potentially majority of emphysematous patients) had more consistent results regarding body composition, than cohorts with patients of broad BMI range or higher average BMI (potentially with a mix of both underlying conditions).

Lack of consensus on the role of body composition in COPD progression could therefore be due to methodological heterogeneity disabling coherent evidence generation. There is a need to bring standardisation into COPD research when assessing and interpreting body composition. There is also a need to better understand the relationship between COPD and body composition in stable disease. To standardise lean depletion definition, it is essential to identify FFMi cut-off points defined as a lean level that increases the risk of poor clinical outcomes, considering that short-term outcomes may require different criteria than long-term outcomes.

This chapter tests the hypothesis that COPD patients with unfavourable body composition (low amount of lean or low amount of lean in proportion to the high amount of fat) are more prone to exacerbations, independent of the disease severity and medical history. In this study the following questions were asked: Are patients with a lower amount of lean more prone to exacerbations and is it independent of the disease severity? Is higher amount of fat protective against exacerbations as suggested by the obesity paradox? Considering lack of standardisation in body composition assessment in previously published studies, an additional question was asked in this chapter ‘Does use of different body composition criteria for lean depletion carry a risk of misclassification?’ To determine if body composition was associated with differences in disease severity, activity, and exacerbations rate, the analysis of lean and fat markers in the AERIS cohort was performed. This included characteristics of the AERIS population, including baseline demographics, markers of disease severity and activity, exacerbations frequency and TTFE. Currently used respiratory markers were assessed for predicting TTFE and AER. Analysis focused on body composition at the stable visit, with an assessment of relationship with clinical outcomes, measured by TTFE and AER in next 12 months.

6.1 Methods

Study design and markers of lung function and clinical outcomes were measured as described in Methods chapter 3.3, page 71. Anthropometric measurements included height, weight, mid-upper arm circumference (MUAC), triceps skinfold thickness (TSF) and waist circumference (WC). All measurements were performed in a standing position in three repetitions with the mean of the three values used for the analysis. Measurements were used to calculate further variables. Height and weight were used to calculate BMI, and standard classification was used for BMI [49], with additional grouping into normal vs. high BMI with cut-point at 25kg/m^2 (WHO criteria) and repeated for cut-point at 27kg/m^2 as suggested disease- and age-adjusted cut-point for well-nourished COPD patients [292].

Mid-upper arm circumference (MUAC) was measured at the midpoint between the acromion and olecranon on the non-dominant side on the relaxed arm. A triceps skinfold (TSF) was measured at the level of the horizontal mark at half mid-upper arm. Based on MUAC and TSF a mid-upper arm muscle area (MUAMA) and mid-upper arm muscle circumference (MUAMC) was calculated [293].

$$MUAMC = MUAC - (\pi * \frac{TSF}{10}) \qquad MUAMA = \frac{((MUAC - (\pi * TSF))^2}{4\pi}$$

Waist circumference was measured at the midpoint distance between lowest rib (lower costal margin) and the highest point of the iliac crest.

Most of the anthropometry measurements (except for height and weight) were used as surrogate markers of body composition. Larger WC and TSF indicate increased fat mass, while decreased arm measurements (especially MUAMC, but also MUAC) suggest decreased lean mass. Fat-free mass (FFM) and fat mass (FM) were assessed using BIA.

Interpretation criteria for anthropometric measurements included:

- arm circumference: MUAC<23cm as a marker of risk of malnutrition [294].
- waist circumference (WC):
    - Men WC≥94cm, Women WC≥80cm as marker of increased risk of metabolic complications ([295])
    - Men WC≥102cm, Women WC≥88cm as marker of substantially increased risk of metabolic complications ([295])

It was recognised that using percentage body weight has a clear disadvantage because the same lean mass is worth less at a higher fat mass [296]. However, there is no evidence to suggest that

FFMi has any advantage in predicting clinical outcomes in COPD over lean expressed in kilogrammes of percentages, therefore lean expressed in all units was used for the analysis.

Based on the results from Chapter 5 (see 5.4, page 117), study- and sex-specific tertiles were defined for FFMi and FMi to compare clinical outcomes between those with the lowest and the highest indexes. Values used to define tertiles are presented in Table 21.

Table 21 Sex-specific cut-points for tertiles (T) of fat-free mass index (FFMi) and fat mass index (FMi)

	FFMi [kg/m^2]		FMi [kg/m^2]	
	T1	T3	T1	T3
Men	<18.4	>20.7	<7.0	>31.1
Women	<14.8	>16.1	<9.6	>43.5

Multiple previously published FFMi cut-offs, which were identified in the systematic review, were used to estimate level of lean depletion and compare prevalence between different criteria used. Overall, there were two cut points for women and five cut-points for men (Table 22).

Table 22 Sex-specific cut-points of fat-free mass index (FFMi) identified in literature and used for analysis of difference in lean depletion prevalence

	FFMi [kg/m^2]				
Cut off original source	[275]	[150]	[276]	[277]	[278]
Men	<16	<17.05	17.4	<16.7	<17.8
Women	<15	<14.62	15.0	<14.6	<14.6

To perform analysis of the differences in the disease markers between patients with low and normal lean mass, one of the cut-points had to be chosen. The cut-offs for further analysis were selected based on the highest cut-point for men (identification of the highest number of lean depleted individuals). The FFMi cut off for men (<17.8kg/m^2) and women (FFMi<14.6 kg/m^2) were defined using the same methods, therefore those values were used as a cut-point in further descriptive analysis.

Increased fat mass is often related to raised inflammation, therefore fat markers were compared between those with normal and elevated inflammation as described in section 3.3.3.2, page 74.

A screening tool for nutritional risk, a 'Malnutrition Universal Screening Tool' (MUST), was calculated using BMI, proportion of unplanned weight loss and acute disease effect (Table 23) [147]. Total score of zero indicate low risk of malnutrition, a score of one indicates medium risk and requires food intake assessment, and a total score of two or more indicate high malnutrition risk and requires treatment.

Table 23 Malnutrition Universal Screening Tool - steps, criteria, and scores

Screening steps	Criteria	Score
BMI score (BMI kg/m^2)	>20 (>30 Obese) 18.5 -20 <18.5	0 1 2
Weight loss score (Unplanned weight loss in past 3-6 months)	<5% 5-10% >10%	0 1 2
Acute disease effects score	If patient is acutely ill and there has been or is likely to be no nutritional intake for >5 days	2

Standard statistical analysis methods were used as described in Methods chapter, section 3.3.6. TTFE was compared between lean depleted and non-depleted patients using different cut-off points for FFMi defined in Table 22. Kaplan-Meier graphs were plotted to compare the proportion of individuals experiencing first exacerbation amongst lean depleted and non-depleted patients defined and compared with log-rank test.

6.2 Results

6.2.1 AERIS cohort description

6.2.1.1 Demographics

Out of 152 screened patients 127 (53.5% men) were successfully enrolled onto the AERIS study. The average age in this cohort was 68.8±8.2y in men and 64.6±8.6y in women with the significantly higher proportion of current smokers in women than in men, but with a smaller number of cigarettes smoked per day in that group (Table 24). Over the years, participants smoked an average of 50±28 pack-years, within a range of 14 up to 216 pack-years (Table 24).

Table 24 Smoking history and smoking status at enrolment (n=125)

Smoking history		Men n=66	Women n=59
Age when started smoking (years)	≤10	9.1%	0.0%
	>10 and ≤18	80.3%	86.4%
	>18 and ≤ 25	9.1%	13.6%
	>25	1.5%	0.0%
Pack-years		54.4±30.1	45.6 ±24.1
Smoking at enrolment		27%	67.3%*
Cigarettes smoked per day at enrolment*	< 5	16.7%	19.4%
	≥5 and ≤10	27.8%	33.3%
	>10 and <20	5.6%	19.4%
	≥20	50.0%	27.8%

* Only current smokers at enrolment: men n=18, women n=36; * p<0.01

Majority of participants (78%), with a greater proportion of men (60%), were retired at enrolment, with only 7.1% participants working full time. Half of the cohort lived with another adult person in the household, while 34.5% participants lived on their own.

Based on the study inclusion criteria, there were no patients without exacerbation in a previous year, 22% of the study population had one exacerbation in that time, 29.1% had two exacerbations and remaining 48.8% had between 3 and 14 exacerbations in 12 months prior the study enrolment. Out of 399 exacerbations identified in the preceding 12 months, the majority (74%) was classified as moderate, while mild and severe exacerbations were less frequent (respectively 14.5% and 11.5%).

Body composition

Most patients had more than one comorbidity. Cardiovascular conditions (hypertension, ischemic heart disease) were predominant, with musculoskeletal and gastrointestinal conditions next most common. Skin and endocrine conditions were less common (mostly diabetes, hypothyroidism), with individual cases of urinary system conditions.

6.2.1.2 Lung function at baseline

Lung function markers at baseline are presented in Table 25. The lowest percent predicted FEV_1 in this cohort was 17% (women) and 18% (men).

Table 25 Lung function markers at baseline in the AERIS cohort (Mean ± SD for normally distributed data, otherwise Median (IQR))

Lung function parameters	Men n=66	Women n=59
FEV_1 (L)	1.3±0.5	0.95 (2)
GOLD 2 (n=57)	1.75±0.34	1.27 (0.58)
GOLD 3 (n=51)	1.08 (0.26)	0.86±0.15
GOLD 4 (n=19)	0.75±0.16	0.49±0.11
FEV_1 (% predicted)	45.3±14.5	47.9±15.8
GOLD 2 (n=57)	54.0 (11)	60.0 (11)
GOLD 3 (n=51)	38.5±6.0	39.4±5.5
GOLD 4 (n=19)	24.5±3.8	24.3±3.9
FVC (L)	3.3 ± 0.7	2.3±0.6
FEV_1/FVC	0.4±0.1	0.4±0.1
Oxygen saturation (%)	95 (2)	95 (2)
TLCO (mmol/kPa/min)	5.3±1.9	4.3 (2.3)

6.2.1.3 AE rate, TTFE and AE severity in the follow-up

Over the first 12 months of the study, there were 355 exacerbations in the whole cohort, of which 107 were classified as first exacerbations. 15% patients have not exacerbated over the 12 months follow-up and in total 52.8 % were classified as infrequent exacerbators [28]. The remaining group experienced between three and nine exacerbations in the follow-up. After the adjustments for the time on the study, 60 patients had AER of less than two, 28 patients had AER between two and four, and 39 patients had AER of more than four. The highest AER was 13.77AE/y.

Severe COPD patients had the highest AER (3.6±2.7 AE/y), followed by very severe COPD (3.2±2.2 AE/y) and moderate COPD (2.6±2.9 AE/y), and the differences between GOLD groups were statistically significant (p=0.043). When patients with moderate COPD (GOLD 2) were compared with severe and very severe patients, combined due to small sample size (GOLD 3+4), the difference in the AER was significant (respectively 2.62.9AE/y vs. 3.5±2.5AE/y, p=0.012). AER was also significantly different between patients with frequent exacerbations in the past compared

with infrequent exacerbators (respectively 4.1±2.9AE/y vs. 2.1±2.2AE/y, p<0.001). AER was similar in men and women, all the age groups, smokers and ex-smokers, but was significantly different between BODE scores (p=0.017).

AER was not correlated with lung function (FEV_1%), either on a cohort level or sex specific. History of exacerbation prior to study enrolment was weakly correlated to AER during the follow-up (Rho=0.376, p<0.001).

In the AERIS cohort, ten patients (7.9%) exacerbated within the first week of the study, another 15 patients (12.2%) had a chest infection within 14 days of the enrolment and the total of 49 patients (38.6%) exacerbated before the end of the first month of the follow-up. Another 22 patients exacerbated for the first time in the second month of the study, summing up to 55.9% of participants exacerbating in the first 62 days of the study. Overall, TTFE ranged from 3 to 371 days.

There was no difference in TTFE between sexes, age groups, smokers, and ex-smokers. The difference in TTFE between frequent and infrequent exacerbators (history of exacerbation) was significant (log rank p<0.001), while neither GOLD nor BODE categories showed the difference in TTFE (Figure 20). Accordingly, the history of exacerbations and TTFE showed a significant correlation (Rho=-0.261, p=0.003) (Figure 19), but TTFE was not related to lung function.

From all 355 exacerbations during the first year 304 needed antibiotics or steroids (moderate exacerbation) and 20 required hospitalisation (severe exacerbation). Mild and severe exacerbations constituted only a small proportion of all exacerbations, 8.7% and 5.6% respectively. Among 107 first exacerbations 84% were classified as moderate, and only 3 cases in that sub-group required hospitalisation.

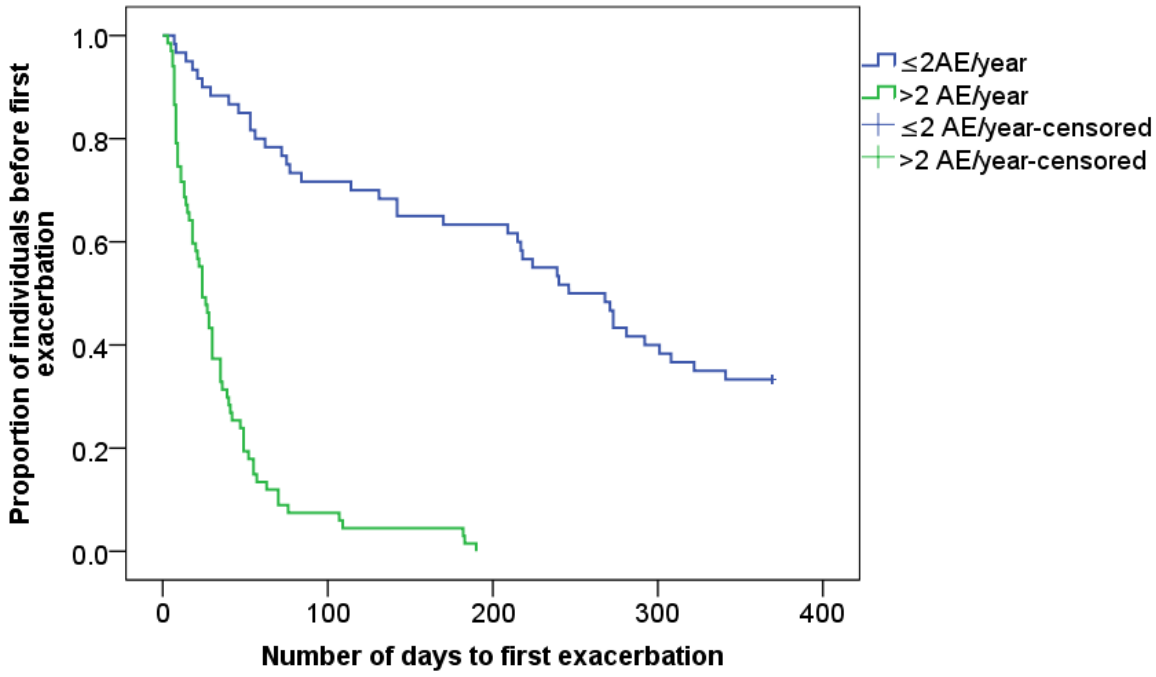


Figure 19 Kaplan-Meier graph of time to first exacerbation infrequent (>2AE/year) and infrequent (≤2 AE/year) exacerbators (log rank p<0.001)

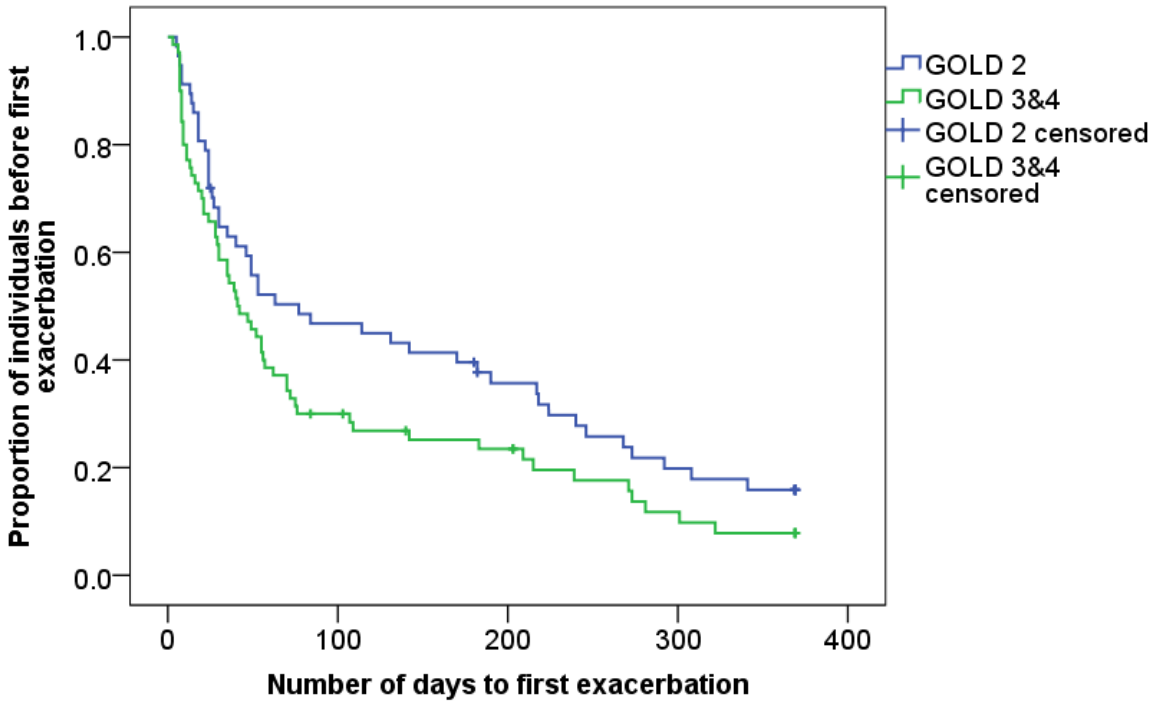


Figure 20 Kaplan-Meier graph of time to first exacerbation in patients with different disease severities (GOLD 2 vs. GOLD 3&4; log rank p=0.113)

6.2.2 Body composition in stable COPD in AERIS

Screening for risk of malnutrition using MUST score showed that 90% of this cohort had a total score of zero, eight patients (6%) had score of one, and five (4%) had score of two. Anthropometry and body composition were significantly different between the sexes, except for BMI and MUAC (Table 26). Only 9 patients (7%) had BMI<20kg/m^{2,} while 29% had BMI>30kg/m^2.

Table 26 Anthropometry and body composition markers at baseline (mean ± SD; t-test)

At enrolment	Women n=56	Men n=67	p-value
BMI [kg/m^2]	27.5 ± 6.1	27.9 ± 4.8	NS
MUAC [cm]	30.5 ± 5.4	30.7 ± 3.6	NS
MUAMC [cm]	23.6 ± 3.4	25.5 ± 3.1	0.001
MUAMA [cm]	45.1 ± 13.0	52.7 ± 12.8	0.001
WC [cm]	98.5 ± 16.1	107.1 ±13.7	0.002
FFM [kg]	40.9 ± 7.4	59.3 ± 10.1	<0.001
FFM [%]	58.3 ± 7.2	71.0 ± 5.0	<0.001
FFMi [kg/m^2]	15.8 ± 2.2	19.6 ± 2.7	<0.001
FM [kg]	29.7 ± 10.5	24.8 ± 7.9	0.004
FM [%]	40.5 ± 7.0	29.1 ± 5.0	<0.001
FMi [kg/m^2]	11.5 ± 4.0	8.2 ± 2.6	<0.001
FFM/FM	1.5 ± 0.5	2.6 ± 0.8	<0.001
IMP50 [kHz]	582.7 ± 84.2	483.1 ± 73.5	<0.001

As body composition differed significantly between sexes, this analysis was performed in men and women separately. Patients with more severe COPD had lower lean mass (FFMi), but this was significant only in men (Table 27). Number of exacerbations in the past year and smoking status had no effect on lean and fat markers in both men and women. Body composition was no different between those with low and high appetite score, as well as between those who stopped or walked uninterrupted during 6-minute walk test.

Table 27 Fat-free mass index (FFMi, kg/m^2) and fat mass index (FMi, kg/m^2) in various groups with significance of the difference between the groups for men and women separately (t-test)

Groups	FFMi in groups	p-value	FMi in groups	p-value
MEN				
GOLD 2 vs GOLD 3&4	20.4±2.5 vs 19.0±2.8	0.027	8.8±2.6 vs 7.8±2.6	NS
Smokers vs. ex-smokers	19.8±2.9 vs. 19.6±2.7	NS	7.7±2.3 vs. 8.4±2.7	NS
HAE ≤2 vs HAE >2	19.7±2.7 vs 19.6±2.8	NS	8.1±2.2 vs 8.4±3.0	NS
BODE≤4 vs BODE>4	20.6±2.8 vs 18.7±2.6	0.015	8.8±2.9 vs 7.9±2.3	NS
AS≤28 vs AS>28	19.2±2.6 vs 20.1±2.8	NS	7.8±2.4 vs 8.6±2.8	NS
6MWTs vs 6MWTui	19.3±2.5 vs 19.9±2.9	NS	8.8±3.0 vs 7.7±2.2	NS
WOMEN				
GOLD 2 vs GOLD3&4	16.3±1.7 vs 15.3±2.5	NS	11.8±3.9 vs 11.1±4.2	NS
Smokers vs ex-smokers	16.0±2.2 vs 15.5±2.3	NS	11.2±4.3 vs 11.7±3.8	NS
HAE ≤2 vs HAE >2	15.8±2.6 vs 15.7±1.8	NS	12.1±4.7 vs 10.8±3.0	NS
BODE≤4 vs BODE>4	15.3±1.5 vs 15.8±3.2	NS	10.1±2.9 vs 12.4±5.7	NS
AS≤28 vs AS>28	15.7±2.1 vs 15.9±2.5	NS	11.2±3.9 vs 11.8±4.3	NS
6MWTs vs 6MWTui	15.9±2.6 vs 15.7±1.9	NS	12.4±4.8 vs 10.7±3.2	NS

HAE – history of exacerbations, AS – appetite score, NS- non-significant, 6MWTs - stopped walking test, 6MWTui – uninterrupted walk test

Prevalence of low lean mass measured with FFMi varied from 30 to 39% in women and from 7% to 25% men, depending on which cut-point was applied (Table 28).

Table 28 Prevalence of low fat-free mass index (FFMi) using various cut-points

	FFMi cut-point (kg/m^2)	16.0	16.7	17.1	17.4	17.8	14.6	15.0
Men N=67	Low FFMi (n)	5	10	14	15	17		
	Low FFMi (%)	7	15	21	22	25		
Women N=57	Low FFMi (n)						17	22
	Low FFMi (%)						30	39

Independent of the criteria used for identification of lean depletion based on FFMi, the majority of patients with BMI>25kg/m^2 were classified as normal lean mass and majority of individuals with BMI<20kg/m^2 as lean depleted (Figure 21). The effect of FFMi cut-off on the prevalence of lean depletion in different BMI groups was evident in men. In normal-weight men, the prevalence of lean depletion varied from 18% (2 out of 11) to 81% (9 out of 11), depending on the FFMi cut off. In women, there was only a minor effect of using different FFMi cut-offs on the prevalence of lean depletion in different BMI groups.

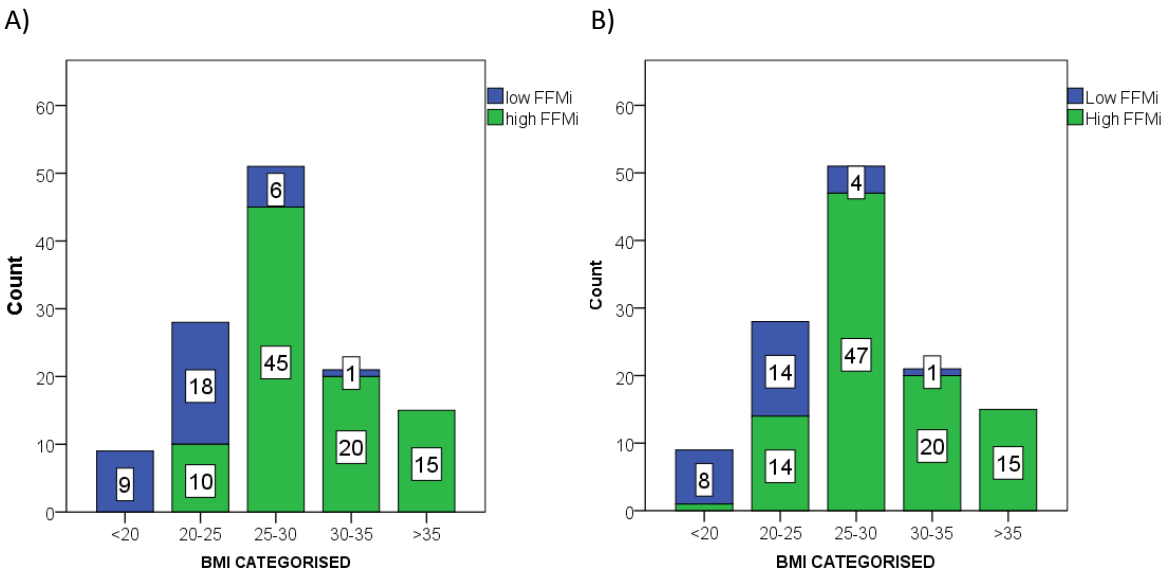


Figure 21 Number of patients with low and high fat-free mass index (FFMi) in different BMI criteria. Low FFMi: A) <14.6kg/m^2 (W) and <17.8 kg/m^2 (M). B)<15 kg/m^2 (W) and <16 kg/m^2 (M)

There was a similar proportion of patients with low FFMi and FMi (21.1%) as with high FFMi and FMi (24%), but some individuals had low FFMi and high FMi (3.3%), while others had the highest FFMi with the lowest FMi (4.9%) (Figure 22).

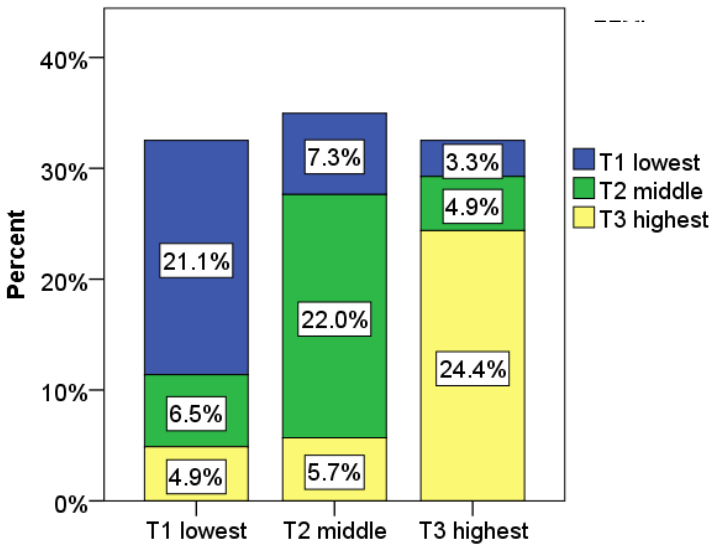


Figure 22 Proportion of individuals in fat mass index (FMi) tertiles with indication of fat-free mass index (FFMi) tertile at baseline (n=123)

Lean mass measured with FFMi was related to age (men only) and strongly related to BMI (both sexes), but was not related to the history of exacerbations (Table 29). Lung function was also related to FFMi, but not inflammation status. The lean marker was not related to survival prediction by BODE and did not show a relationship with the extent to which COPD affected patients' everyday life (CAT).

Table 29 Correlations of fat-free mass index (FFMi) with disease markers

	Men			Women		
	n	Correlations	p-value	n	Correlations	p-value
Age [y]	67	R=-0.248	0.043	57	X	NS
HAE [AE/year]	67	X	NS	57	X	NS
BMI [kg/m^2]	67	R=0.912	<0.001	57	R=0.832	0.00
FEV1 [%]	67	R=0.262	0.032	57	R=0.312	0.018
FEV/FVC	67	R=0.428	<0.001	57	R=0.412	0.001
TLCO [%]	38	R=0.394	0.014	26	X	NS
BODE	51	Rho=-0.286	0.042	41	X	NS
CAT [pts]	67	X	NS	56	X	NS
CRP [mg/L]	67	x	NS	57	Rho=0.271	0.041
Fibrinogen [g/L/L]	59	X	NS	52	X	NS
IL-6 [pg/L]	51	X	NS	35	X	NS

x – no significant correlation, NS – not significant; r=Pearson's correlation, Rho=Spearman's' correlation

Patients with low lean mass had significantly lower lung function and BMI than those with normal lean mass (Table 30). There was a trend showing higher risk of mortality predicted with BODE score and higher inflammation measured with CRP in those with lower FFMi. Patients' disease perception (CAT) was not different between those with low and normal lean mass.

Table 30 Difference in disease markers in patients with low (n=34) and normal (n=90) fat-free mass index (FFMi) using sex-specific cut-points: men FFMi<17.8kg/m^2; women FFMi<14.6 kg/m^2

	Low FFMi Vs normal FFMi	P-value
Age [y]	68.1±9.3 vs 66.0±8.2	NS[a]
HAE [AE/year]	3.0±1.7 vs 3.2±2.5	NS[a]
BMI [kg/m^2]	22.4±3.1 vs 29.8±4.8	<0.001[a]
FEV1 [%]	37.7±12.9 vs 49.5±14.6	<0.001[a]
FEV/FVC	0.37±0.09 vs. 0.45±0.11	<0.001[a]
TLCO [%]	50.3±14.2 vs. 63.2±18.7	0.005[a]
BODE	4.9±2.6 vs 3.8±2.4	0.069[b]
CAT [pts]	18.1±6.7 vs 16.1±7.8	NS[a]
CRP [mg/L]	8.4±14.6 vs 7.5±6.2	0.067[b]
Fibrinogen [g/L]	4.8±1.0 vs 4.8±0.9	NS[b]
IL-6 [pg/L]	26.3±94.8 vs 5.5±7.6	NS[b]

[a]T-test, [b]- Mann-Whitney test;

Lean to fat ratio was related to age in women only (R=-0.271, p=0.043), but there was no relationship with lung function markers or CAT score in either women or men. Lean to fat ratio was correlated with BMI in both sexes, but not very closely (men r=-0.506, p<0.001; women r=-0.684, p<0.001). Lean to fat ratio was related to inflammation, but only in women (CRP Rho=-0.301, p=0.024, fibrinogen Rho=-0.357, p=0.010).

The amount of fat was related to lung function (FEV/FVC) and inflammation level (CRP) in both men and women, but not with the history of exacerbations, age or disease markers like BODE or CAT score (Table 31).

Table 31 Correlations of fat mass index (FMi) with disease markers in men and women

	Men			Women		
	n	Correlations	p-value	n	Correlations	p-value
Age [y]	67	x	NS	56	X	NS
HAE [AE/y]	67	X	NS	56	X	NS
BMI [kg/m^2]	67	R=0.908	<0.001	56	R=0.889	<0.001
FEV1 [%]	67	X	NS	56	X	NS
FEV/FVC	67	R=0.280	0.022	56	R=0.391	0.003
TLCO [%]	38	X	NS	26	X	NS
BODE	51	X	NS	40	X	NS
CAT [pts]	67	X	NS	55	X	NS
CRP [mg/L]	67	Rho=0.257	0.036	56	Rho=0.324	0.015
Fibrinogen [g/L]	59	x	NS	51	Rho=0.301	0.032
IL-6 [pg/L]	51	X	NS	34	X	NS

x – no significant correlation, NS – not significant; r=Pearson's correlation, Rho=Spearman's' correlation

There was a borderline difference in fat markers between women with normal or elevated fibrinogen levels, and similarly, between normal and elevated CRP level groups (Appendix K, Table 86), but there was no difference in men (Appendix K, Table 85). Waist circumference, a proxy for fat distribution, was related to CRP level (Rho=0.210, p=0.019), and the difference in inflammation between low and high waist circumference was borderline significant (p=0.045). However, low and high waist circumference groups presented wide distribution of CRP levels (8.2±13.6mg/L vs. 7.6±6.2mg/L).

Body composition

There was significant difference in CRP level (not fibrinogen) between study & sex-specific tertiles of fat markers when expressed as FM and FM%, but after comparison of CRP concentrations in each tertile, results were not showing clinically relevant differences, as presented on the FM example (T1 12.2±17.9kg, T2 7.8±7.0kg, T3 9.3±6.3kg; p=0.038).

Anthropometry markers of lean (e.g. MUAMC) and fat (e.g. waist circumference) were compared with disease markers and results are presented in the Appendix K. Physical capacity markers did not show correlation with body composition (Appendix K, Table 88).

6.2.3 Body composition and clinical outcomes – time to first exacerbation

None of the body composition markers showed a relationship with TTFE in either men or women. TTFE was also similar in those with normal and high BMI, whether 25kg/m^2 or 27kg/m^2, was used as a cut point of high BMI. Approximately 30% of those who exacerbated within first 30 days had obesity. Independent of criteria used for lean depletion, low FFMi group had longer TTFE, however no significant differences were found (Table 32).

Table 32 Comparison of time to first exacerbation (days) in low and normal fat-free mass index (FFMi) (Mann-Whitney test) with low FFMi defined using different criteria

	N (low FFMi)	low FFMi vs normal FFMi	p-value
Criterion 1	124 (27)	128 ± 123 vs 102 ± 117	0.3
Criterion 2	124 (37)	134 ± 126 vs 97 ± 113	0.1
Criterion 3	124 (27)	135 ± 125 vs 100 ± 115	0.2
Criterion 4	124 (31)	134 ± 123 vs 99 ± 115	0.2
Criterion 5	124 (34)	134 ± 128 vs 98 ± 113	0.1

Criterion 1: FFMi<16.0kg/m^2 men FFMi <15.0 kg/m^2 women
Criterion 2: FFMi<17.4kg/m^2 men FFMi <15.0 kg/m^2 women
Criterion 3: FFMi<16.7kg/m^2 men FFMi <14.6 kg/m^2 women
Criterion 4: FFMi<17.1kg/m^2 men FFMi <14.6 kg/m^2 women
Criterion 5: FFMi<17.8kg/m^2 men FFMi <14.6 kg/m^2 women

The ROC curve analysis showed that FFMi cut-off <14.6kg/m^2 was borderline significant in women (AUC=0.656, Std Er 0.076, p=0.064, Figure 23), while no other cut-off in women had significant results. The highest area under the curve for men was seen when FFMi cut-off <17.4kg/m^2 was used (AUC=0.633, Std Er 0.078, p=0.118, Figure 24), but none of cut-offs for men had significant results.

Sensitivity and specificity of cut-offs were analysed, and 14.6kg/m^2 had a relatively high sensitivity (approximately 70%) but with 30-50% of false positive findings. Cut-offs for men had a sensitivity of 90% for values below 16.1kg/m^2 showing no false positive findings, but there were only six

patients with FFMi below that cut-point in this cohort. Based on ROC analysis, none of the FFMi cut points for men and women were a good separator of patients with different TTFE.

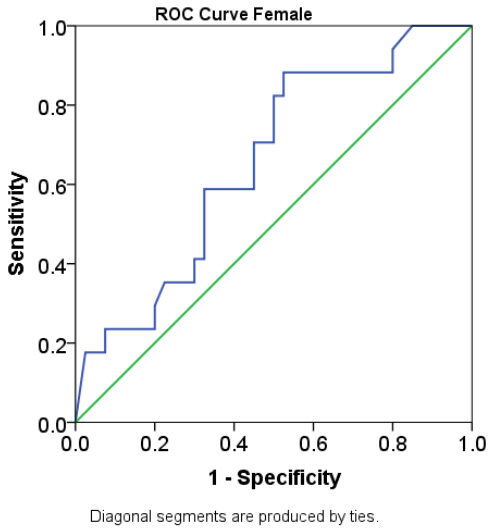


Figure 23 Area under the curve (10^3) for time to first exacerbation using fat-free mass index (FFMi) cut-point of 14.6 kg/m^2 for female

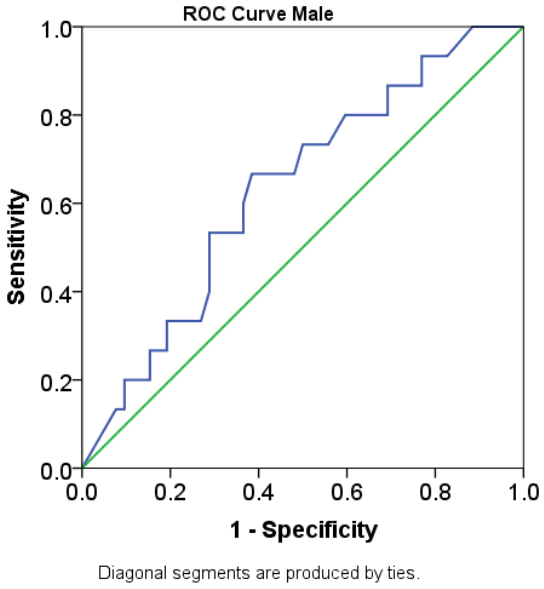


Figure 24 Area under the curve (10^3) for time to first exacerbation using fat-free mass index (FFMi) cut-point of 17.4 kg/m^2 for male

Cohort and sex-specific tertiles of FFMi showed that patients with the lowest FFMi (T1) had lower hazard ratio than patients with the highest FFMi (T3) (Table 33). Tertiles were sex-specific, and adjustment for age and lung function did not change hazard ratio of FFMi. As results were opposite to anticipated, data coding and results were crosschecked and re-analysed, which only confirmed originally obtained results. Also, when the fat mass index was analysed using the same approach, there was no relationship between FMI and TTFE.

Table 33 Hazard ratio (HR) of time to first exacerbation for fat-free mass index (FFMi) tertiles (T1, T2) compared with the highest FFMi tertile (T3) (Cox regression)

N=124	p-value	HR	95% CI
FFMi T1 vs T3	0.023	0.579	0.36-0.93
FFMi T2 vs T3	0.053	0.635	0.40-1.01

A significant difference in TTFE between tertiles of FFMi (Figure 25a) and Imp50 (data not shown) were seen. In both markers, two out of three tertiles were overlapping, so those groups were merged. In FFMi the lowest and the middle tertiles were merged (T1+T2, Figure 25b). As a result, the significance of the difference between compared groups increased. Individuals with the lowest FFMi (T1+T2) had the longest TTFE (T1+T2 mean=72 days, 95% CI 44-100 days, T3 mean=27days, 95%CI 16-38 days, p=0.012). Detailed results for each tertile and merged tertiles are presented in Appendix K (Table 89, Table 90).

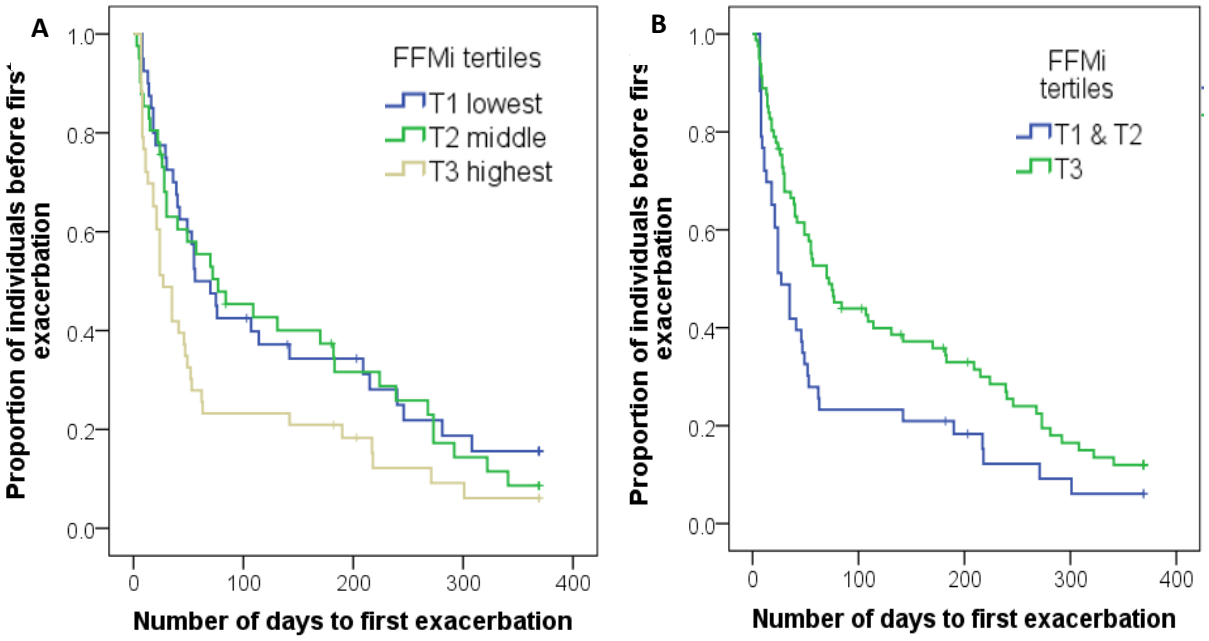


Figure 25 Kaplan-Meier graph of time to first exacerbation in A) sex-specific tertiles of fat-free mass index (FFMi) (log rank p=0.041) B)) sex-specific tertiles with lowest and medium fat-free mass index tertile combined (log-rank p=0.012)

Except for FFMi and FMI, other anthropometric measurements were also analysed for relationship with TTFE, but none have shown relevance (Appendix K).

6.2.4 Body composition and clinical outcomes – exacerbation rate

None of the body composition markers showed a relationship with AER in either men or women (Table 34). The AER was similar in those with normal and high BMI. Most anthropometry markers showed lack of relationship with AER (Appendix K).

Table 34 Significance of the differences between frequent and infrequent exacerbators as well as three groups of exacerbation frequency, for main body composition markers

	Frequent vs infrequent exacerbators*		Between three categories ** (≤2AE/y, 2-4AE/y, >4AE/y)	
	Men	Women	Men	Women
BMI	0.689	0.808	0.861	0.123
FFMi	0.668	0.652	0.816	0.089
FMi	0.659	0.737	0.893	0.206

*Mann-Witney U test; **Kruskal Wallis test

6.3 Discussion

It has been demonstrated that as health deteriorates and body composition worsens, the ability to recover from acute illness also deteriorates and risk of mortality rises [66, 133]. Many have investigated body composition in COPD, but the evidence is inconclusive to state how abnormal body composition influences COPD status and progression. Even though lean depletion has been identified as an important element of COPD course [66, 67, 185], the process of lean tissue wasting is still not fully understood, or easy to identify, quantify or prevent. Moreover, there is no consensus on how abnormal body composition should be defined and if the amount of lean or fat tissue is more relevant, or proportion between the two compartments determines nutritional wellbeing in COPD.

This analysis focused on evaluation of the relationship between body composition, especially FFMi and FMi, and COPD markers, especially TTFE. The main findings were: high amount of lean tissue was related to shorter TTFE, which was independent of history of exacerbations; criteria chosen for interpretation of body composition markers lead to differences in the prevalence of lean depletion and none of the tested criteria discriminated patients with different TTFE. A number of surrogate markers of lean and fat were used, including anthropometry measures of the arm and waist, however anthropometry markers showed much lesser relevance with disease status and clinical outcomes than FFMi or FMi.

Initial analysis of demographics and basic respiratory parameters in the AERIS study showed that this cohort was similar to other UK based COPD cohorts (Appendix A) [62]. AERIS cohort consisted of 127 mostly retired, moderate to very severe COPD patients, who, over 12 months follow-up, had in total over 350 exacerbations (mostly moderate) with an average of three AE per patient. Half of the studied cohort was classified as frequent exacerbators (≥2AE/y), which represents greater proportion than in other cohorts [30, 297].This could have been partially due to daily monitoring of symptom changes, highlighting even mild exacerbations, an overall AE rate was higher than in other studies [38]. The analysis of the TTFE has revealed that TTFE ranged from 3 to 371 days and 20% of patients have exacerbated within two weeks from the baseline, which indicates that study baseline in those patients could have already been reflecting a pre-exacerbation state, rather than a stable condition. Only a few body composition markers were related to lung function markers, which was more common in women than men.

In this study, patients with the highest FFMi had the shortest TTFE, while no other body composition markers showed a relationship with TTFE or AER. COPD patients with a lower FFMi had longer TTFE and 42% lower hazard ratio for AE compared to those with a higher FFMi. Also, a

higher amount of fat and higher BMI did not show a protective effect from exacerbations. The number of exacerbations in the past did not relate to body composition, suggesting that previous exacerbations did not influence current body composition, and current body composition was not determining future outcomes.

This is in contrary to underlying hypothesis and to what other studies have shown, as the majority of studies demonstrated a protective effect of higher lean mass against exacerbations and mortality [66, 67, 150]. Moreover, results seem physiologically unlikely, as there are no known mechanisms by which high amount of lean tissue would have an adverse effect on health. Considering these unexpected findings, various analytical approaches and crosscheck of the database were carried out, all of which have confirmed the unforeseen findings.

The unexpected direction of the relationship between the FFMi and clinical outcomes could have been caused by various factors, like difference in inflammation and fat mass. Patients with a higher amount of lean had also higher levels of inflammation markers, and the lowest inflammation was seen in those with the lowest FFMi. Others have shown that patients with lower FFMi had lower inflammation [298], and the highest CRP levels were seen in COPD patients with the highest FFMi (quartile) [197]. In agreement with the current results, Hitzl et all showed that COPD patients from 20th FFMi centile had lower death hazard ratio than those from the 50th FFMi centile, suggesting similar trend seen in this cohort, but Hitzl's studied cohort of patients receiving home mechanical ventilation [65]. It could be that high lean was a proxy for the high amount of fat tissue, as fat tissue would be considered to have pro-inflammatory activity. In this cohort, 70% of patients within the highest FFMi tertile were also in the highest FMi tertile. A positive relationship between the FMi and CRP was seen in this cohort and previously reported [197]. However, there was no relationship between the high FMi and TTFE, suggesting that relationship between the high FFMi and short TTFE was not driven by the high amount of fat tissue. As no other available data could explain the unusual results, potential causes were discussed next.

Findings in this chapter could have been confounded by several factors. Results demonstrated a relationship between FFMi and TTFE, while FFMI showed no relationship with AER. Because body composition is essentially constant over 12 months and year-to-year exacerbation frequency is similar, it would follow that the positive relationship with TTFE should be reflected in a positive relationship with AER as well. However, this was not observed in the relationship with AER, hence, this casts doubt over the TTFE positive findings. This could suggest that the relationship between lean mass and TTFE was a false positive finding (type I error).

Secondly, the relationship between FFMi, CRP and TTFE could have been indirect. High FFMi was related to higher CRP, more severe COPD, and shorter TTFE. It could be that high FFMi was not directly related to shorter TTFE, but it is the combination of higher inflammation and more severe disease that determined the TTFE, while lean marker was correlated with TTFE indirectly, through its relationship to disease severity and inflammation.

Also, those unexpected findings could be related to the higher disease activity compared with other COPD studies. A minimum of one exacerbation in 12 months before the enrolment was one of the inclusion criteria, which to some extent, limited participants to those with reoccurring exacerbations and more active disease. The majority of other studies did not include recent exacerbation as an inclusion criterion (Appendix A). This would be supported by the higher average AER (over 3 AE/year) in this study, compared to other cohorts, where AER ranged from less than one to two exacerbations per year [38, 62, 268].

Next, this cohort appeared to have lower proportion of visibly malnourished patients than other cohorts. The average BMI at enrolment in this cohort (BMI 27.9±4.8 kg/m^2 men and BMI 27.5±6.1 kg/m^2 women) was higher than in many other COPD studies [66, 264, 299], with only nine patients classified as malnourished at enrolment. Average lean mass (FFMi) in this cohort was 15.8kg/m^2 in women and 19.6 kg/m^2 in men, which was similar to some cohorts [64], but higher than others [184]. Prevalence of low FFMi in this cohort was three times higher than the prevalence of low BMI, which was higher than observed previously [184]. It should be noted that in that study prevalence of low BMI <20kg/m^2 was much higher than in this study (28% vs 7%), which again emphasises the difference between AERIS cohort and previously studied cohorts.

Relationship between FFMi and TTFE was independent of the criteria for lean depletion, as tertiles model was used in the analysis. Since standard practice is to use FFMi cut-offs, evaluation of various cut-offs and their relationship with TTFE was performed. Lean depletion in women ranged between 30 to 39%, similar to other studies [178]. Prevalence of lean depletion in men varied more drastically depending on the FFMi cut-off ranging from 7% to 25%. Such big variance has been seen before, as Rutten et al have shown that this variability of calculations, when combined with different cut-offs for lean depletion, led to variation in lean depletion from 11% to 70% in men and from 31% to 59% in women [299].

All the above results were in opposition to nutritional screening tool (MUST) results, with only 4% patients identified as being at risk of malnutrition, which is significantly less than the prevalence of lean depletion identified by any FFMi cut-off. That discrepancy in results suggests that MUST is not sufficient to identify the risk of poor nutritional status in COPD patients, but the methodology of identifying lean depletion can significantly influence generated results.

Considering a variety of FFMi cut-offs, it was important to compare how well different cut-offs distinguish patients with different clinical outcomes. None of the published cut-offs were determined using the relationship between lean mass and TTFE. This analysis demonstrated that none of the cut-offs related to TTFE or AER in COPD, (only FFMi<14.6 kg/m^2 in women showing some borderline relevance to TTFE). This, in light of significant difference in TTFE between those in different tertiles of FFMi suggests that relationship between TTFE and FFMi requires different cut-off than previously published. However, lack of statistical evidence for a relationship between lean mass and TTFE or AER could have been related to small sample size. The size of the AERIS cohort might not have been powered to detect differences between low and high lean groups when to compare TTFE and AER. It is also possible that there is no difference in clinical outcomes when considering lean mass and, regardless of sample size, lean markers will not be able to discriminate between those with better and worse clinical outcomes and hence results in this cohort were of type I error.

Previously, it has been observed that overweight and obesity had a protective effect on relative risk of mortality when compared to normal weight COPD patients, which was independent of FEV and age [148]. Severe COPD patients, who are overweight or obese according to BMI, were shown to have greater muscle mass, muscle strength and exercise capability [139]. So-called “obesity paradox” was described in COPD patients several times [119, 187, 288] and meta-analysed [148] showing a protective effect of weight, similarly to a paradox in cardiovascular diseases [149]. However, in this study there was no difference in TTFE or AER between those with normal or high BMI. It should be noted, that protective effect of higher BMI was seen against hospitalisations and mortality, while in this cohort majority of exacerbations did not require hospitalisation and mortality was infrequent. Overall, high body weight did not show a protective effect against TTFE or AER. It would be feasible to assume that obesity paradox is valid only amongst people with certain fat distribution, as abdominal fat contributed to increased systemic inflammation and was associated with mortality in COPD patients [300, 301]. In this cohort waist circumference was used as a proxy of fat distribution, but there was only a small difference in the inflammation status between those with different waist circumference. It suggests that accumulation of fat around the waist was not related to increased inflammation; hence, fat distribution and inflammation were not linked.

Currently, there are no guidelines including consideration of body composition in COPD, with only low BMI used for screening of malnutrition. Therefore, before introducing and implementing COPD management guidelines including body composition markers, there is a need to thoroughly and systematically assess nutritional status amongst all disease phenotypes, including patients without obvious malnutrition, who were often overlooked. Results suggesting sarcopenic obesity

[61, 140] have already stressed the need to go beyond BMI and classification into malnourished and not malnourished patients. Current and previous results suggest that COPD patients could be considered in several subgroups – visibly malnourished with low disease activity potentially benefiting from higher body and lean amount; not visibly malnourished and potentially benefiting from higher body and lean amount; not visibly malnourished with high disease activity potentially not benefiting from higher body and lean amount. Further studies should assess feasibility of those subgroups and establish what are the phenotypes that could be essential for appropriate and efficient clinical care.

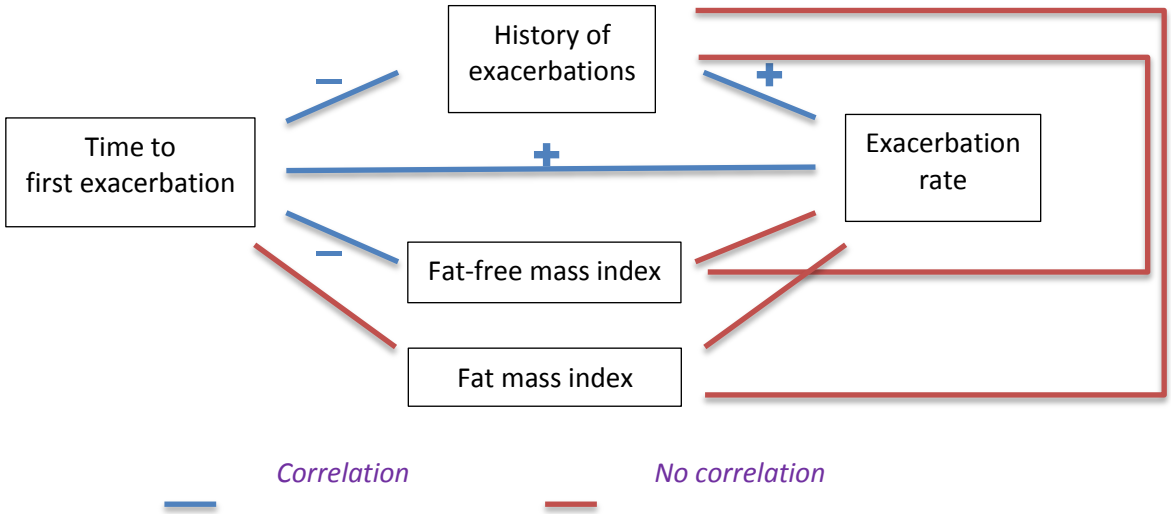


Figure 26 Summary of correlations between exacerbation history, clinical outcomes and body composition at baseline, '+' positive correlation, '-' negative correlation

This chapter aimed to explore if body composition markers are related to TTFE or AER in COPD and whether this relationship is independent of disease severity or medical history. Results showed that body composition was not related to the history of previous exacerbations, while there was some link between body composition and disease severity. Results showed that only FFMi has potential to aid in prediction of TTFE (Figure 26), but unexpectedly, those with a higher amount of lean had worse clinical outcomes. This could have been a false positive finding, but without further exploration, this can be neither confirmed nor refuted. The higher amount of fat did not show a protective effect against exacerbations. There is a need to understand why in this cohort higher lean mass had an adverse effect on clinical outcomes, which was in contrary to underlying hypothesis and previous findings. Differences between the studied group and other cohorts could have been related to disease activity or disease pathomechanism. Previous studies focused on visibly malnourished COPD patients, while this study focused on patients with higher disease activity. If this difference was proven to be a valid explanation of the different, or even opposite, effect on clinical outcomes, that would require extensive exploration. This novel finding requires further investigation and begs the question of the validity of universal nutritional criteria

for different disease phenotypes. Also, considering controversial results around body composition in COPD, other nutritional markers require investigation to understand if these unexpected results are also evident in other nutritional domains. As higher body weight (and higher FFMi) would require increased food intake, a higher appetite would be also expected in those patients. Also, increased body weight could have a limiting effect on physical capacity, however higher lean mass could suggest the opposite. Therefore, both appetite and physical capacity should be explored in this cohort.

6.4 Limitations

History of exacerbations appears to be a good discriminator of TTFE in frequent and non-frequent exacerbators. This difference is statistically significant, however, has limitations when to consider the clinical use of it. The relevance of this results in this cohort was strong due to a thorough evaluation of exacerbation history based on patients reported frequency and comparison with medical records. In everyday practice, distinguishing between 2 and 3 exacerbation in past 12 months is more challenging, and requires highly motivated patients who keep records of their exacerbations, and considering exacerbations of all severity. The second challenge of exacerbation history is its dichotomous nature and no consideration of patients who over last 12 months have not presented as frequent exacerbators, yet they present rapid disease progression in recent past. Therefore, exacerbation history as a single marker is valuable in identifying the overall risk of exacerbation in future for those who frequently exacerbated in the past, but does not provide a precise timeframe of the next exacerbation and could be insensitive for rapidly deteriorating patient.

The diversity of techniques available to measure body composition requires a careful selection of an adequate method for the purpose, especially that differences in results was dependent on which technique was used [176]. For ethical reasons (but also cost-effectiveness), using several techniques and performing multiple measurements, that provide very similar results, should be avoided. The simplicity and versatility of BIA assessment allow monitoring of changes over time but has its limitations. Skin conductivity is important to consider, as the information derived from a BIA measurement are based on the flow of an electric current between electrodes placed on hand and foot. Poor skin conductivity (e.g. very dry hands) may give inaccurate results suggesting lower levels of total body water, while increased perspiration may overestimate hydration level [250].

The electrodes should be placed on the same body side (usually on the right side) to avoid electrocardiogram artefacts [250]. The positioning of electrodes has been analysed in the past,

and standard placements have been agreed [254]. However, as presented by Hungwe (unpublished data) change in electrodes placement of less than 3cm may alter the reading of resistance at 50kHz by 70 ohms (average readings are 450ohms) which represent 15% of the reading. In addition, the positioning of the electrodes on the inner side or on the back of the hand, as well as using sole or front top of the foot affects readings, mainly due to the difference in conductivity of soft skin and corneous tissue.

Another challenge relates to metal implants. Metal is an excellent current conductor, therefore measurements of body composition in a patient with a metal implant can artificially increase lean mass. A small amount of metal, like a single screw in an elbow or a metal plaque supporting finger bone, should not have a great effect on readings (metal implants above the neck technically do not influence BIA readings as electric circuit does not include head and neck in natural current flow). It is important to consider that COPD population consists of elderly who often have multiple metal implants e.g. knee implants and hip replacements. In that case, measuring body composition using BIA may be unreliable and other methods should be considered.

Water balance is also a factor that should be considered before performing a BIA measurement, again, particularly in this population of patients. BIA is based on current flow so the greater the amount of water, the faster the current flow, and consequently, the higher the value for lean mass. With this in mind, results from patients with oedema are likely to be inaccurate, particularly because excessive water is often located in the legs, which proportionally contribute considerably to the results.

Readings and interpretation of body composition results using BIA have multiple limitations. The most importantly, there is a lack of outcome-based interpretation criteria. Current reference ranges are generated from healthy populations and are established based on arbitral cut-off points (e.g. below the fifth centile). None of the available criteria relates to disease-specific clinical outcomes, leading to pure number-focused interpretation, with no clinical relevance.

In addition, the interpretation of body composition in weight gain, weight loss or in very high total body weight remains a challenge and requires caution. To illustrate this issue, a case scenario follows. Fat-free mass (FFM) is on average 75% total weight [172]. Therefore, for a person with total body weight 70kg, approximately 52.5kg is FFM. For each kilogramme of extra body weight, a 20-25% weight gain is due to increase in FFM. Therefore, if a person gained 60kg, only 12-15kg of the excessive body weight would be FFM. In total, for a person with 130kg body weight, the proportional lean mass would be 67.5kg (52.5kg+15kg). If the ideal FFM was estimated based on the ideal body weight (70kg), ideal FFM would be 52.5kg. If the actual body weight was used to estimate FFM (130kg), then ideal FFM would be 67.5kg. Hence, if the ideal FFM is estimated based

on the ideal body weight, rather than actual body weight, then significant lean mass depletion (22%) may be missed.

Regardless the described caveats of the BIA, if used with caution and following the protocol carefully, this method can generate a reliable estimation of lean and fat mass. In addition, BIA is the simplest, cheapest and relatively easy method of body composition assessment, therefore, if proven useful in COPD management, meets the cost-efficiency criteria for potential implementation into clinical practice to aid COPD management.

7 Results - Exploration of appetite and its relevance in COPD – AERIS study

Patients with COPD commonly report appetite loss along with weight loss, general weakness, fatigue and lack of energy [302]. Weight loss, poor appetite and low intake are often used interchangeably to describe the same problem, but the three processes should be considered independently as components within a complex process. There is an understanding of a causal link between low appetite and low food intake [187, 303]. There is some evidence that loss of appetite in COPD is apparent during acute exacerbation with appetite and food intake improving at a resolution of exacerbation [304]. However, no studies to date have objectively described the longitudinal changes in appetite in stable COPD patients or the extent to which change in appetite relates to clinical outcomes.

Long-term observation of appetite in haemodialysis patients led to the identification of 'Malnutrition-inflammation complex syndrome' [1] and provided evidence that patients with poorer appetite have shorter survival (Figure 27). The role of inflammation in COPD suggests that appetite could play a similar role in COPD and that measurement of appetite in COPD could serve as an important clinical sign of disease activity and predict future clinical events.

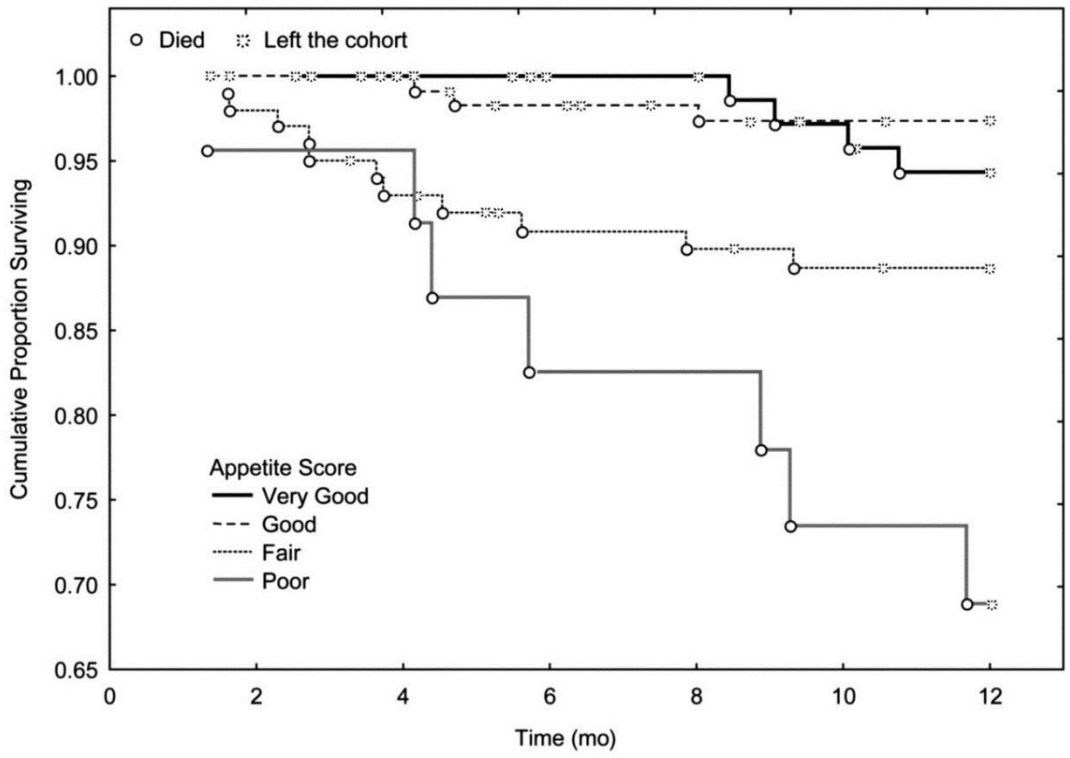


Figure 27 Survival in haemodialysis patients shown for different levels of appetite [1]

Change in appetite can occur suddenly, as a consequence of systemic changes preceding the AE. Assuming that loss of appetite can be identified earlier than respiratory symptoms of AE, change in appetite could be used as an early indicator of AE. On the other hand, change in appetite could be slow, with change over an extended period. Slow deterioration in appetite could be unnoticed, but result in progressively decreasing food intake. That, in turn, would lead to stepwise deterioration of nutritional status. Progressive limitation in nutrient intake would influence body metabolism and result in selective insufficiencies. In response to an extensive period of depleted nutrients intake, the body would then activate mechanisms of retrieving necessary nutrients from body storage, causing tissue catabolism. This process, along with ongoing systemic inflammation present in many COPD patients [305], could cause systemic decompensation and increase the risk of AE. Monitoring food intake on a regular basis is challenging. Therefore, regular monitoring of appetite would provide information on long-term changes in appetite as a proxy for changes in food intake, could help identify patients at higher risk of exacerbation.

The question arises: how to standardise and measure appetite objectively? Most doctors ask COPD patients about their appetite in an unstructured way, as a part of general history taking and examination, but appetite is rarely assessed objectively or quantitatively. Currently, there are no recommendations regarding appetite monitoring and the lack of standardised and objective methods of measuring appetite limit its use in disease management.

Feeding behaviour is regulated by complex mechanisms, a balance of stimulating and inhibiting forces in the central nervous system, in particular, the hypothalamus. There is a number of appetite inhibitory signals (cholecystokinin [199], glucagon-like peptide-1 [200], peptide YY [201]). Hormones derived from the pancreas (glucagon [202], insulin [203], and amylin [204]) and adipose tissue-derived signals (leptin [205]), adiponectin [206]) also exhibit anorexigenic actions. Gut-derived ghrelin is the only example of a peripheral hormone with orexigenic actions to counteract anorexic signalling [205].

However, food intake is moderated not only by a desire to eat but also by various other, non-hormonal factors. Breathing difficulties during eating associated with hypoxia, hypercapnia and dyspnoea [210] can decrease food intake in COPD patients. Also, early or prolonged satiety or nausea (potentially related to stomach compression by enlarged lungs) can limit food intake. Pain and discomfort were shown to impair appetite in elderly [306], while the change in taste perception (either due to smoking or medications) and other dietary problems can dramatically change food intake [209, 302]. There are a number of practical aspects influencing food intake, which should also be considered in elderly. Socio-economical status influences everyday food-related activities like grocery shopping and cooking [210], limiting access to food, while living

alone, limited social interactions could decrease pleasure from eating and cause a change in food intake. Hence, appetite should be considered as a multidimensional marker and not a surrogate marker of food intake.

A tool that incorporates multiple dimensions of food intake regulation was needed to assess appetite in the most comprehensive way, considering patients feelings and experiences. Therefore, an appetite questionnaire was chosen as a straightforward and affordable alternative that could be implemented to objectively measure appetite

COPD exacerbations are recognised to be most dangerous and detrimental to patients' health, therefore there is a great interest in understanding how each exacerbation influence patient's health. It was shown that prevention from exacerbations remains a challenge and current therapies and treatment focus on exacerbation resolution, from first respiratory symptoms, rather than prevention prior to symptoms occurrence. One of the reasons for that is a lack of a marker that would enable recognition of incoming exacerbation prior to respiratory symptoms development. There is broad empirical evidence that patients lose their appetite at exacerbation, but there is no evidence when that change begins, whether it starts earlier in severe exacerbations and how long it takes to recover it back to pre-exacerbation level, if ever.

The hypothesis was that low appetite occurs in patients with COPD who are more prone to exacerbation independently of the disease severity, activity or medical history. To test this hypothesis, following questions were asked: Firstly, does appetite score (AS) relate to clinical outcomes independently and beyond currently recorded respiratory symptoms, including the history of exacerbations. Secondly, have patients with a greater change in AS at exacerbation shown different clinical outcomes to those with smaller changes, and was that change reversed at the exacerbation resolution. Lastly, can previously validated criteria used for identification of risk of malnutrition be used to identify risk of worse clinical outcomes. To determine whether differences in AS were associated with differences in disease severity, activity or exacerbations frequency and severity, the analysis of the appetite in the AERIS cohort was performed. This included analysis of baseline appetite in stable COPD and change in appetite around exacerbations. Analysis comprises of a description of appetite score and its individual components, and its relationships with disease markers and clinical outcomes.

7.1 Methods

The appetite score (AS) was calculated using patient administered CNAQ questionnaire – multiple choice, 8-items questionnaire with five answers to each question. Each answer was given points (from a=1 for answers representing 'very bad' to e=5 for answers representing 'very good') with the maximum score of 40 points. The AS was in the original publications validated, which identified a cut-off of 28 points out of 40 to be capable of identifying patients at risk of weight loss (low AS≤28 points) [211]. In the analysis reported here the same cut-off was used, together with analysis of cut-offs between 26 and 29 points to explore which one would be most relevant as a potential predictor of exacerbation risk.

The questionnaires were filled out by the patients at three-monthly visits and at every exacerbation visit. Compliance with questionnaire instruction and rate of non-participation with questionnaire were monitored over 12 months. The AS was analysed at the single time point (enrolment) and change was calculated a) between the last stable visit with available appetite data (prior to exacerbation) and the first exacerbation, as well as between the first exacerbation and the first available AS for the subsequent stable visit within the first year. Not all patients had results available on all three occasions (stable-exacerbation-stable) therefore in a different analysis various number of cases were included.

The analysis was performed using standard statistical methods (as described in section 3.3.6). Also, to test sensitivity and specificity of different AS interpretation criteria (cut-offs) a receiver operating characteristic (ROC curve) was performed. The exacerbation hazard curves based on time-to-event analysis using Kaplan–Meier method, stratified by AS (low and high), were plotted with log-rank analysis.

To estimate what proportion of variance in AS can be explained by various markers stepwise regression modelling was used. First, demographics (sex, age, smoking status at enrolment, total number of exacerbations in past 12 months) and lung function markers (baseline FEV_1% predicted, FEV_1/FVC) were used. Second model used nutritional parameters (BMI, FMi, FFMi, waist circumference) and sex. Lastly, first two models were combined including demographic, lung function and nutritional components.

To compare risk of time-to-event between AS and other markers, Cox proportional hazard regression analysis for TTFE was done using single variable models.

The analysis was performed using AS in several forms: a total score of individual questions (total AS); low and high score (using established cut-off ≤28 points); tertiles representing cohort and sex-specific AS results – lowest (T1, men AS<27pts, women AS<25pts) and highest (T3, men

AS≥30, women AS≥29); individual questions from the CNAQ questionnaire including self-reported appetite. To embrace appetite and non-appetite driven limitations of food intake, three selected questions (self-reported appetite, satiety and number of meals at enrolment) were analysed together, representing ‘eating limitations’.

The severity of exacerbations was classified by clinicians according to patient information and prescribed treatment into mild, moderate or severe (for details see 3.3.3.6.3, page 76).

7.2 Results

7.2.1 Appetite in stable COPD

7.2.1.1 Appetite score and COPD markers

Out of 1024 questionnaires filled out by patients during the first year, only 26 questionnaires (2.5%) from 19 different patients were not available. Questionnaires were excluded if they had one or more answers missing (15 cases), had more than one answer given in a single question (7 cases) or patient refused to fill the questionnaire (4 cases).

All 127 of the enrolled patients were included in the baseline characteristics of patients' appetite. The AS ranged from 12 to 37 points with the average of 27±4 points. Total AS was significantly different between sexes and between current and ex-smokers (Table 35). Patients with more severe COPD (GOLD 3 and 4) had significantly lower AS than patients with moderate COPD, which was also reflected in BODE score groups – those with the higher mortality risk (BODE>4) had lower AS. The AS at enrolment was no different between frequent and infrequent exacerbators in 12-months prior to study enrolment.

Table 35 Appetite score (points) in various groups with the significance of the difference between the groups (t- test)

Groups	Appetite scores in groups	p-value
Men vs Women	28.2 ±3.7 vs. 26.0±4.8	0.004
Smokers vs. ex-smokers	26.2±4.1 vs. 28.1±4.4	0.02
GOLD 2 vs GOLD3&4	28.1±4.2 vs. 26.4±4.3	0.020
BODE≤4 vs BODE>4	28.3±4.4 vs 25.9±3.9	0.006
HAE ≤2 vs. HAE >2	27.1±4.3 vs. 27.3±4.4	NS

HAE – history of exacerbations, NS- non-significant

On individual question basis, questions 3,5 and 6 had most negative answers with over 50% answers classified as neutral or worse (Table 36). 60% patients reported feeling full after they have most of their meal suggesting early satiety to be an infrequent problem, and nausea was not a problem for over half of this group. Women were more often giving 'very poor' results than men, while men were more often giving 'very good' answers.

Table 36 Prevalence of answers detailed for each CNAQ question [%]

CNAQ individual questions	Very poor	Poor	Neutral	Good	Very good
Total cohort n=127					
1. My appetite is…	4.8	7.2	32.8	28.8	26.4
2. When I eat, I feel full …	4.8	14.4	13.6	60.0	7.2
3. I feel hungry …	16.8	32.0	44.0	7.2	0.0
4. Food tastes…	0.8	5.6	36	35.2	22.4
5. Compared to when I was younger, food tastes…	2.4	20.0	56.8	12.8	8.0
6. Normally I eat…	2.4	21.6	44.8	30.4	0.8
7. I feel sick or nauseated when I eat…	0.8	2.4	17.6	26.4	52.8
8. Most of the time my mood is…	1.6	6.4	28.0	49.6	14.4
Men n=68					
1. My appetite is…	1.5	4.5	28.4	31.3	34.3
2. When I eat, I feel full …	6.0	4.5	7.5	70.1	11.9
3. I feel hungry …	14.9	37.3	41.8	6.0	0.0
4. Food tastes…	0.0	1.5	32.8	37.3	28.4
5. Compared to when I was younger, food tastes…	3.0	20.9	53.7	13.4	9.0
6. Normally I eat…	0.0	22.4	50.7	25.4	1.5
7. I feel sick or nauseated when I eat…	0.0	0.0	11.9	26.9	61.2
8. Most of the time my mood is…	1.5	3.0	29.9	47.8	17.9
Women n=59					
1. My appetite is…	8.6	10.3	37.9	25.9	17.2
2. When I eat, I feel full …	3.4	25.9	20.7	48.3	1.7
3. I feel hungry …	19.0	25.9	46.6	8.6	0.0
4. Food tastes…	1.7	10.3	39.7	32.8	15.5
5. Compared to when I was younger, food tastes…	1.7	19.0	60.3	12.1	6.9
6. Normally I eat…	5.2	20.7	37.9	36.2	0.0
7. I feel sick or nauseated when I eat…	1.7	5.2	24.1	25.9	43.1
8. Most of the time my mood is…	1.7	10.3	25.9	51.7	10.3

Prevalence of low AS was on average 53.5%, but it was different between sexes, and at the same time related to COPD severity. Two third of women had low AS and it was independent of GOLD class, while there was significant ($p<0.001$) difference in the proportion of low AS in men with GOLD 2 compared to GOLD 3 and GOLD 4 (Table 37).

Table 37 Appetite score (AS) and proportion of patients with low AS (AS≤28pts) in GOLD classes for men and women (mean ± SD, n=127)

		All (n=127)	GOLD 2 (n=57)	GOLD 3 (n=51)	GOLD 4 (n=19)
Men (n=67)	Total AS	28.4±3.7	*29.8*±2.9	27.2±3.8	27.2±4.3
	Low AS n (%)	33 (49.3)	7 (22.6)	18 (72.0)	8 (72.7)
Women (n=58)	Total AS	26.2 ± 4.8	26.7±4.7	26.3±4.7	24.5±5.0
	Low AS n (%)	35 (60.3)	15 (60.0)	15 (60.0)	5 (62.5)

In moderate and severe GOLD groups the AS was clustered around mean (Figure 28). The group average for AS in severe patients was significantly lower than in moderate patients (p=0.013).

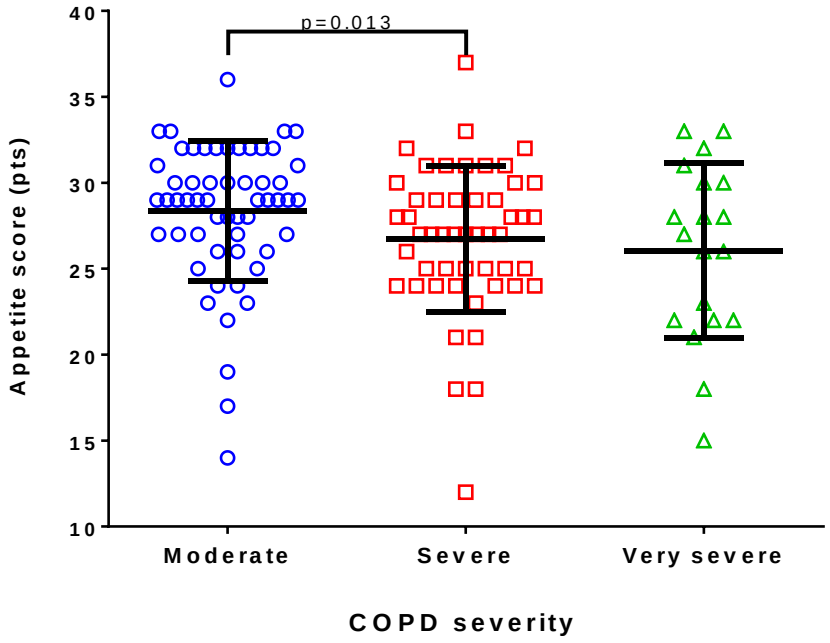


Figure 28 Individual appetite scores at baseline categorised by COPD severity

Table 38 Comparison of standard characteristic between low and high appetite groups (t-test)

	n	low AS (n=68) vs. high AS[z] (n=59)	P-value
Age [y]	125	66±8.5 vs. 67.8±8.9	NS[A]
FEV1 [%]	125	42.7±14.5 vs. 50.8±15.0	0.003[A]
FEV/FVC	125	0.40±0.1 vs. 0.46±0.1	0.004[A]
TLCO [%]	63	51.2± 13.0 vs. 70.1± 18.7	<0.001[A]
BODE	92	5±2 vs. 3±2	<0.001[B]
CAT [pts]	124	17.6±7.5 vs. 15.7±7.6	NS[A]
HAE [AE/y]	125	3.4±2.7 vs. 2.8±1.8	NS[A]
CRP [mg/L]	125	8.1±11.0 vs. 7.1±6.2	NS[B]
Fibrinogen [g/L]	113	4.8±0.9 vs. 4.8±0.9	NS[B]
IL-6 [pg/L]	88	15.9±66.1 vs. 5.0±3.4	NS[B]

[z]Low AS≤28pts, ns – not significant; [A]T-test,

[B] Mann-Whitney test; HAE – history of exacerbations

Lower appetite score was related to significantly worse disease status – lower lung function (FEV, FEV/FVC), and lower survival prediction (BODE) (Table 38), but was not related to factors that would be considered to have an adverse effect on appetite, like inflammation and previous exacerbations. There was a trend showing the worse effect of COPD on patients' life (CAT) and higher inflammation status (CRP, IL-6) amongst patients with lower AS, but those were not statistically significant. Out of four inflammatory markers, there was no significant difference in their levels between patients with low and high appetite scores.

BODE score showed higher scores (shorter predicted survival) in those with lower AS. However, there was sex effect – majority of men with high AS (79.2%) had higher predicted survival (BODE≤4) while only 25.9% men with low AS had higher predicted survival (BODE≤4) and that difference was statistically significant ($p<0.001$). This pattern was not evident in women, there was a similar proportion of women with high AS in both low and high BODE score groups.

Regression model with demographic and lung parameters has shown that gender and EV% statistically significantly predicted total appetite score $F(2,122)=7.000$, $p=0.001$, but only 10% of the variance in total AS was explained by this model ($R^2=0.103$).

7.2.1.2 Appetite scores and nutritional markers

None of lean or fat markers were showing the difference between low and high appetite groups (Table 39), but there was a tendency for patients with low AS to have lower lean markers, than high AS group. Participants with low AS had significantly lower physical capacity measured by 6MWT (20%, p=0.005) and grip endurance (40%, p=0.03), than patients with high AS.

Table 39 Comparison of body composition markers in low and high appetite group (t- test)

	n	low AS (n=68) vs high AS[z] (n=59)	p-value
BMI [kg/m2]	125	27.2±5.3 vs. 28.3±5.7	NS[a]
MUAC [cm]	126	30.0±4.3 vs 31.2±4.6	NS
MUAMC [cm]	124	24.2±3.4 vs 25.1±3.1	NS
MUAMA [cm^2]	124	47.4±13.5 vs 50.8±12.3	NS
FFM [kg]	124	49.2±12.7 vs 52.7±13.0	NS
FFMi [kg/m^2]	124	17.4±2.9 vs 18.3±3.4	NS
FFM [%]	124	65.0±9.6 vs 65.6±8.0	NS
Imp50 [kHz]	124	541.1±93.9 vs 514.0±90.9	NS
Waist [cm]	124	101.9±15.4 vs 104.2±15.6	NS
FM [kg]	123	26.2±9.3 vs 27.8±9.7	NS
FMI [kg/m^2]	123	9.5±3.6 vs 9.9±3.8	NS
FM [%]	123	34.0±8.6 vs 34.4±7.9	NS
TSF [mm]	124	18.6±8.5 vs 17.9±7.7	NS
6MWT [s]	125	270.8±108.6 vs 327.0±108.8	0.005
Grip endurance [s]	117	41.0±20.8 vs 58.7±55.7	0.023
Max grip [N]	110	243.5±93.2 vs 241.8±82.4	NS

[z]Low AS≤28pts, NS – not significant

To adjust for that limitation, a tertile approach was used to focus on those with very low (T1) and very high AS (T3). There was a statistically significant difference in FFM between T1 and both T2 (14.7%, 7.87kg, p=0.014) and T3 (14.65%, 7.82kg, p=0.016), with no statistical difference between T2 and T3. Similar pattern was apparent for FFMi, but not for the FFM%. Body fat was not significantly different between tertiles. The 6MWT showed a significant difference between T1 and T3 (21.9%, 73.9m, p=0.008). Regardless the significant findings between the tertiles, the range of results in each tertile were broad and overlapping between all tertiles for both sexes as shown in the example of FFM (Figure 29).

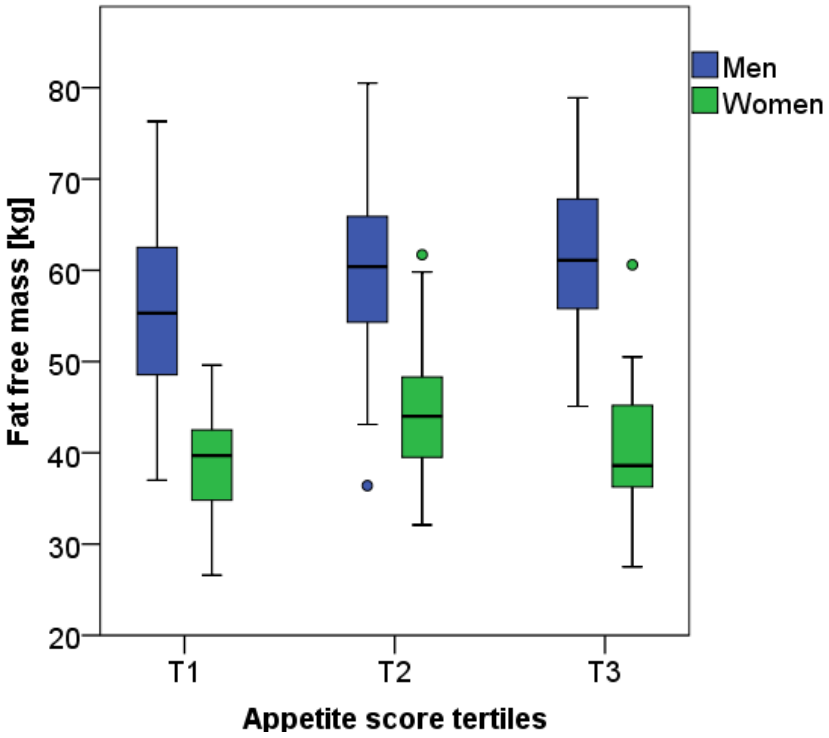


Figure 29 Fat-free mass (kg) in appetite score tertiles for men and women (T1 – lowest AS)

Taking into account that there was a significant difference in appetite score between men and women, the analysis of body composition markers was repeated to explore sex difference. The difference in lean and fat markers between low and high appetite groups was similar in men and women and almost all correlations were statistically insignificant (Appendix F, Table 80).

Regression model with nutritional markers and gender has shown that FFMi statistically significantly predicted total appetite score $F(1,119)=10.164$, $p=0.002$, but only 8% of the variance in total AS was explained by this model ($R^2=0.079$). When this model was combined with the demographics model, FFMi and age statistically significantly predicted total appetite score $F(2,118)=7.944$, $p=0.001$, but only 12% of the variance in total AS was explained by this model ($R^2=0.119$).

7.2.1.3 Appetite scores and clinical outcomes

7.2.1.3.1 Appetite Scores and time to first exacerbation

The AS correlated significantly with TTFE (rho=0.251, p=0.005). When considered men and women separately, there was a relationship between TTFE and AS in men (rho=0.328, p=0.007), but not in women. There was a significant difference between low and high AS groups in the TTFE (low AS 85±104 days vs. high AS 147±133 days; p=0.004). Similarly, the difference was evident in men (p=0.002), but not in women when to analyse each sex separately.

In Kaplan-Meier time-to-event analysis there was a significant difference between low and high AS groups (Figure 30) with 50% of individuals experiencing AE within 40 and 77 days respectively. To compare those with the highest AS and the lowest AS the analysis was performed using tertiles (Figure 31). The difference between groups was still significant (p=0.036), with a clear difference between T1 and T3 (T1 median=28 days, 95% CI 18-38 days, T3 median=142 days, 95%CI 0-286 days). When medium AS (T2) was excluded from the analysis, the significance of the difference between T1 and T3 was greater (p=0.012).

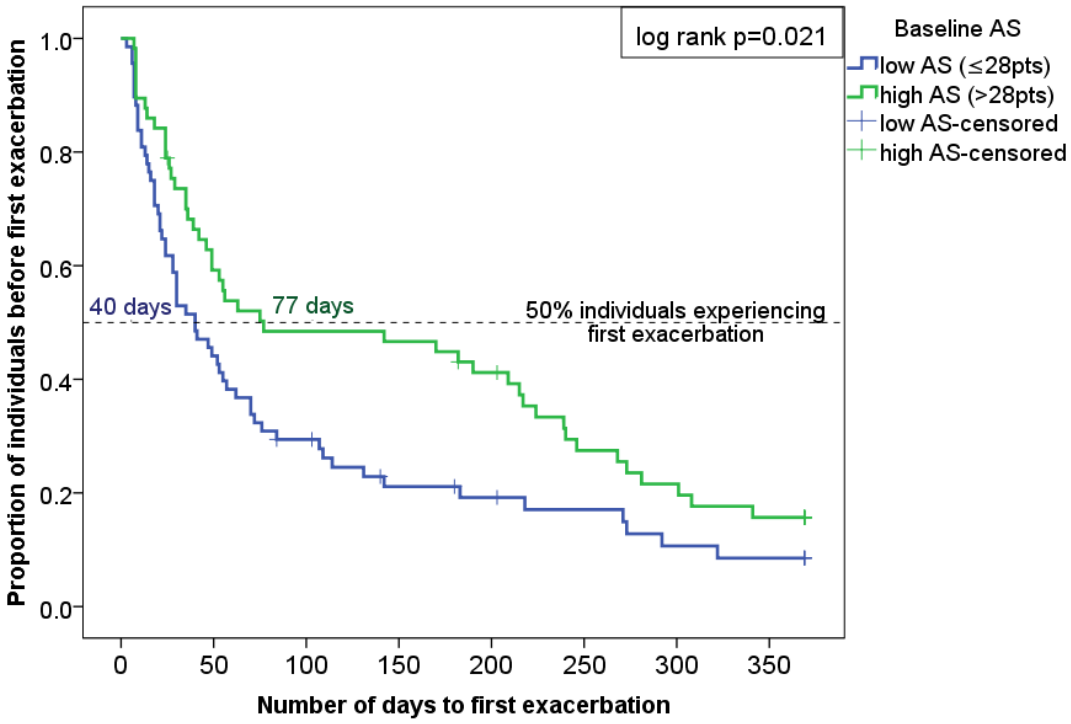


Figure 30 Kaplan-Meier graph of time to first exacerbation in low and high appetite score groups with indication of number of days 50% individuals experienced first exacerbation

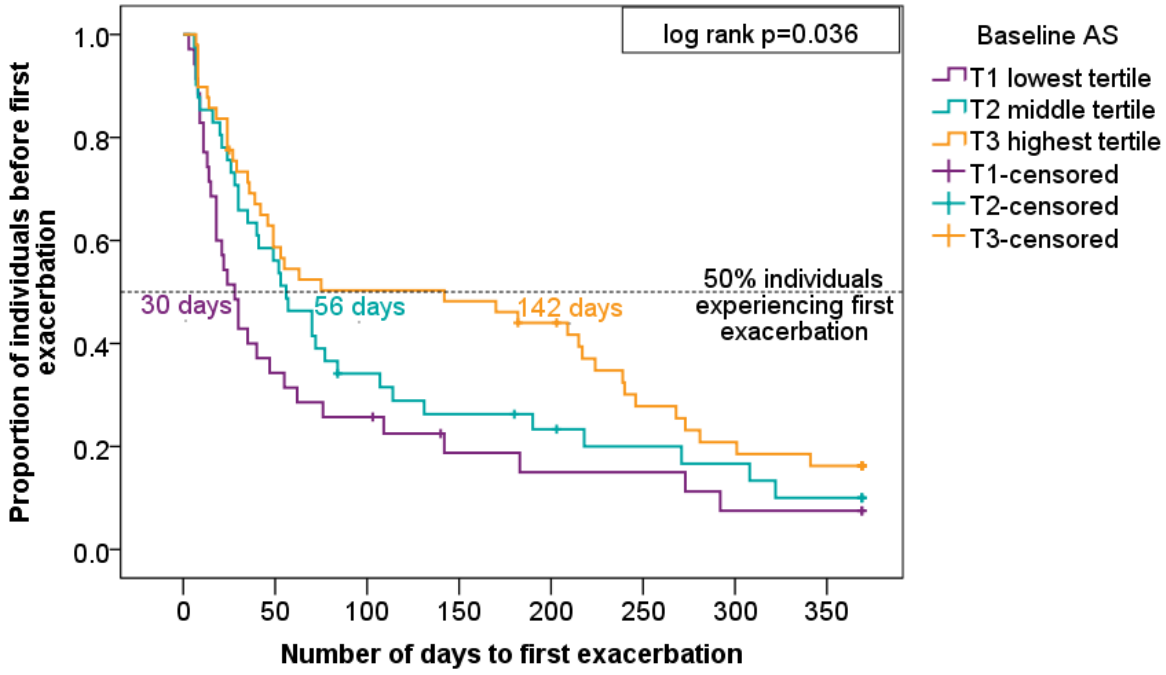


Figure 31 Kaplan-Meier graph of time to first exacerbation in sex-specific tertiles of appetite score with indication of number of days to 50% individuals experienced first exacerbation in each tertile

To estimate if there is a higher risk of exacerbation amongst those with lower AS COX regression modelling was used. The categorised AS showed 58% increased hazard ratio for those with the low AS and 83% for those from the lowest AS tertile in time-to-event modelling (Table 40). It was the second strongest model after exacerbation history (frequent and infrequent exacerbators) with a hazard ratio of 87%. All models consist of a single variable, because neither age, sex, lung function nor BMI increased the strength of the model.

Table 40 Cox proportional hazard regression analysis for time to first exacerbation using single variable models

Models		Hazard ratio (95% CI)	P value
Model 1	AS (cut-off at ≤28pts)	1.575 (1.066, 2.326)	0.023
Model 2	AS (T1 vs T3)	1.827 (1.132, 2.949)	0.014
Model 3	Total AS	0.966 (0.927, 1.006)	0.096
Model 4	FEV1	0.991 (0.979, 1.004)	0.175
Model 5	GOLD 2 vs GOLD 3*	1.459 (0.962, 2.212)	0.075
Model 6	Exacerbation history	1.196 (1.101, 1.299)	<0.001
Model 7	Exacerbation history (cut-off at ≤2)	1.868 (1.267, 2.753)	0.002

*GOLD 2 vs. GOLD 4 had lower significance, CI – confidence interval

7.2.1.3.2 Individual CNAQ questions and time to first exacerbation

Assessment of each CNAQ question individually was performed to explore if any individual question or combination of few questions had a good predictive value of TTFE. There was no statistical difference in TTFE between individuals reporting poor status (answers 'very poor' and 'poor' considered together) compared to good status (answers 'very good' and 'good' considered together) for every CNAQ question (patients with middle answers were excluded from the analysis) [Table 41). When the analysis was repeated for men and women separately mood and a number of meals a day were related to significantly different TTFE, while in women only feeling of fullness was showing the difference in TTFE.

Table 41 Time to first exacerbation (TTFE) (mean ± SD) for 'poor', neutral and 'good' answers for each CNAQ question with the significance of the difference in TTFE between 'poor' and 'good' answers (Mann-Whitney test)

CNAQ individual questions	'poor' (answers 1 or 2)		Neutral (answer 3)		'good' (answers 4 or 5)		p-value
	n	TTFE	n	TTFE	n	TTFE	
My appetite is…	15	89±104	41	105±124	69	123±124	NS
When I eat …	24	84±116	17	80±64	84	128±130	NS
I feel hungry …	61	109±127	55	114±117	9	134±125	NS
Food tastes…	8	125±134	45	82±111	72	131±124	NS
Compared to when I was younger, food tastes…	28	89±114	71	129±125	26	96±117	NS
Normally I eat…	30	99±114	56	108±119	39	132±132	NS
I feel sick or nauseated when I eat…	4	93.5±121	22	60±88	99	126±126	NS
Most of the time my mood is…	10	124±123	35	68±98	80	132±127	NS

When analysis of relationship between individual questions and TTFE was repeated for men and women separately, there was no difference compared to whole cohort results except for significantly shorter TTFE amongst men who have smaller number of meals a day and those with poor mood, as well as in women with earlier satiety (data not shown). However, the size of 'poor' and 'good' groups in each case was very different (e.g. three vs. 44 for men in the mood question), influencing statistical results.

The mood question was considered to have potentially high importance in a group of patients with chronic disease, but the effect of poor mood on appetite could not have been tested as only ten people self-reported poor mood. Out of those three self-reported poor appetite, 3 reported good appetite and 4 assessed their appetite to be neither good nor bad, therefore further statistical analysis was not appropriate.

There was weak, but significant correlation between eating limitations score (combined score of three selected questions looking at self-reported appetite, satiety and a number of meals a day) and TTFE (Rho=0.194, p=0.030), but this was not significant when considered for men and women separately. Kaplan-Meier graph of time-to-event showed no relationship between those with eating limitations score above or below the mean for those three questions (10.2 points).

7.2.1.3.3 Appetite score and exacerbation rate

The AS correlated significantly with AER (Rho =-0.232, p=0.009), which was also statistically significant in women and men considered separately. There was no significant difference between low and high AS groups in the AER (low AS 3.4±2.7AE/y vs. high AS 2.7±2.7AE/y; p=0.111), either on cohort level or for men or women.

When AS was compared between frequent and infrequent exacerbators (two or less AE vs. more than two AE) there was significant difference in AS on cohort level (infrequent 28.5±3.9pts vs. frequent 26.4± 4.6pts, p=0.001), but also for men (infrequent 29.3± 3.4 pts vs frequent 27.6± 3.8 pts, p=0.010) and women (infrequent 27.6±4.2 pts vs frequent 24.8±5.1 pts, p=0.016). When frequent exacerbators were divided into further two categories (two to four or more than four AE) the difference between categories remained significant (p=0.002) (Figure 32). In women average AS decreased with increase in AER (27.6 pts vs. 26.7 pts vs. 24 pts, p=0.015) while in men this pattern was not clear (29.3 pts vs. 26.8 pts vs. 28.1 pts, p=0.054).

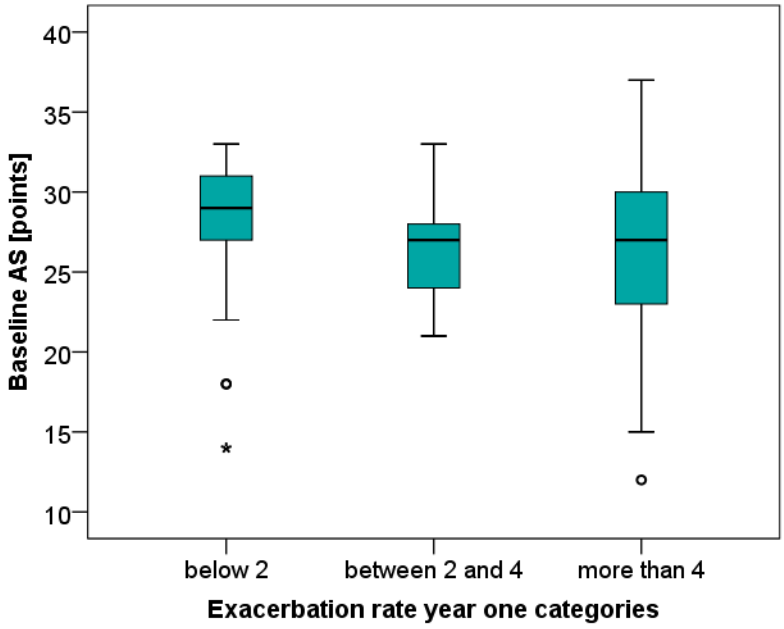


Figure 32 Baseline appetite score (AS) in three categories of exacerbation rate during the follow-up (median, error bars)

7.2.1.3.4 Individual CNAQ questions and exacerbation rate

There was no significant difference in AER between those with 'poor' or 'good' answers to each CNAQ questions (patients with middle answers were excluded from the analysis). However, there was a number of questions that showed the difference in AER if considered only in women (Table 42). There was weak, but significant negative correlation between eating limitations score (selected three questions on appetite, satiety and number of meals a day) at baseline and AER (Rho=-0.185, p=0.039).

Table 42 Exacerbation rate (AER) (mean ± SD) for 'poor', neutral and 'good' answers for each CNAQ question with the significance of the difference AER between 'poor' and 'good' answers (Mann-Whitney test)

CNAQ individual questions	'poor' (answers 1 or 2)		Neutral (answer 3)		'good' (answers 4 or 5)		p-value
	n	AER	n	AER	n	AER	
My appetite is…	15	4.6±3.5	41	3.0±2.5	69	2.8±2.7	0.051
When I eat …	24	4.0±3.5	17	3.1±2.3	84	2.8±2.6	ns
I feel hungry …	61	3.3±2.4	55	3.1±2.0	9	1.8±1.9	ns
Food tastes…	8	4.0±3.5	45	3.4±2.6	72	2.8±2.7	ns
Compared to when I was younger, food tastes…	28	3.3±2.4	71	3.0±2.9	26	3.1±2.6	ns
Normally I eat…	30	3.3±2.5	56	3.0±2.3	39	3.0±3.5	ns
I feel sick or nauseated when I eat…	4	5.2±3.1	22	4.2±3.4	99	2.8±2.5	0.063
Most of the time my mood is…	10	3.2±3.2	35	3.3±2±2	80	3.0±2.9	ns

When analysis of the relationship between individual questions and AER was repeated for men and women separately, there was no difference in clinical outcome between men who gave 'poor' or 'good' answers to questions analysed one by one. In women, several questions showed a significant difference in AER between those with 'poor' or 'good' answers, but the size of 'poor' and 'good' groups in each case was very different (e.g.5 vs. 26 in question about hunger) being the main reason for the statistical significance of findings.

7.2.1.3.5 Appetite score – does original cut-off relate to clinical outcomes in COPD?

The AS was able to statistically discriminate those with and without AE within 30 days (AUC=0.649, p=0.005, SE=0.051) which was stronger than results for 100 or 365 days(Figure 33). Almost 40% of first exacerbations happened within first 30 days, and by 100 days it increased to 65%, which supports the significance of AUC in both time categories.

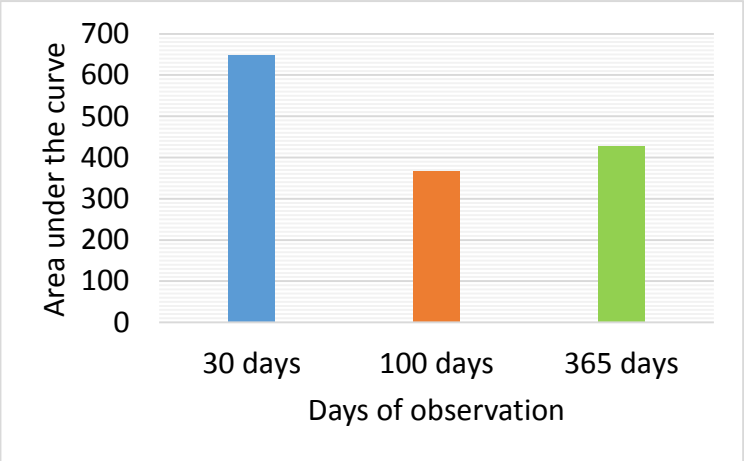


Figure 33 Area under the curve values (10^3) for appetite score for patients with and without exacerbation within 30 days (p=0.005), 100 days (p=0.012) and 365 days (p=0.3)

Cut-points between 25 and 28 points showed statistically significant differences in TTFE between low and high AS, with a cut-off at AS≤27pts showing the highest significance (log rank p=0. 010) (Figure 34).The difference in population falsely identified by the original cut-off of ≤28 points, compared to the most statistically significant difference at 27 points, was 10%. The decision was made to use the original cut-off of ≤28 points in the majority of analyses.

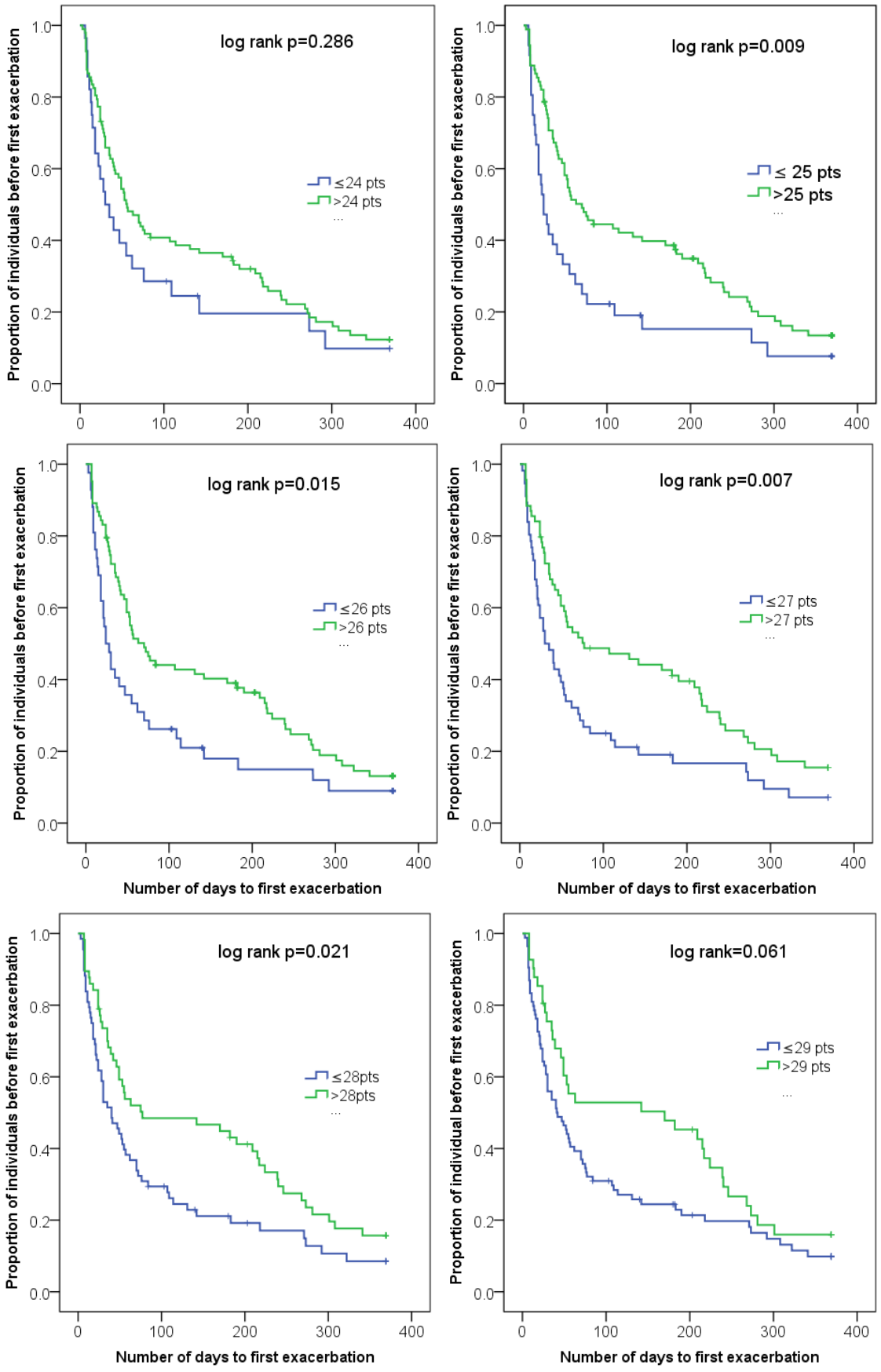


Figure 34 Kaplan-Meier graphs of time to first exacerbation in low and high appetite groups defined by different cut-off points (a=24, b=25, c=26, d=27, e=28, f=29 points)

7.2.1.4 Appetite scores in stable COPD - Summary

7.2.2 Change in appetite around exacerbation

7.2.2.1 Change in appetite scores at exacerbation

Out of 107 first exacerbations during 12 months 76 had data for a stable-exacerbation-stable complex, of which 62 were moderate, 12 mild and only two required hospitalisation. Inflammation markers were increased, compared to pre-exacerbation visit, in majority of patients: 69% had significantly increased CRP (average increase of 16.0±34.2mg/L, $p<0.001$) and 62% had increased fibrinogen (average increase of 0.66±1.13g/L, $p<0.001$). The degree of change in CRP and fibrinogen was not related to the exacerbation severity.

Change in AS around exacerbation was evident when the pre-exacerbation AS was compared with the AS at exacerbation (Figure 35). On average, the AS at exacerbation decreased by 1.1 ± 3.7pts compared to the pre-exacerbation and increased again in the post exacerbation period (mean 63 ± 33 days) by 1.6 ± 3.3pts.

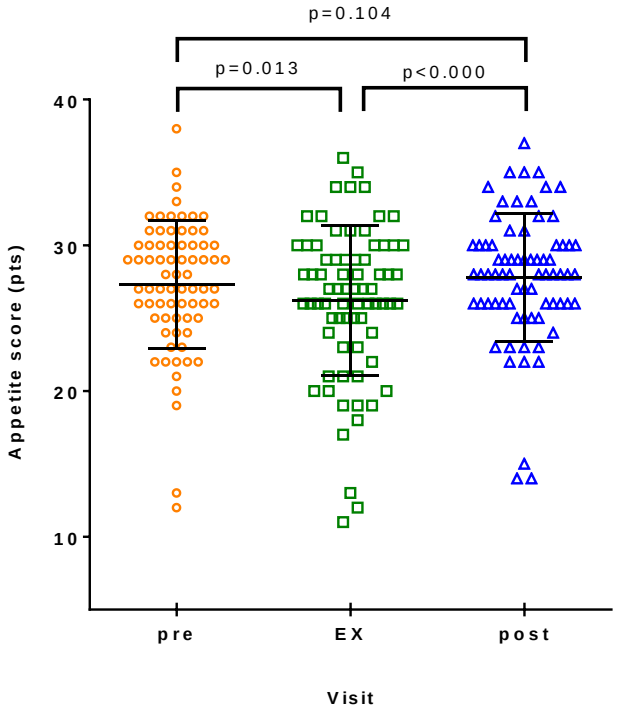


Figure 35 The appetite score (pts) at pre-exacerbation visit (pre), at exacerbation (EX) and post-exacerbation visit (post), (paired sample T- test, n=73)

The overall small decrease in AS at exacerbation was not always evident on an individual level (Figure 36 and Figure 37). 28 patients increased AS by 1 to 4 points and nine patients showed no change in AS between pre-exacerbation and exacerbation visit. Decrease in AS was in a range

from 1 to 19 points, with 13 patients presenting a change in AS of one point, eight patients reporting a change of two to four points and 16 patients showing a change of more than 4 points.

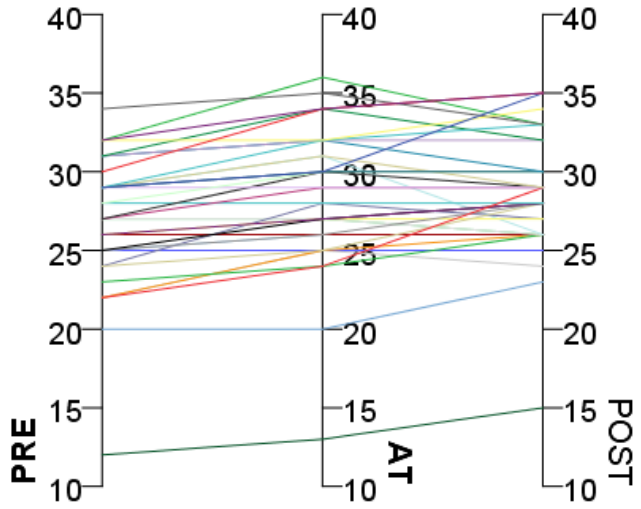


Figure 36 Appetite score at pre-exacerbation visit (PRE), at exacerbation (AT) and post-exacerbation (POST) separately for those who A) increased or maintained appetite score between pre- and exacerbation visit (n=37)

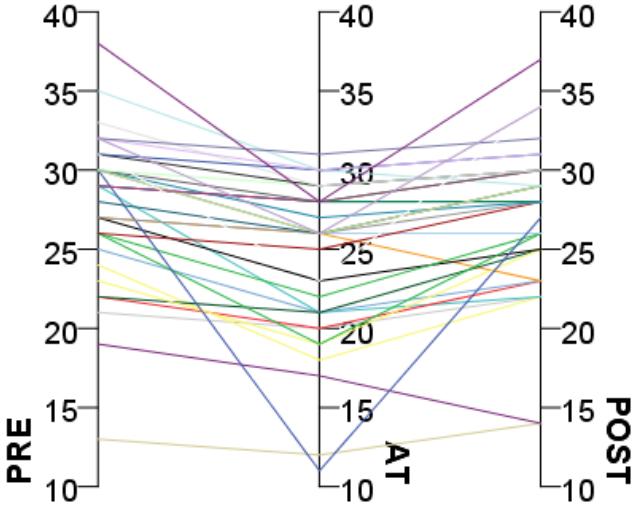


Figure 37 Appetite score at pre-exacerbation visit (PRE), at exacerbation (AT) and post-exacerbation (POST) separately for those who decreased appetite score between pre- and exacerbation visit (n=37)

Patients with the lowest (T1) AS experienced smaller loss of appetite at exacerbation than those with initially the highest baseline AS (T3) (T1 ΔAS 0.7±2.9pts vs. T3ΔAS 2.4±5.0pts, p=0.257).

Self-reported appetite (individual question) did not change at exacerbation in 42% patients, and answers related to hunger, satiety or mood did not change in approximately 60% of patients (data not shown).

The time between last stable and the first exacerbation visit varied from 7 to 169 days. Those who have exacerbated in less than 14 days had the lowest pre-exacerbation score, compared to those who exacerbated in the first or second month (Table 43). There was no significance difference between pre-exacerbation and exacerbation score in all time categories; however appetite score was significantly different between exacerbation and post-exacerbation in three out of four categories. Change in AS was evident in all groups that exacerbated within two months from the last stable visit, but in patients who exacerbated later than in 60 days, there was no difference between pre-, at- or post-exacerbation scores.

Table 43 The average appetite score (AS, points) (mean ±SD) in groups of different time between the last stable and the first exacerbation visit

Number of days between pre- and exacerbation visit	n	CNAQ score ± SD			*p
		Pre-exacerbation	Exacerbation	Post-exacerbation	
≤14	10	25.6 ± 5.4	23.8 ± 5.6	26.7 ± 5.9	0.036
15-29	18	26.8 ± 5.8	25.1 ± 6.2	27.8 ± 4.5	0.015
30-59	25	28.6 ± 3.1	27.1 ± 4.3	28.8 ± 3.7	0.009
≥60	20	27.1 ± 3.8	27.1 ± 4.5	27.2 ± 4.4	NS

**P values for difference between exacerbation and post-exacerbation score, other differences were NS; Wilcoxon signed ranks test*

7.2.2.2 Change in appetite scores at exacerbation and COPD markers

There was no relationship between the degree of changes in AS at first exacerbation and exacerbation history prior to study or AER during the follow-up, showing an insignificant decrease of AS by -0.05 point with each new exacerbation.

Lung function from the last stable visit (FEV_1, FVC, FEV/FVC, TLCO) and standard COPD markers (GOLD, BODE, CAT) were not related to change in AS at first exacerbation. In addition, the degree of change in AS at exacerbation was not related to the exacerbation severity or number of days between the last stable visit and the exacerbation visit (data not shown).

7.2.2.3 Change in appetite scores at exacerbation and inflammation

Inflammation (CRP or fibrinogen) was not directly related to the level of AS at exacerbation, and the degree of change between stable and exacerbation visit were not related either. 54 patients had CRP level lower at the post-exacerbation visit (Figure 38), and 53 patients improved the AS in the same time. However, in four cases both CRP and AS changes were opposite to expected direction of change.

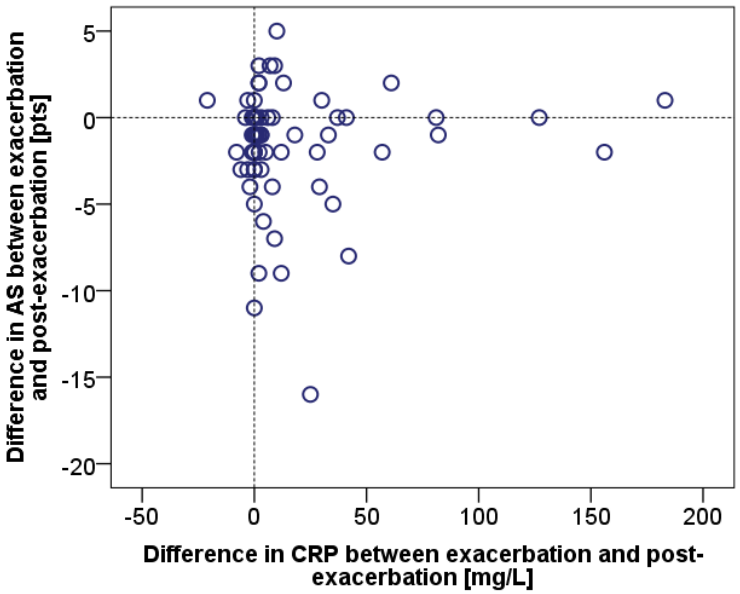


Figure 38 Difference in CRP and appetite score between exacerbation visit and post-exacerbation visit (negative values indicate higher value at post-exacerbation visit), n=73

The degree of change in AS at exacerbation was independent of BMI. Despite this, change in AS between pre-exacerbation and post-exacerbation score was significantly different between those with normal and high BMI (in normal BMI decrease of 0.9±2.2pts vs. high BMI increase of 1.2±2.2 pts, p=0.003). Smokers showed a significant reduction in AS at exacerbation (p=0.032) and had significantly lower AS at the resolution of the exacerbation than ex-smokers (smokers 26.3±4.5 pts vs. ex-smokers 28.9±4.1 pts, p=0.018).

Statistical relevance of BMI in AS change at exacerbation was not evident, however, there was an emerging pattern. Individual changes in AS and CRP between the pre-exacerbation and post-exacerbation visit for low and high BMI groups were plotted (Figure 39). There was a group of patients who decreased AS at the end of recovery period when compared with the pre-exacerbation score (Figure 39, top half). 50 patients had higher AS at the post-exacerbation visit compared to the pre-exacerbation visit of whom 38 (76%) were patients with high BMI (Figure 39, bottom half). Amongst patients with normal BMI (n=36) 14 had post-exacerbation AS lower than pre-exacerbation score, amongst whom ten had CRP elevated beyond the baseline levels (Figure 39, top left quarter, blue dots).

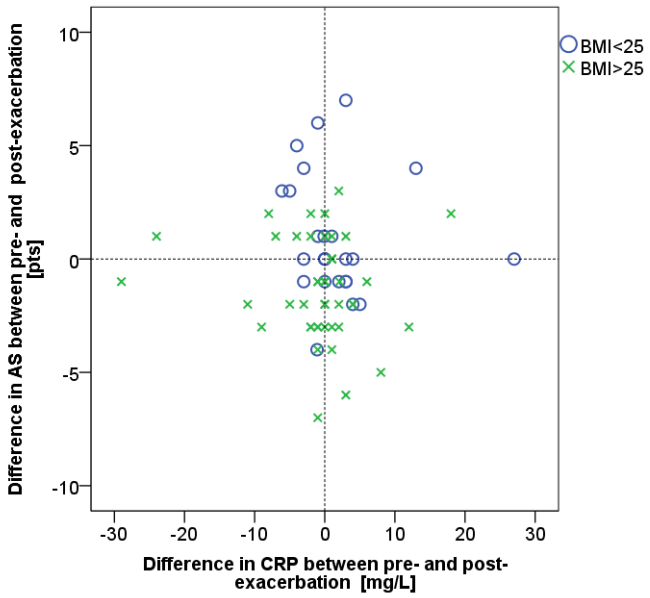


Figure 39 Change in AS and CRP between pre-exacerbation and post-exacerbation visit with BMI categories (BMI≤25 and BMI>25), some points overlap, n=98

Change in inflammatory markers between the pre-exacerbation visit and exacerbation visit, as well as exacerbation visit and post-exacerbation visit, were different between patients with different AE severity (Table 44). However, only 14 out of analysed first exacerbations were mild, while 86 were moderate and only three patients experienced a severe exacerbation, therefore analysis included only mild and moderate exacerbations. Changes in AS between pre-exacerbation and exacerbation visit were no different between severity groups, while inflammation markers were. The increase in appetite score at recovery was significantly higher in those with moderate exacerbation compared to those with mild exacerbation, which was in line with the decrease in CRP and fibrinogen. The degree of loss in AS pre-exacerbation was similar to the level of increase in AS post-exacerbation for both mild and moderate exacerbation groups.

Table 44 Change in appetite score (AS, point) and inflammatory markers (mean±SD) in patients experiencing mild (n=14) or moderate (n=86) exacerbation with comparison of the difference between the severity groups (Mann-Whitney)

Change between two visits	Mild AE (n=14)	Moderate AE (n=86)	p-value
ΔAS PRE-AE	-0.2±2.6	-1.5±3.4	0.278
ΔAS AE-POST	0.1±1.1	1.8±3.5	0.034
ΔCRP PRE-AE	0.6±4.4	16.5±32.3	0.026
ΔCRP AE-POST	2.2±10.6	-17.7±35.1	0.010
Δfibrinogen PRE-AE	0.03±0.7	0.7±1.2	0.021
Δfibrinogen AE-POST	0.2±0.9	-0.6±1.3	0.070

PRE-AE – change from pre-exacerbation to exacerbation; AE-POST – change from exacerbation to post-exacerbation

7.3 Discussion

Appetite is often considered an obvious symptom of COPD and many patients often complain about the lack of appetite. However, this apparent symptom was considered as an inevitable consequence of the disease hence was often overlooked in research. There was a need to understand if appetite can help with prediction of clinical outcomes in COPD and to explore how AS changes around exacerbation. Therefore, the aim of this chapter was to explore if appetite in COPD patients is related to TTFE and AER and to understand how appetite changes around exacerbations. Additionally, considering that interpretation criteria for AS were validated for different purpose, an evaluation of appetite threshold that relates to TTFE was set up.

COPD patients with lower AS had a shorter TTFE and higher AER in the follow-up compared with those with higher AS. Lower appetite score was also related to significantly increased the risk of exacerbation. Individual CNAQ questions did not show any better predictive value either for TTFE or AER, suggesting AS based on total score of eight question is required for identification of patients with increased risk of the poor clinical outcome.

This study provides unique evidence: low appetite score was related to increased risk of exacerbation, and this was independent of the disease severity or history of exacerbations; appetite score changed significantly at exacerbation; original interpretation criteria for CNAQ was related to clinical outcome, especially TTFE. Appetite score measured with CNAQ was straightforward and convenient to use by patients, which offers an easy, clinically relevant tool, an adjunct to current clinical assessments in COPD.

Individuals with low appetite score had a shorter TTFE but there was no difference in AER (Figure 40). Results suggest that appetite score measured with CNAQ questionnaire helps identify people with a risk of an exacerbation within next 30-40 days. This was evident for appetite score interpreted by previously validated cut-off (28 points), regardless the fact that almost 30% of the cohort had AS between 27 and 29 points. 50% of those with the lowest appetite score experienced first exacerbation within less than 30-40 days.

At stable COPD the AS differed between smokers and ex-smokers and those with low AS had worse respiratory status. Sex played a major role in AS, but the prevalence of low appetite was similar in men and women. Lack of relationship with the history of exacerbations suggests its value as an independent marker. The AS was correlated with BMI, but patients with low AS did not have significantly lower BMI, suggesting that in this cohort low appetite was a short-term condition, which has not influenced total body weight Results have shown lack of relationship between AS and inflammation, which could be potentially caused by wide distribution of the

inflammation results and small distribution of the AS. Although AS was related to various disease and demographic markers, results suggest it was not determined by those. Greater proportion of variance in AS remains unexplained as demographic and disease markers explained only 10% of the variance in the AS. It is remarkable to note that in absence of relationship with markers of lung function or other disease markers such strong relationship between categorised appetite and TTFE was found. This could potentially by a type I error, but considering consistency and strength of the relationship between appetite and TTFE, it suggest that this observation could be marking a true relationship. Given the innovative nature of these results, there is a limited body of evidence to compare against.

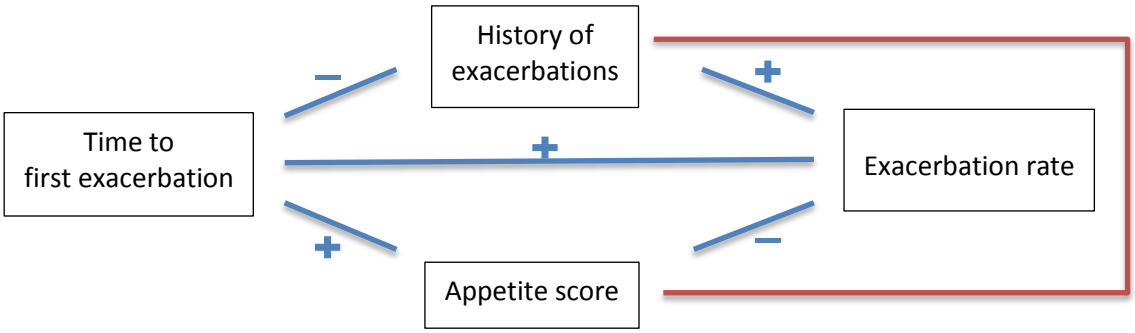


Figure 40 Summary of correlations between exacerbation history, clinical outcomes and appetite score at baseline, '+' positive correlation, '-' negative correlation

The AS was not dependent on the history of exacerbations, suggesting its potential predictive value for both frequent and infrequent exacerbators. To date, history of exacerbations was the best predictive marker of the future exacerbations [38]. However simple and useful it is, there are two major limitations to using history of exacerbations as a risk predictor. Firstly, it is only informative for patients who already have experienced frequent exacerbations, missing individuals who are currently deteriorating, but who have previously not experienced multiple exacerbations. This group of patients requires the most attention, as a potentially minor intervention at that time could protect those patients from rapid deterioration. Secondly, classifying patient as a frequent exacerbator does not give any indication to when to expect next exacerbation, only indicates overall high risk of exacerbation. The appetite score could answer both of those challenges, considering that AS interpreted with tertiles showed as high hazard ratio in Cox model as exacerbation history (83% vs. 87%). Also, AS (both as tertiles or standard cut-off interpretation) was able to differentiate individuals with risk of an exacerbation within 30-40 days. To the author's knowledge, this is the first tool that allows identifying individuals with high risk of exacerbation within next month irrespective of previous exacerbations.

Appetite decreased during the first exacerbation and increase at recovery, as previously suggested [304]. Wang et al. investigated the change in appetite using simplified CNAQ (SNAQ) at exacerbation between the admission to hospital and discharge and showed that even within an average of 12 days from admission to discharge AS increased significantly. In this cohort, there was no significant difference between pre- and post-exacerbation appetite score, suggesting that most patients returned to the pre-exacerbation appetite after recovery from the exacerbation. Patients with higher appetite at baseline have lost appetite to a greater extent during the exacerbation, than those with initially lower appetite. However, this study was unable to estimate how soon after the identification of the exacerbation the appetite returns to baseline.

In some cases, better appetite score was seen in the post-exacerbation visit compared with pre-exacerbation. This could be due to various reasons, including a number of days in between the pre- and post-exacerbation days. The CNAQ score was collected on a three-monthly basis and at ever exacerbation. If patient exacerbated soon after the stable three-monthly visit (in 10 cases it was less than 14 days), the appetite score might have already been altered by the systemic changes prior to exacerbation. In addition, in some cases, the first available post-exacerbation CNAQ score was after 170 days post exacerbation (cases where the patient did not attend the first three monthly visit following the exacerbation or was not considered stable at that visit). This may have been a much longer period of clinical stability, than the one before the exacerbation. Those who have exacerbated soon after the pre-exacerbation assessment (within 14 or 30 days), had on average lower pre-exacerbation score than those who exacerbated later, but they decreased CNAQ score at exacerbation to a similar extent as those who exacerbated later than 30 days after the pre-exacerbation assessment. This suggests that overall change in CNAQ score in this group could be even higher than assessed, if the pre-exacerbation score would have been obtained earlier, prior to systemic changes before the exacerbation. Further analysis of those who have recovered AS to a lesser extent compared with those who fully recovered AS could help understanding the effect of frequent exacerbations on clinical outcomes.

The CNAQ questionnaire that was used in this study, has previously been validated to monitor the risk of weight loss in elderly [211]. In this study the CNAQ was used to explore its relationship with clinical outcomes therefore the cut-off, which was previously validated for different purpose, was evaluated and results suggested that use of the original threshold could also be useful for clinical outcomes in COPD.

Individual components of AS were explored for relationship with TTFE and AER. Self-reported appetite was not as informative as full appetite score during both stable and exacerbation visits, suggesting that one question about appetite during a medical review is not sufficient. Change in

appetite at exacerbation was caused by a change in different questions and, in some cases, self-reported appetite has not changed, while other aspects measured by CNAQ questionnaire did.

The aim of this chapter was to explore if appetite in COPD patients is related to TTFE or AER and to understand how appetite changes in relation to exacerbations. This analysis provides foundations of the role of appetite in the natural course of COPD and shows the area of research that could help in better understanding and management of the disease in the future. Presented findings support the hypothesis that COPD patients with higher appetite score had significantly longer TTFE than those with lower appetite score, therefore appetite score could aid in the identification of individuals at risk of an exacerbation within 30-40 days. Change in AS at exacerbation was significant and showed potential to indicate the development of exacerbation before evident respiratory symptoms. Further investigation should include monitoring of appetite score on a weekly basis over an extensive period to identify when appetite score starts to change prior to exacerbation. This would mark the last 'opportunity window' when the introduction of an appropriate intervention could protect or curtail the exacerbation. In addition, potentially a multicomponent approach could have been more informative than appetite alone and a combination of appetite with body composition or physical capacity could be of a greater value in predicting clinical outcomes like TTFE.

7.4 Limitations

Measurement of the appetite using a patient administered questionnaire is a simple and inexpensive approach, but has several limitations. It requires patient cooperation - to understand and follow instructions accordingly. This showed not to be a challenge within studied cohort, however, it should be acknowledged that this was a group of highly motivated and determined patients, who were determined to provide high-quality data.

The size of the cohort was one of the major limitations of this analysis. There were 127 patients at enrolment, but only 73 had data for stable-exacerbation-stable complex. Measuring appetite on the 3-monthly basis and at exacerbations limits the analysis around the exacerbation and exploring the pre-exacerbation change in detail. Only data obtained on a daily basis would allow detailed analysis and identify exact timelines of appetite change prior to exacerbation. This, however, was beyond the capacity of this study. Regardless that limitation, monitoring appetite on a regular basis with multiple occasions and at every exacerbation is without a doubt exceptional and already provides greater insight into the appetite change in COPD patients, compared with current knowledge than was known up to date.

Physical capability

Using eight questions to measure appetite allows a limited exploration and understanding of appetite modulating factors. Questions, simple and short, require an objective opinion on appetite-related factors and intake-determining factors, which cannot be compared with the information that would be obtained by measuring appetite hormones. The level of appetite-related hormones would be more objective, however, food intake is determined by more factors than the balance between anorexic and orexigenic hormones, which was captured by the questionnaire. Therefore, however, limited and bias it could be, use of a questionnaire to assess the level of parameters influencing food intake appears to be a good alternative to a highly costly and involved assessment of blood markers.

Measuring appetite on a 3-monthly basis and at exacerbations limits the analysis around the exacerbation and identifying a pre-exacerbation change in detail. Data obtained on a weekly basis would allow more detailed analysis and identifying more exact timelines of appetite change prior to exacerbation. This, however, was beyond the scope of this study. Also, a number of factors that could potentially influence change in appetite at exacerbation have not been monitored, including pain and discomfort, which were shown to impair appetite in elderly [306].

The appetite score changes at exacerbation, however, it may not be sensitive enough to accurately assess the level of changes in comparison to stable status. Potentially exacerbation-specific CNAQ question could enable more objective assessment of changes in appetite, if questions were to compare status at exacerbation with the last stable period e.g. ‘Compared to your last stable period, your appetite has changed…’. Such approach would enable to see the difference in appetite related aspects in the more detailed way and possibly relate the change in specific domains (e.g. mood or satiety) with inflammation.

Results presented in this chapter should be considered with caution. Cohort size and study design was not set up to explore the value of AS in predicting TTFE. Therefore, type I error should be considered, when interpreting positive relationship between AS and TTFE.

8 Results – Exploration of physical capability and its relevance in COPD-AERIS study

It has been shown multiple times that COPD patients experience muscle dysfunction in the form of atrophy, weakness and decreased endurance [214, 307], which can lead to reduced physical activity and sedentary lifestyle. Together with limitations in everyday activities and withdrawal from the social activities, it deteriorates the quality of life [265], but also increases the risk of mortality [308]. Taken together the evidence suggests higher mortality and morbidity among those with the worse physical capability, and this was sufficient to encourage international institutions (ATS, ERS) to recommend monitoring of muscle function in COPD patients. In 2014 the evidence was compiled and Maltais et al. concluded that "limb muscle dysfunction is a key systemic consequence of COPD" but there was not enough evidence to explain mechanisms of its development [307]. Moreover, there was, and still is, neither clear guidance what test to use, how often the measurements should be repeated, what level of change over time should instigate an intervention, nor what the successful intervention should consist of.

Selection of exercise test in COPD is currently driven by technical limitations or the personal preferences of clinicians. This often lacks consideration of the differences in tests or specificity of what each test represent. The most commonly used exercise test in COPD, a 6-minute walk test (6MWT), measures lower extremity function, muscle strength, resistance to fatigue and balance. From a physiological perspective, multiple factors contribute to 6MWT results, including body composition (muscle mass and function), respiratory capacity (potential breathlessness) and cardio efficiency, but also non-physiological components may influence the results, like personal speed preferences or space to perform the test. The 6MWT results were thoroughly reviewed by ERS/ATS and shown to be a robust marker of functional capability. Poor 6MWT was associated with increased mortality [221], but there are no unified criteria defining 'poor 6MWT' based on a single measurement. Recent systematic review [REF] defined only criteria for minimal important difference (MID) between two tests for COPD patients and it was determined to be between 25 and 33 m. However, it was not specified what is the minimum time between the two assessments for which this criteria would be applicable. It would be expected to see different magnitude of the effect of the same intervention continued for a month, three months or 12 months period.

Variability of results obtained by different research groups (diverse populations, recruitment sites, included covariates and minor differences in methodology) restricted meta-analysis of the data from ERS/ATS review, limiting ability to define clinically relevant interpretation criteria. This variability and lack of consensus about which criterion represents the greatest relevance to

clinical outcome resulted in studies using randomly selected criteria. Previously used criteria were based on statistical methods (median [309], statistical modelling [290]), distance-based categories [310], distance expressed as speed [311] or MID [312]. Stopping during the walk test was explored in one study [311]. However, none of the interpretation criteria were derived based on its predictive value for clinical outcomes and no analysis has used 6MWT to evaluate the risk of TTFE. Nevertheless, some of previously published criteria, when applied to COPD cohorts, showed relationship with mortality or risk of hospitalisation, as shown in ATS/ERS systematic review [221].

Regardless the limitations of test utility, also stressed in the ERS/ATS review, the 6MWT was the only recommended exercise capacity tests. In clinical practice, that recommendation can sometimes be difficult to implement. Crowded and tight corridors, lack of additional 10 minutes for such assessment and lack of clear interpretation criteria, are probably the most common limitations of 6MWT use by GPs and in hospitals. Therefore, physical capability as simple as 6MWT, but with clear interpretation criteria based on clinical outcomes, would be required.

Hand grip offers an alternative physical capability test [307], as it has relatively low equipment requirements, short test time and is acceptable to older people [137, 179, 269]. Handgrip test can be performed to obtain maximal endurance or muscle strength. Endurance was defined as 'the ability to sustain a specific task' followed by fatigue, known as a failure of force generation that is reversible by rest [307]. Grip endurance depends on the oxygen delivery and motivation, while muscle strength depends on a number of fibres, the cross-sectional area of the muscle, muscle fibre recruitment and fibre type [313]. Change in any of these aspects, especially cross-sectional area, could result in a decrease of muscle strength and response to fatigue. Grip test results can be limited by non-muscle dependent factors like arm fractures, shoulder injuries, arthritis, neurological dysfunction or simply, through lack of motivation. Previous results of handgrip strength were inconclusive, showing similar handgrip strength in healthy control and stable COPD patients [137], while others have shown the relationship between grip strength and hospitalisations due to COPD exacerbations [179]. Interpretation of grip strength, similarly to 6MWT, varied between studies in values and methodologies using a percentage of predicted value [265], arbitrary cut-offs [186] or distribution in large cohorts [314], with no consensus on interpretation criteria.

Whichever test to use, there is a lack of consistent evidence on how exercise capability changes in time and to what extent COPD influences the loss of physical capability, or how a change in exercise capability relates to clinical outcomes. Currently, the evidence is not sufficient to recommend a single test with clear interpretation criteria to predict health outcomes in COPD or identify patients with high risk of exacerbation. There is a need to find simple and equipment-

undemanding capacity tests that could be used in hospitals and GP clinics for every COPD patient, in order to monitor changes and disease progression on a regular basis. Therefore, grip strength and grip endurance were used, and compared with 6MWT results, to explore which of these tests could be used to objectively and in a standardised way measure exercise capability for identification of patients at high risk of poor outcomes and, therefore, a better understanding of COPD.

This chapter tests the hypothesis that low physical capability is common in patients with COPD who are more prone to exacerbations, independently of COPD severity or medical history. Therefore, the primary question of this analysis was 'Do patients with worse physical capability have worse clinical outcomes when measured by exacerbations and is it independent of disease severity or medical history?'. To determine if physical capability was associated with differences in disease severity, activity or exacerbations rate, the analysis of 6MWT, grip strength and grip endurance was performed. This included analysis of physical capability at the stable visit with an assessment of relationship with TTFE and AER over a year.

8.1 Methods

Physical capability tests were performed as described in the Chapter 3.3.4.1. Lack of consensus on interpretation criteria led to the inclusion of various approaches, that could help understand obtained results and to explore the extent to which the interpretation can change depending on the applied criteria.

Six different criteria were used to categorise 6MWT. First, results were categorised for those who stopped (6MWTs) or performed 6MWT uninterrupted (6MWTui), as used in one other study [311]. Next, baseline 6MWT was categorised into the low and high distance by three criteria: 6MWT <350m [290]; 6MWT <300m (based on the visual distinction between 6MWTs and 6MWTui in this cohort) and 6MWT <286m (mean distance of the 6MWTs group plus one SD). Following the international consensus on sarcopenia in elderly, a walking speed was calculated (total distance divided by time) and two criteria, 0.8m/s [315] and 0.9m/s [311], were used.

An effort that individual has to put into a walking exercise depends, to some extent, on the total body weight and is related to the muscle mass. The 6-minute walk work (6MWW) was calculated (total body weight kg x 6-minute walking distance [311]) and a threshold at 6MWW >27000 was used to define high walk work [311].

Grip strength (GS) results obtained in this study were provided in Newtons, while previously published criteria were expressed in kilogrammes, therefore used criteria were recalculated to be expressed in Newtons as well (100N=10.197kg [316]). Grip strength of <30kg (294.2N) for men and <20kg (196.1N) for women [140], along with GS<26kg (255.0N) for men and GS<16kg (156.9N) for women [191] were used.

Grip endurance of 300 seconds should be considered as physiologically improbable and excluded from the analysis. When applying 50% of the maximal grip strength, the maximal endurance would be no longer than 100 seconds [317]. There are no published references for grip endurance test (GE) so sex-specific tertiles (T1 – the lowest results) and quartiles (Q1 – the lowest results) were used (detailed cut-points in Appendix L, Table 91).

Statistical analysis was performed as described in Methods Chapter, section 3.3.6, page 81. Additionally, to investigate relationship between each physical capacity marker and TTFE a Kaplan-Meier plots of TTFE were used with log rank test. Categories for each Kaplan-Meier test were used as specified on each graph.

Physical capability

Cox regression model for GS, GE and 6MWT and TTFE was first performed first for single variable (unadjusted) and hazard ratio with 95%CI was reported. Next, to adjust for sex, smoking at enrolment, age and BMI (high vs. normal based on BMI<25kg/m^2) and lung function (FEV1% predicted) cox regression was repeated with all the above components. Additionally, for 6MWT, a COX regression model adjusted for previous exacerbations (frequent vs infrequent) was performed.

8.2 Results

8.2.1 Physical capability in stable COPD

8.2.1.1.1 Grip strength and COPD markers

Out of 127 enrolled patients 125 grip strength results from baseline were qualified for the analysis. Men had significantly higher grip strength than women. There was no difference in the average grip strength between different disease severities (Table 45) and frequency of exacerbations in the past 12 months did not result in different grip strength. Patients with poor appetite score had the similar grip strength to those with good appetite score.

Table 45 Grip strength (GS) in various groups with significance of the differences between the groups (t-test)

Groups	GS [N] in groups	p-value
Men vs Women	291.1±77.6 vs 180.2±56.6	<0.001
GOLD 2 vs GOLD3&4	241.8 ± 89.4 vs 241.6±87.9	NS
Smokers vs. ex-smokers	242.9±96.6 vs. 241.0±83.4	NS
HAE ≤2 vs. HAE >2	243.8±85.1 vs. 239.5±92.1	NS
BODE≤4 vs. BODE>4	241.2±94.2 vs. 238.5±79.4	NS
AS≤28pts vs. AS>28pts	241.8 ± 93.2 vs. 241.8 ± 82.4	NS

HAE – history of exacerbations, AS – appetite score, NS- non-significant

Previously, two different grip strength thresholds were used to define low GS in COPD patients. In this cohort the prevalence of low GS was very different depending on the used threshold (Table 46). Taking a pragmatic approach, lower cut-points were used for the description of the cohort, showing an approximately third of the cohort having low GS.

Table 46 Prevalence of low grip strength (GS) in men and women using two different criteria (n=110)

		Criterion 1	Criterion 2
Men	Low GS (n)	34	22
	Low GS (%)	55.7	36.1
Women	Low GS (n)	29	17
	Low GS (%)	59.2	34.7

Criterion 1: GS<30kg men GS <20kg women
Criterion 2: GS<26kg men GS <16kg women

Grip strength was deteriorating with age and inflammation markers, but none of the respiratory markers showed a correlation with GS. Grip strength was not related to the history of

exacerbations in past 12 months or patients' perception of the disease severity (CAT) either (Table 47).

Table 47 Characteristic of the grip strength (GS) with correlations and difference between low and high GS groups `

	n	Correlations	p-value	Low GS (n=39) Vs normal GS (n=71) [z]	P-value
Age [y]	125	r=-0.234	0.014	63.8±8.4 vs 71.2±7.0	<0.001[a]
FEV1 [%]	110	x	NS	47.3±16.4 vs 46.3±14.2	NS[a]
FEV/FVC	110	x	NS	0.42±0.12 vs 0.44±0.11	NS[a]
TLCO [%]	53	x	NS	58.9±18.4 vs 61.4±19.7	NS[a]
BODE	80	x	NS	4.6±2.3 vs 3.8±2.6	NS[b]
CAT [pts]	109	x	NS	15.6±7.6 vs 18.2±8.3	NS[a]
HAE [AE/y]	110	x	NS	3.1±2.5 vs 3.2±2.0	NS[a]
CRP [mg/L]	110	Rho=-0.188	0.049	9.7±7.5 vs 6.8±10.4	0.009[b]
Fibrinogen [g/L]	102	r=-0.210	0.034	5.0±0.8 vs 4.6±0.8	0.021[b]
IL-6 [pg/L]	77	Rho=-0.234	0.041	20.9±79.0 vs 4.7± 4.8	0.007[b]

[z]Low GS≤26kg for men and 16kg for women; x – no significant correlation, NS – not significant
[a]T-test, [b]- Mann-Whitney test; r=Pearson's correlation, Rho=Spearman's' correlation

Patients with low GS were significantly older but showed no difference in lung function when compared to those with higher GS (Table 47). In addition, those with low GS had a higher level of inflammatory markers (CRP, fibrinogen, IL-6). When GS cut-off was changed to the higher values (30kg for men and 20kg for women) only CRP remained significantly different between low and high grip strength (data not shown).

BODE score includes physical capability measured with 6MWT. There was no relationship between BODE score and GS, but patients with low GS had insignificantly lower predicted survival according to BODE score interpretation.

8.2.1.1.2 Grip endurance and COPD markers

Baseline grip endurance (GE) of 117 patients qualified for the analysis. There was no difference in the GE between sexes, but those with more severe COPD had borderline significant lower GE, which showed the same pattern in BODE score groups – those with the higher mortality risk (BODE>4) had significantly lower GE (Table 48). GE was no different between frequent and infrequent exacerbators (HAE). Grip endurance was significantly lower in those with lower appetite score.

Table 48 Grip endurance (GE, second) in various groups with significance of the difference between the groups (t-test)

Groups	GE [N] in groups	p-value
Men vs. Women	49.3±42.8 vs. 49.6±40.7	NS
GOLD 2 vs. GOLD3&4	57.5±57.4 vs. 43.0±20.9	0.062
Smokers vs. ex-smokers	44.3±19.3 vs. 52.7±50.9	NS
HAE ≤2 vs. HAE >2	53.1±38.5vs. 45.6±44.8	NS
BODE≤4 vs. BODE>4	60.0±57.2 vs. 39.9±22.5	0.027
AS≤28pts vs. AS>28pts	39.7 ± 21.7 vs. 56.7 ± 55.7	0.034

HAE – history of exacerbations, AS – appetite score, NS- non-significant

There are no published criteria for low/high endurance, therefore lowest grip endurance group (Tertile 1) was compared with the highest grip endurance group (Tertile 3) in this cohort. Such approach limits the analysis of the prevalence of low grip endurance.

Grip endurance was independent of age and BMI but showed a weak relationship with FEV/FVC ratio and BODE score (Table 49). There was a relationship between GE and previous exacerbation number and current inflammation status, but only when measured by CRP.

Table 49 Characteristic of grip endurance (GE) with correlations and difference between low and high GE groups

	n	Correlations	p-value	Low GE (n=39) vs. high GE (n=38) [z]	P-value
Age [y]	117	x	NS	68.3±8.5 vs 65.3±9.7	NS[a]
FEV1 [%]	117	x	NS	45.5±14.0 vs 46.9±16.2	NS[a]
FEV/FVC	117	0.204	0.027	0.42±0.11vs 0.43±0.12	NS[a]
TLCO [%]	62	x	NS	61.3±14.5 vs 58.7±22.8	NS[a]
BODE	88	Rho=-0.220	0.040	4.5±2.1 vs3.6±2.6	NS[b]
CAT [pts]	116	x	NS	18.4±7.5 vs 16.1±7.4	NS[a]
HAE [AE/y]	117	r=-0.208	0.024	3.8±2.9 vs 2.5±1.3	0.011[a]
CRP [mg/L]	117	Rho=-0.189	0.042	8.8±7.0 vs 5.8±5.9	0.026[b]
Fibrinogen [g/L]	105	x	NS	5.0±1.1 vs 4.7±0.8	NS[b]
IL-6 [pg/L]	79	x	NS	19.9±82.7 vs 4.5±2.8	NS[b]

[z]Low GE = lowest tertile, high GE = highest tertile, x – no correlation, NS – not significant
[a]T-test, [b]- Mann-Whitney test r=Pearson's correlation, Rho=Spearman's' correlation

There was no significant difference in disease severity markers, predicted mortality (BODE) or patients disease perception (CAT) between low and high GE groups. Those with lower grip endurance had a significantly higher history of exacerbations and inflammation status measured by CRP.

8.2.1.1.3 Six-minute walk test and COPD markers

125 walk tests were performed at enrolment in this cohort, of which 69 participants had to stop during the test. The majority of individuals who stopped during the 6MWT did so due to breathlessness (n=41) or pain (n=8). The average distance walked in his cohort was 297±111m, but there was a significant difference in the mean 6MWT distance between those who performed the test uninterrupted and those who stopped during the test (Table 50). Similarly, those with more severe disease (GOLD 3&4), frequent past exacerbations (HAE>2AE/y) and lower predicted survival (BODE>4) had significantly lower 6MWT. In addition, those with lower appetite score had significantly lower 6MWT. Similar to walking distance, there was a significant difference in walking work (6MWW) between men and women (p=0.001), between those who stopped during 6MWT or not (p<0.001) and between those who had a good or poor appetite (p=0.002) (data not shown).

Table 50 Six-minute walk test (6MWT, meter) in various groups with significance of the difference between the groups (t-test)

Groups	6MWT in groups	p-value
Men vs. Women	308.6±121.5 vs. 282.6±96.6	NS
GOLD 2 vs. GOLD3&4	333.3±103.7 vs. 267.1±108.8	0.001
Smokers vs. ex-smokers	301.6±101.2 vs. 293.6±117.7	NS
HAE ≤2 vs. HAE >2 AE/y	329.8±109.5 vs. 268.1±105.2	0.002
BODE≤4 vs. BODE>4	352.7±86.0 vs. 205.4±83.7	<0.001
AS≤28 vs. AS>28 pts	270.8 ± 108.7 vs. 327.0 ± 108.7	0.005
6MWT stopped vs. uninterrupted	210.7±76.7 vs. 366.7±81.9	<0.001

HAE – history of exacerbations, AS – appetite score, NS- non-significant

Several of the most commonly used interpretation criteria for 6MWT showed that approximately half of men had poor walking results, independent of used criteria (Table 51). In women, results varied more than in men, showing poor results amongst 41% up to 71% depending on the criteria. Stopping during 6MWT was selected as the criteria of interest because of its simplicity, objectivity and potential easiness of application.

Table 51 Prevalence of poor result in 6-minute walk test (6MWT) depending on distance, pace, stopping criteria and 6-minute walking work (6MWW)

Poor 6MWT		Distance in 6MWT			Pace in 6MWT		Stopping	6MWW
		<350m	<300m	<286m	<0.8m/s	<0.9m/s		<27000kgm
Men (n=68)	(n)	38	32	29	29	34	32	39
	(%)	55.9	47.1	42.6	42.6	50.0	47.1	57.4
Women (n=57)	(n)	42	32	28	28	37	24	48
	(%)	71.2	54.2	47.5	47.5	62.7	40.7	81.4

Walking distance was not related to age or BMI, but showed a relationship with respiratory markers (Table 52). Distance walked by patients was not related to the number of past exacerbations (HAE). Patients' perception of the disease severity (CAT) and inflammation status did not show any correlation with walked distance. As expected, there was a very strong relationship between BODE score and 6MWT, as the walking distance is one of the components of the BODE score.

Table 52 Characteristic of six-minute walk test (6MWT) with correlations and difference between those who stopped and those who walked uninterrupted

	n	Correlations	p-value	stopped (n=56) vs. uninterrupted (n=69) 6MWT	P-value
Age [y]	125	X	NS	66.9±7.4 vs 66.3 ±9.4	NS[a]
FEV1 [%]	125	0.348	<0.001	42.4±15.9 vs 50.0±13.5	0.005[a]
FEV/FVC	125	0.235	0.008	0.41±0.12 vs 0.44 ± 0.11	0.068[a]
TLCO [%]	65	0.346	0.005	54.4±14.0 vs 62.5±30.4	0.078[a]
BODE	94	-0.796	<0.001	5.7±2.1 vs 2.8±1.9	<0.001[b]
CAT [pts]	124	X	NS	17.7±7.9 vs 15.5±7.0	NS[a]
HAE [AE/y]	125	X	NS	3.5±2.3 vs 2.9±2.3	NS[a]
CRP [mg/L]	125	X	NS	9.0±11.7 vs 6.9±6.5	NS[b]
Fibrinogen [g/L]	112	X	NS	4.9±1.0 vs 4.8±0.8	NS[b]
IL-6 [pg/L]	88	X	NS	15.3±64.7 vs 5.1±4.4	NS[b]
AS [pts]	123	0.249	0.006	26.3±4.4 vs 28.3±4.1	0.010

x – no correlation, ns – not significant

[a]T-test, [b]- Mann-Whitney test r=Pearson's correlation, Rho=Spearman's' correlation

As previously shown, there was a significant difference in distance walked by patients who stopped and did not stop (Table 50) and significantly higher BODE (lower predicted survival) in those who stopped, compared to those who did not stop, was present (Table 52). Those who stopped during the 6MWT had also significantly more severe respiratory changes (FEV, FEV/FVC, TLCO%), than those who did not stop during the walk test. This was in line with results showing that majority of patients who stopped during the walking test have done so due to breathlessness. There was no difference in inflammation status between patients who stopped and did not stop during the 6MWT, but appetite score was significantly lower in those who stopped. Having poor result in one test was not an indicator of a poor result in any other physical capability test (Appendix L).

8.2.1.2 Physical capability and nutritional markers

8.2.1.2.1 Grip strength and nutritional markers

Taking into account that there was a significant difference in grip strength between men and women (Table 27, page 130), the analysis of body composition markers was performed separately for men and women to explore sex difference (Table 53 and Table 54). There were fewer significant relationships between body composition markers and grip strength for each sex. Only FFM and FM% were significantly related to grip strength in both men and women. When compared low and normal grip strength groups, only one lean marker and one fat marker were significantly different between the groups in both men and women. As expected, women had lower lean markers and higher fat markers than men in both low and high grip strength groups.

Table 53 Pearson's correlations between grip strength (GS), body composition and physical capability markers at enrolment and comparison of body composition markers in low and normal GS groups (t-test) – women only

	n	Correlations	P-value	low GS (n=17) vs normal GS[z](n=32)	p-value
MUAC [cm]	49	X	NS	28.5±3.7 vs 30.7±5.7	NS
MUAMC [cm]	48	X	NS	22.9±2.5 vs 23.8±3.7	NS
MUAMA [cm^2]	48	X	NS	42.3±9.0 vs 46.1±14.8	NS
FFM [kg]	49	0.362	0.011	37.6±5.9 vs 41.5±7.4	0.050
FFMi [kg/m^2]	49	x	NS	15.0±2.0 vs 15.9±2.2	NS
FFM [%]	49	X	NS	57.3±5.7 vs 59.3±8.0	NS
Imp50 [kHz]	49	X	NS	609.9±89.1 vs 577.0±75.9	NS
Waist [cm]	49	X	NS	96.5±12.3 vs 97.2±16.7	NS
FM [kg]	48	X	NS	28.7±7.8 vs 28.4±10.5	NS
FMI [kg/m^2]	48	X	NS	11.5±3.4 vs 10.8±3.9	NS
FM [%]	48	-0.290	0.046	42.7±5.8 vs 38.5±7.2	0.030
TSF[mm]	48	0.284	0.050	18.9±6.5 vs 20.5±7.9	NS
Appetite score [pts]	48	X	NS	27.1±4.9 vs 25.6±5.2	NS

[z]Low GS≤26kg for men and 16kg for women, x – no significant correlation, NS – not significant

Table 54 Pearson's correlations between grip strength (GS), body composition and physical capability markers at enrolment and comparison of body composition markers in low and normal GS group (t-test) – men only

	n	Correlations	P-value	low GS (n=22) vs normal GS[z](n=39)	p-value
MUAC [cm]	61	X	NS	30.8±3.3 vs 30.9±3.5	NS
MUAMC [cm]	61	X	NS	25.4±2.7 vs 25.6 ±3.2	NS
MUAMA [cm^2]	61	X	NS	51.8±11.3 vs 52.8±13.1	NS
FFM [kg]	60	0.280	0.030	57.2±9.9 vs 61.1 ±9.2	NS
FFMi [kg/m^2]	60	X	NS	19.6±2.6 vs 19.8±2.7	NS
FFM [%]	60	0.413	0.001	68.6±4.3 vs 71.9±4.9	0.008
Imp50 [kHz]	60	X	NS	492.3±83.9 vs 479.3±62.5	NS
Waist [cm]	59	x	NS	107.9±12.1 vs 107.5±14.6	NS
FM [kg]	60	X	NS	26.5±6.9 vs 24.8±8.4	NS
FMI [kg/m^2]	60	-0.250	0.054	9.1±2.3 vs 8.0±2.7	NS
FM [%]	60	-0.395	0.002	31.5±4.3 vs 28.3±4.8	0.012
TSF[mm]	61	X	NS	17.3±7.0 vs 17.1±9.0	NS
Appetite score [pts]	61	X	NS	28.6±4.5 vs 28.4±3.4	NS

[z]Low GS≤26kg for men and 16kg for women, x – no significant correlation, NS – not significant

8.2.1.2.2 Grip endurance and nutritional markers

None of the body composition markers was related to grip endurance, and there was no difference in nutritional markers between those with low or high grip endurance (Table 55). Patients with low grip endurance had some lean markers lower than high GE group (e.g. MUAMA), but other lean markers would be higher in low grip endurance group than in high GE group (e.g. FFMi). This was also evident when to consider fat markers.

Table 55 Pearson's correlations between grip endurance (GE), body composition and physical capability markers at enrolment and comparison of body composition markers in low and high GE groups (t-test)

	n	Correlations	P-value	low GE (n=38) vs high GE [z](n= 39)	p-value
BMI [kg/m2]	117	x	NS	27.9±4.6 vs 26.4±4.7	NS[a]
MUAC [cm]	117	X	NS	30.2 ±4.0 vs 30.3±3.4	NS
MUAMC [cm]	115	X	NS	24.5±3.4 vs 24.1±2.7	NS
MUAMA [cm^2]	115	X	NS	47.0±10.2 vs 48.6±13.2	NS
FFM [kg]	115	X	NS	51.8±13.5 vs 50.8±13.7	NS
FFMi [kg/m^2]	115	X	NS	18.2±3.2 vs 17.5±3.2	NS
FFM [%]	115	X	NS	65.5±7.8 vs 67.0±7.7	NS
Imp50 [kHz]	115	X	NS	522.3±103.9vs 532.1±82.1	NS
Waist [cm]	115	X	NS	104.6±14.9 vs 99.3±15.0	NS
FM [kg]	115	X	NS	27.1±8.3 vs 24.5± 7.8	NS
FMI [kg/m^2]	115	X	NS	9.7±3.1 vs 8.6±2.7	NS
FM [%]	115	X	NS	34.6±7.8 vs 32.3±6.8	NS
TSF [mm]	115	X	NS	17.9±8.0±18.5±8.3	NS
Appetite score [pts]	115	X	NS	27.4±4.5 vs 27.8±4.7	NS

[z]Low GE=lowest tertile, high GE=highest tertile, x – no significant correlation, NS – not significant

8.2.1.2.3 Six-minute walk test and nutritional markers

Distance walked during 6MWT was negatively correlated to fat markers, but FFM% was the only lean marker that correlated with 6MWT (Table 56). When comparing those who stopped with those who did not stop during 6MWT there was no significant difference in body composition, except for borderline significant higher FMI in those who stopped.

Table 56 Pearson's correlations between 6-minute walk test (6MWT), body composition and physical capability markers at enrolment and comparison of body composition markers in 6MWT stopped and 6MWT uninterrupted groups (t-test)

	n	Correlations	P-value	stopped (n=56)vs. uninterrupted (n=69)6MWT	p-value
BMI [kg/m^2]	125	X	NS	28.5±6.4 vs 27.2±4.7	NS[a]
MUAC [cm]	125	X	NS	30.8±5.4 VS 30.5±3.7	NS
MUAMC [cm]	123	X	NS	24.6± 3.6 VS 24.6±3.1	NS
MUAMA [cm^2]	123	X	NS	49.4±14.8 VS 49.1±12.4	NS
FFM [kg]	123	X	NS	50.2±12.3 vs 51.7±13.2	NS
FFMi [kg/m^2]	123	X	NS	17.8±3.0 vs 17.9±3.3	NS
FFM [%]	123	0.306	0.001	63.9±9.1 vs 66.5±8.3	NS
Imp50 [kHz]	123	-0.184	0.041	536.9±85.9 vs 520.2±97.6	NS
Waist [cm]	123	-0.177	0.050	105.0±17.1 vs 101.6±14.0	NS
FM [kg]	122	-0.238	0.008	28.4±10.7 vs 25.8± 8.2	NS
FMI [kg/m^2]	122	-0.284	0.002	10.4±4.2 vs 9.1±3.1	0.064
FM [%]	122	-0.318	<0.001	35.5±8.3 vs 33.0±7.8	NS
TSF [mm]	123	X	NS	17.8±8.0 vs 18.6±8.2	NS
Appetite score [pts]	123	0.249	0.006	26.3±4.4 vs 28.3±4.1	0.010

x – no significant correlation, NS – not significant

Additionally, walking distance was plotted against lean markers with an indication of stopping/uninterrupted test (Figure 41). There was the evident difference in distance walked by those who stopped and did not stop with a wide spread of FFMi in both groups confirming that lean markers were related neither to distance nor to stopping during 6MWT.

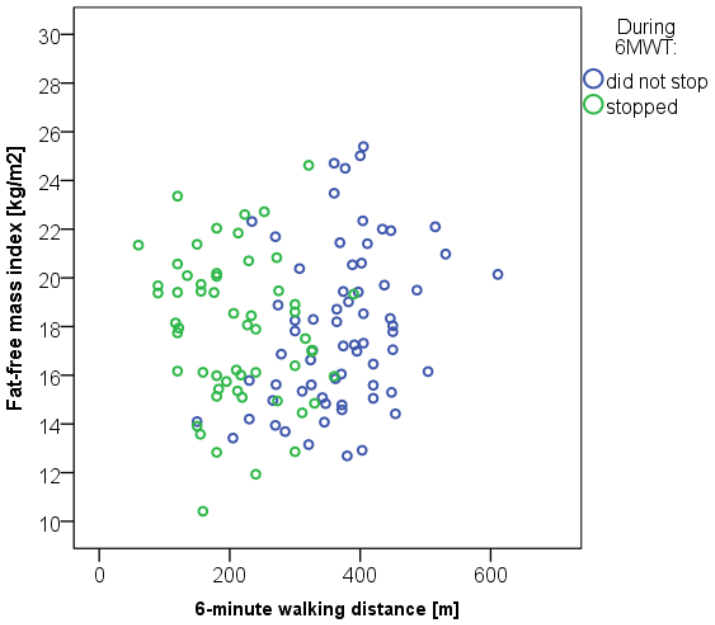


Figure 41 Distance walked during 6-minute walk test compared with fat mass index and categorised by stopping or not-stopping during the walk test

8.2.1.3 Physical capability and clinical outcomes

8.2.1.3.1 Grip strength, grip endurance and clinical outcomes – time to first exacerbation

None of the baseline grip tests showed a correlation with TTFE, not for the whole cohort or sexes separately. When categorical approach was applied for GS, no difference in TTFE between low and high GS groups was found, irrespective of used interpretation criteria (Table 57).

Table 57 Comparison of time to first exacerbation (days) in low and normal grip strength (GS) groups (Mann-Whitney test) with low GS defined using two different criteria

	N (low GS)	low GS vs normal GS	p-value
Criterion 1	110 (63)	105±126 vs 129±116	NS
Criterion 2	110 (39)	114±122 vs 116±123	NS

Criterion 1: GS<30kg men GS <20kg women
Criterion 2: GS<26kg men GS <16kg women

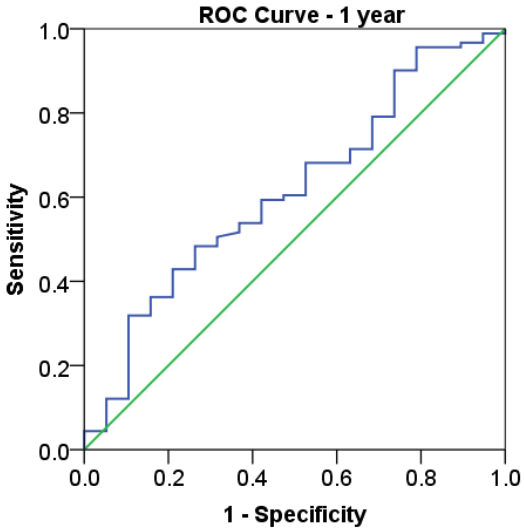


Figure 42 A ROC curve for grip strength and exacerbation occurrence within one year (AUC=0.613, p=0.123)

To test if GS or GE has qualities to discriminate those with and without exacerbation, a ROC analysis was used. The area under the curve (AUC) was very low in all cases, regardless of analysing GS or GE effect on exacerbation occurrence within 30 days (AUC_{30}), 100 days (AUC_{100}) or one year (AUC_{365}) (GS AUC_{30}=0.502, AUC_{100}=0.530, AUC_{365}=0.613; GE AUC_{30}=0.554, AUC_{100}=0.581, AUC_{365}=0.450). Low specificity and sensitivity were evident in each case as shown in the example of ROC for grip strength for exacerbation occurrence in 1 year (Figure 42). In all cases, identifying cut-offs to discriminate those with or without exacerbation was futile, considering that the event was predicted by GS and GE to the similar extent as it would be by chance.

There was no significant difference in the TTFE between those below and above the 50th centile (data not shown). When GS was categorised into tertiles (T1 – the lowest grip), individuals in the middle tertile (T2) had shorter TTFE than both T1 and T3 (Figure 43– Median T1 55±11 days, T2 28±9 days, T 70±47 days). The pattern changed after 200 days, when individuals from the T3 presented a rapid increase in the first exacerbations rate. Analysis of GS quartiles showed that Q1 line (the lowest GS) was above the Q4 line (the highest results, Figure 44). A 50% of individuals experienced first exacerbation within 77±50 days (Q1), 40±8 days (Q2), 108±111 days (Q3) and 62±10 days (Q4) from enrolment (median ± SD), showing no pattern in TTFE, when to consider an increase in GS

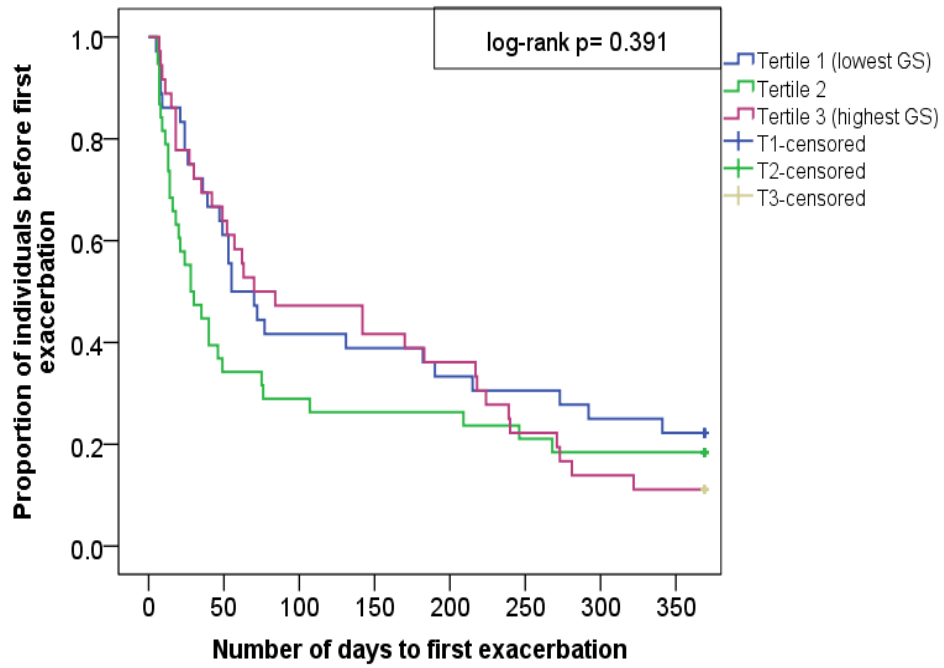


Figure 43 Kaplan-Meier graph of time to first exacerbation in sex-specific tertiles of grip strength at enrolment

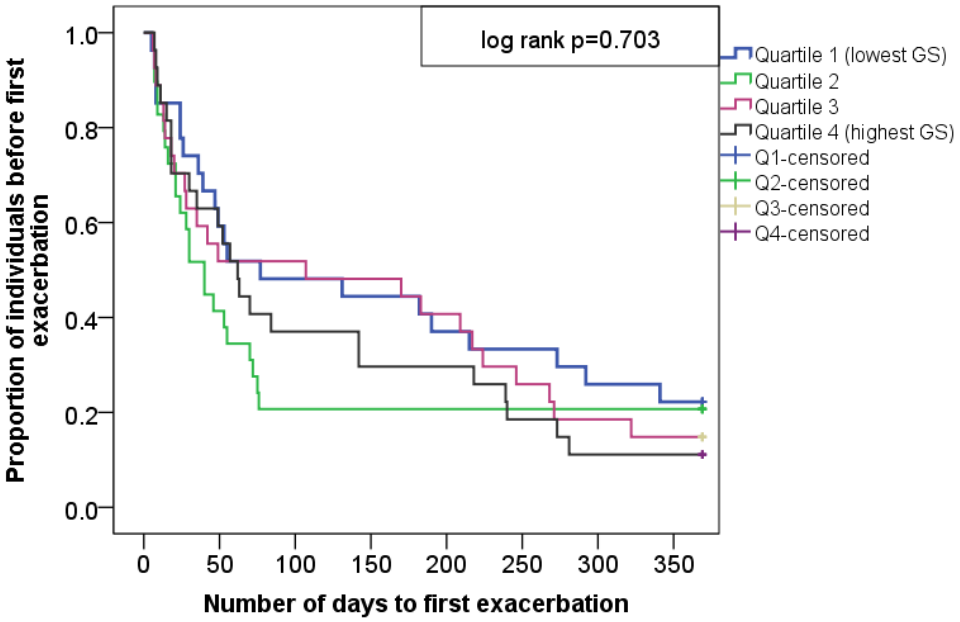


Figure 44 Kaplan-Meier graph of time to first exacerbation in sex-specific quartiles (Q) of grip strength at enrolment

Grip endurance was also plotted against TTFE and difference between those below sex-specific 50th centile and above it were not significant (log rank p=0.276), even though median TTFE was different (below 50th centile median TTFE 40±9 days vs. above 50th centile median 75±15 days). At any time, those with the highest GE (T3) had the lowest proportion of patients who experienced first exacerbation (Figure 45). The TTFE was increasing with the increase in the grip endurance – T1 41±8 days, T2 52± 25 days, T3 70±22 days (median ± SD). The same pattern was evident when

grip endurance was categorised into tertiles, and those with the highest endurance (Q3 and Q4) had longer TTFE (Figure 46).

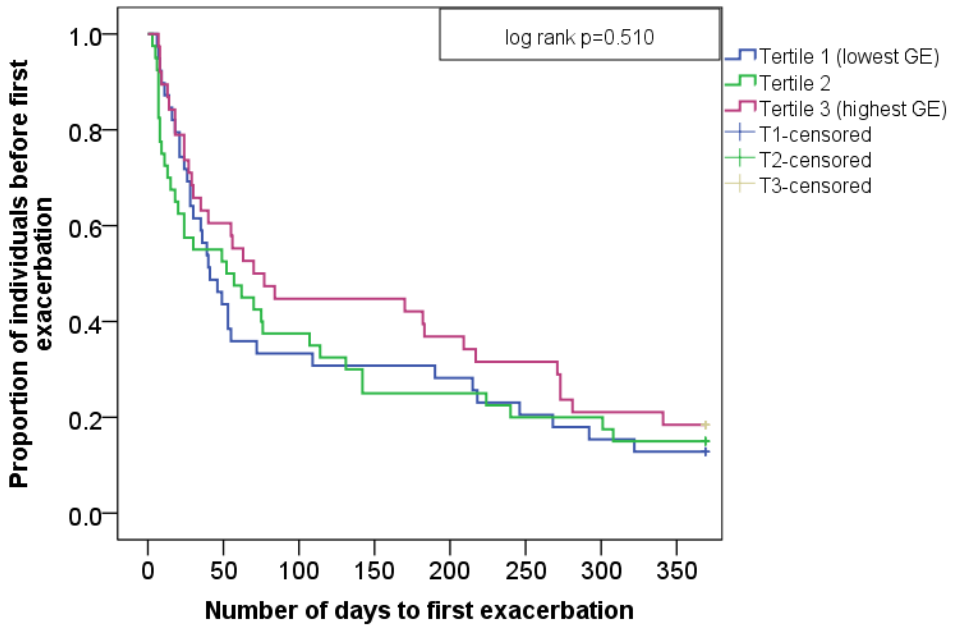


Figure 45 Kaplan-Meier graph of time to first exacerbation in sex-specific tertiles (T) of grip endurance at enrolment

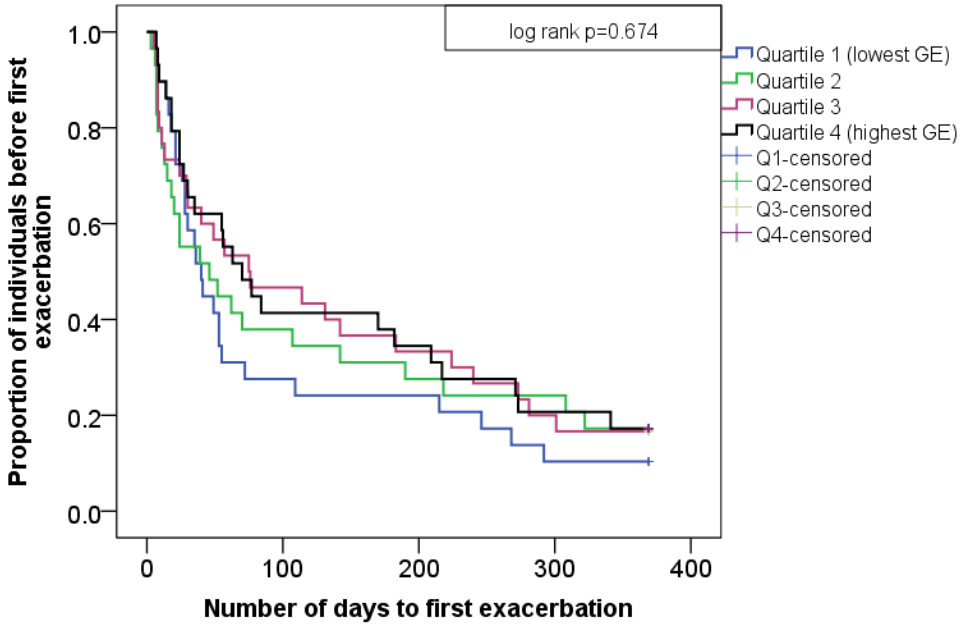


Figure 46 Kaplan-Meier graph of time to first exacerbation in sex-specific quartiles (Q) of grip endurance at enrolment

Cox regression model with grip tests confirmed the lack of relevance of GS and GE in predicting TTFE (Table 58). When Cox regression model was adjusted for sex, smoking and age, none of the parameters showed significance or changed the effect of GS or GE on the risk of exacerbation (data not shown).

Table 58 Cox regression model for grip strength (GS) and grip endurance (GE) (non-adjusted)

	HR	95% CI	p-value
GS [N]	1.001	0.998-1.003	0.534
GS low vs normal (criterion 1*)	1.065	0.702-1.614	0.767
GS low vs normal (criterion 2**)	0.905	0.586-1.398	0.654
GE [sec]	0.997	0.991-1.003	0.299
GE low vs high (T1 vs T3)	1.315	0.807-2.145	0.272

HR – hazard ratio, CI – confidence interval

*Criterion 1: GS<30kg men GS <20kg women

**Criterion 2: GS<26kg men GS <16kg women

8.2.1.3.2 Grip strength and endurance and clinical outcomes – exacerbation rate

Grip strength and endurance were also compared against AER, but none of baseline grip tests showed a correlation with AER, not for the whole cohort or sexes separately. GS and GE did not differ between the AER categories either. The mean values of GE were deteriorating with increasing categories of AER, but the range of results was broad and overlapping in between the categories (Figure 47 and Figure 48).

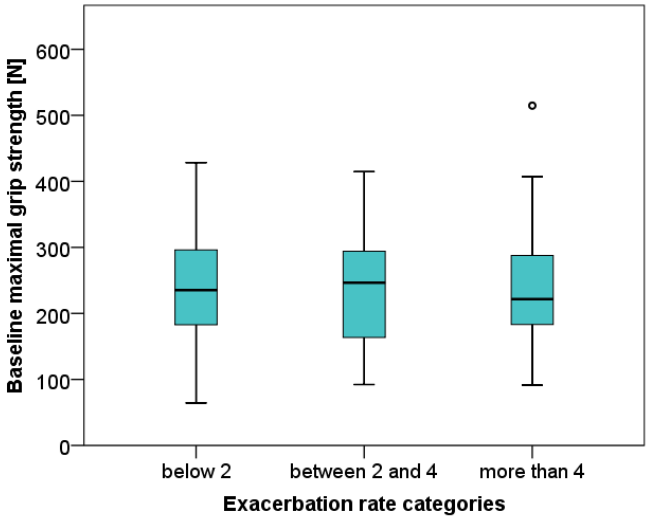


Figure 47 Baseline grip strength in three categories of exacerbation rate (median, error bars)

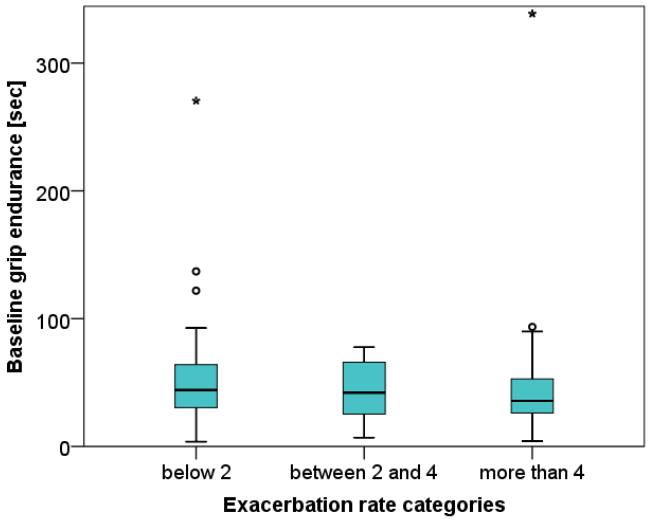


Figure 48 Baseline grip endurance in three categories of exacerbation rate (median, error bars)

When categorical approach was applied for GS, using previously published cut-off values (criterion 1 and criterion 2), no difference in AER between low and high GS groups was found (data not shown).

8.2.1.3.3 6-minute walking distance and relationship to clinical outcomes – time to first exacerbation

The 6MWT was correlated with TTFE, but only in women (Rho=0.378, p=0.004). All applied criteria, walking distance, walking speed and discontinuation of walking test, showed a significant difference in TTFE (Table 59). For the majority of 6MWT categorization criteria, poor 6MWT was related with median TTFE of 35 days, while good 6MWT result was related with TTFE between 75 up to 170 days. When classical categorisation method (tertiles, quartiles) were used, there was no significant difference in TTFE between the groups (data not shown).

Table 59 Relationship between 6-minute walk test (6MWT) (various criteria) and time to first exacerbation (TTFE)

Criteria for poor 6MWT	TTFE in poor vs. good 6MWT*	Difference in TTFE**	Log-rank
6MWT <350m	39±10 vs 170±89	p=0.011	p=0.001
6MWT <300m	35±6 vs 77±26	p=0.039	p=0.023
6MWT <286m	35±6 vs 75±29	p=0.026	p=0.014
6MWT <0.8m/s	35±6 vs 75±29	p=0.009	p=0.014
6MWT <0.9m/s	39±6 vs 84±43	p=0.015	p=0.009
6MWT stopping	36±10 vs 75±28	P=0.024	p=0.001

*median ±SD; ** Mann-Whitney Test

Because the average distance of those who walked uninterrupted (6MWTui) or stopped during 6MWT (6MWTs) was significantly different, the relationship between 6MWTui and 6MWTs with clinical outcomes was investigated (Figure 49). The individuals who stopped during the 6MWT showed significantly shorter TTFE (mean=77.1, 95% CI 53.1 – 100.8 days) than those with uninterrupted walking test (mean=147.0, 95% CI 113.5 – 180.5 days; p=0.01).

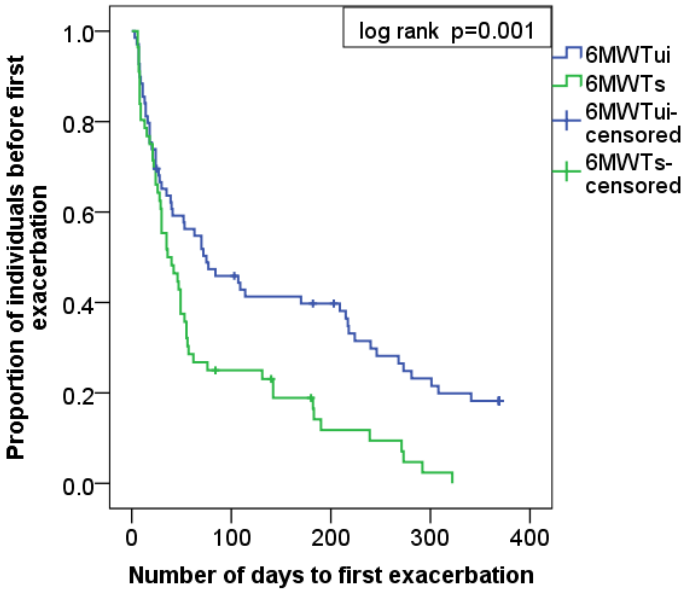


Figure 49 Kaplan-Meyer graph of time to first exacerbation in those who stopped during 6-minute walk test (6MWTs) or did not stop (6MWTui)

Cox regression model was used to test the risk of exacerbation using various 6MWT criteria (Table 60). From all of the 6MWT interpretation criteria, the 350m and uninterrupted/stopped tests were the most significant criteria for identifying high risk of exacerbation. The hazard ratio for 6MWT (uninterrupted vs. stopping group) increased to 102.3% (p=0.001) when adjusted for age, sex, smoking, and BMI>25kg/m^2 (high vs. normal) (see Appendix L, Table 92). The hazard ratio for 6MWT (uninterrupted vs. stopping group) was 1.896 and decreased to 1.676 (p=0.011) when adjusted for history of exacerbations (HR for HAE in this model was 1.164, p=0.001).

Table 60 Cox regression model for 6-minute walk test (6MWT) presented as different criteria

	HR	95% CI	p-value
6MWT [m]	0.998	0.996 - 1.000	0.029
6MWTs vs 6MWTui	1.896	1.279 - 2.812	0.001
6MWT<350m vs 6MWT>350m	1.947	1.282 - 2.956	0.002

HR – hazard ratio, CI – confidence interval; 6MWTs – walk test stopped, 6MWTui – walk test uninterrupted

8.2.1.3.4 6-minute walking distance and clinical outcomes – exacerbation rate

The 6MWT showed weak, but significant negative correlation with AER for both men (Rho=-0.254, p=0.036) and women (Rho=-0.274, p=0.039). The mean values of 6MWT were similar in AER categories, especially when individuals who stopped or not during the test, were considered separately (Figure 50).

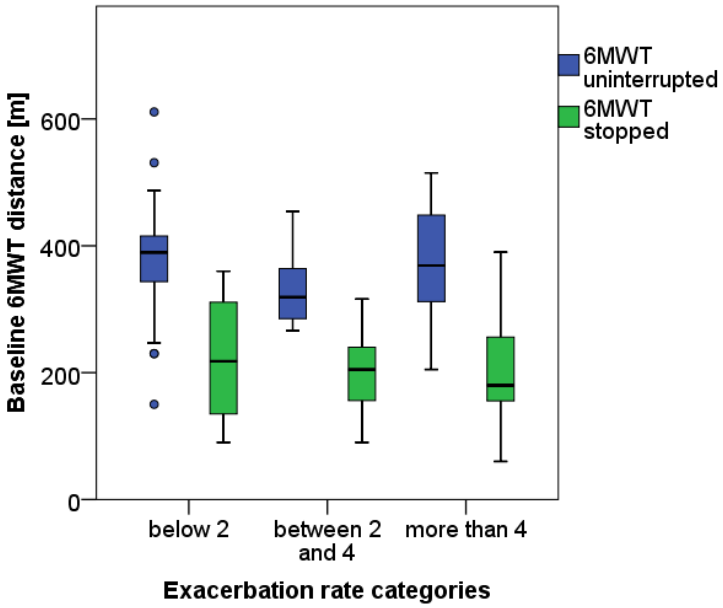


Figure 50 Baseline 6MWT distance in different exacerbation rate categories presented separately for those with uninterrupted 6MWT and those who stopped

Independent of used 6MWT criteria for poor results (distance, speed, and stopping) all walking distance criteria showed a significant difference in AE rate between poor and good 6MWT groups (Table 61).

Table 61 Difference in exacerbation rate (AER) between those with poor or good 6-minute walk test (6MWT) results by various criteria

Criteria	AER in poor vs. good 6MWT*	Difference in AER *
6MWT <350m	3.7±3.0 vs 2.2±2.0	p=0.004
6MWT <300m	3.8±2.9 vs 2.4± 2.3	p=0.004
6MWT <286m	3.9±2.9 vs 2.5± 2.4	p=0.003
6MWT <0.8m/s	3.9±2.9 vs 2.5±2.4	p=0.003
6MWT <0.9m/s	3.7±2.8 vs 2.4± 2.4	p=0.003
6MWT stopping	4.0±3.1 vs 2.4 ±2.2	p=0.003

*Median±SD ** Mann-Whitney Test

8.3 Discussion

Many studies have shown that COPD patients have the impaired physical capability and some have indicated a relationship between low physical capability and increased mortality both in healthy [318, 319] and in COPD patients [308, 320]. The use of physical capability as a disease marker has been recognised by ATS/ERS [214, 221, 307], but detailed guidelines were only available for 6MWT, while other tests, like grip strength, have limited guidelines on measurement techniques and interpretation criteria or even its value in COPD. Therefore, the aim of this section of the study was to explore the usefulness of different physical capability markers in predicting TTFE and AER. This study provided evidence for two important findings : poor grip strength or endurance were not related to different TTFE or AER; several criteria of 6MWT, including stopping during test or walked distance, showed significant difference in TTFE.

This study provided evidence for lack of statistically significant relationship between grip tests (endurance and strength) and clinical outcomes (TTFE and AER). Results of 6MWT suggested that many of previously used interpretation criteria discriminate between those with worse and better clinical outcomes. The simplest approach of looking at uninterrupted/stopped 6MWT was as sensitive as distance- or speed-based interpretation criteria. Those who performed 6MWT uninterrupted had almost twice as long TTFE comparing to those who stopped during the test. Walking test was related to respiratory markers, GS was related to body composition and age, while GE was related to appetite. Having poor result in one test was not an indicator of a poor result in any other physical capability test.

Previously, poor physical capacity was shown to be associated with hospitalisations due to exacerbations [179, 265], and current analysis showed a weak association with the history of exacerbations for 6MWT and GE, which was in contrary to results by Spruit [290]. Disease severity had no significant effect on the grip or walking test results, like in several studies previously, including COPD gene study and ATS review [320-322]. Men had higher results in physical capability tests than women, as would be physiologically expected and previously shown [321, 323]. Walking test results were significantly lower in individuals with worse lung function, higher BODE score and higher history of exacerbations, which to some extend could have confounded results.

The 6-minute walking tests showed a relationship to TTFE and AER independent of applied interpretation criteria and independent of disease severity. In addition, there was a negative association between fat mass and 6MWT, similar to what others have shown [324], while in contrary to previous results 6MWT was not related to age [325]. Many of previously used 6MWT interpretation criteria discriminated between those with better and worse clinical outcome. The

simplest approach, looking at uninterrupted 6MWT, was as sensitive as distance- or speed-based interpretation criteria and it was exposed to a lesser error than distance criteria. Those who performed 6MWT uninterrupted had TTFE almost twice as long comparing to those who stopped, which was the only time it has been repeated since the ECLIPSE study [311]. This criterion offers great potential, as according to 6MWT SOP, discontinuation of the test should always be recorded, which significantly increases chances for introducing this as a quick interpretation criteria in clinical practice. It has to be considered that stopping was caused by breathlessness or pain, which indicates that stopping during walk test captures both respiratory capacity and nutritional status.

In opposition to the hypothesis, results have shown no relevance of baseline grip strength or grip endurance tests and exacerbations. Grip endurance showed less relevance with clinical outcomes than grip strength, which potentially explains the lack of published grip endurance data from COPD studies, due to publication bias (negative results do not get published). Interpretation of capacity tests based on simple statistical approach (tertiles and quartiles) showed that those with the lowest capacity scores did not always have the worse clinical outcome, which was in contrary to the biological assumptions.

Grip tests have been recognised as a potential marker of functional capacity and indirectly of lean mass in COPD patients [214, 307]. In most COPD studies grip strength was not related to respiratory function [178, 269], but showed associations with lean mass measured by fat-free mass index [178, 180, 269], same as in this cohort. Also, there was a negative association between fat mass and GS as others have also shown [324]. This suggests that GS could be used as a surrogate marker of body composition, but only if consider limitations like age, because GS decreased with age in both men and women, same as in other studies [222, 323, 325]. The importance of good grip could also be related to the quality of life rather than to clinical outcomes [326], but in this cohort, GS was not related to subjective disease severity score (CAT), and no other quality of life marker was available at the time of the analysis.

This analysis aimed to explore if worse physical capability was related to worse health outcomes in COPD patients. Some, but not all tested capacity markers showed a relationship with TTFE or AER. From all the examined measures and various interpretation approaches, stopping during the 6MWT appeared to be the most unbiased and relevant marker of TTFE, which should be further investigated. Also, shorter walking distance was related to sooner exacerbation. Grip strength and grip endurance did not show relevance to TTFE or AER.

Further investigation should explore walking discontinuation criteria and its relationships with clinical outcomes not measured in this study, like mortality and hospitalisation. In addition, future

research in length of the walking test and time of tests discontinuation should be explored, to verify if stopping occurs at the first or second half of the test and whether this information relates to clinical outcomes. A multicomponent approach could explore whether patients with poor physical capability, who experience worse clinical outcomes, differ in nutritional status measured with body composition or appetite.

8.4 Limitations

Physical capability in COPD can be measured with different tests, all of which have similar caveats. First, exercise testing is often a complex marker of physical capability depending on both body composition (muscle mass) and muscle strength, as well as on the lung function, breathlessness and pain.

Many exercise tests should be considered as patient-driven measurements, where investigators role is to provide instructions, encouragement and monitor the performance. Patient's motivation (or its lack) and learning process play a role. Learning how to perform an exercise, can lead to improvement in results, but only until the maximum level is reached (followed by a plateau). For some participants, once the technique is learned, the effort and focus could decrease, causing a reduction in motivation and test quality. Also, if the test is repeated consequently too many times, the physical capacity could deteriorate due to tiredness and boredom.

Exercise tolerance can be measured by muscle strength using grip strength or grip endurance tests. Even though these techniques are widespread in research, there are many discrepancies in the methodologies. Grip tests are performed with manual or computerised machines, and there is a lack of standardised procedures or rejection criteria, which can lead to low confidence in results. Standardisation is required even with basic elements, such as choosing a consistent hand to perform grip test (i.e. left, right, dominant, non-dominant, both). Rejection criteria are also necessary to secure exclusion of incorrectly performed tests. Interpretation of grip tests is also challenging, as recently there were various reference ranges published but most of them for a healthy population. Knowing the average grip strength in a certain age group in healthy adults does not provide any understanding of results in contexts of chronic disease.

Grip endurance was performed using computerised dynamometer and dedicated software, which some patients found difficult to understand. The high sensitivity of the dynamometer on several occasions caused misrepresentation of the results, as software considered the test to be started at the first touch of the dynamometer, rather than at the start of grip. This caused abnormal results, which required evaluation and implementation of steps, which would limit the prevalence of such circumstances.

9 Results - Multicomponent analysis of the exacerbation risk in COPD – AERIS study

9.1 Introduction

COPD has demonstrated to be a complex, challenging to manage and life changing condition. Traditional COPD classification, based on the airflow obstruction, was proven to be insufficient in guiding treatment [30, 31]. Current understanding of this limitation is related to phenotypical heterogeneity amongst COPD patients. Therefore, other symptoms were investigated in order to group patients with similar disease presentation into disease phenotypes. The aim was to identify patients with similar underlying pathomechanism demonstrated by similar symptoms, hence potentially similar response to treatment.

Historically 'blue bloaters' and 'pink puffers' model was introduced [55], emphasising that patients with emphysema and bronchitis look differently, and have different symptoms on a daily basis. This approach was useful, but not specific enough to manage disease efficiently, as many patients were falling in between the two phenotypes. For the last twenty years researchers and clinicians were making an enormous effort and many attempts in identifying COPD phenotypes [54, 58, 59, 327]. However, the majority of phenotypes focused on presence or absence of one factor e.g. frequent exacerbators, rapid FEV decliners, emphysema-hyperinflation phenotypes [34, 54]. However true those phenotypes are, they have limited ability to guide disease treatment as treatment based on single component phenotype, will only address the problem of that single component and not improve patient's status from a holistic perspective. This is clear when compare two patient with the same rate of FEV_1 decline – they could present with many other differences (body weight, appetite, physical capacity) because of different underlying pathomechanism of the disease. Most recent studies have turned the focus towards multifactorial phenotypes e.g. inflammatory phenotype, systemic phenotype [59]. As useful and informative those phenotypes are, they have limitations when focus on protection and prevention from exacerbations or improvement of patients' quality of life. Phenotyping COPD remains challenging and nutritional phenotyping was emerging as a novel approach that could improve patients' wellbeing. Recently, ERS published four phenotypes focused on assessment of body composition and metabolic risk [61]. Acknowledging the importance of nutritional status in COPD by the international respiratory body is a grand step, however now there is a need to review which nutritional markers have the greatest importance in COPD. So far, the focus was on lean wasting (cachexia and sarcopenia phenotypes) and fat mass redistribution, but in clinical practice, relying

on assessments like DXA or MRI is not feasible. Moreover, previously described phenotypes including a nutritional marker had been determined in relation to mortality or hospitalisation. The validity of this approach is irrefutable, however, like previous respiratory-focused phenotypes; it has limitations in guiding disease management on an everyday basis and carries a risk of extending patients life without improving its quality. Therefore, there is a need to explore widely available nutritional assessment methods for their relationship with clinical outcomes like TTFE and understand differences between nutritional phenotypes. Defining a nutritional risk score based on nutritional phenotypes and their relationship with TTFE could aid in disease management and help with the development of treatments and therapies that focus on resilience to COPD exacerbations. This could have direct effect on patients and potentially improve their wellbeing and quality of life.

In this thesis two clinical outcomes were of major interest: TTFE and AER (AER). Using standard demographics or simple clinical markers, was not helpful in identifying patients at risk of the worse clinical outcome (Table 62). Only history of previous exacerbations (two or less vs three or more) was showing relevance in differentiating between those with low and high TTFE and AER. Overall though, monitoring disease severity in this cohort was not providing sufficient evidence to predict TTFE or AER.

Table 62 Summary of the evidence for difference in clinical outcomes measured with time to first exacerbation (TTFE) and acute exacerbation rate (AER) between the groups

Groups	Difference in TTFE	Difference in AER
Men vs Women	NO	NO
Age groups	NO	NO
Frequent vs infrequent exacerbators (past year)	YES	YES
Smokers s ex-smokers	NO	NO
FEV categories	NO	NO
GOLD classes	NO	NO
BODE categories	NO	YES

To explore the relevance of nutritional markers and clinical outcomes, a number of nutritional markers were compared with disease status and TTFE. Focusing on three core research questions in this thesis, results from previous chapters were compiled and summarised in Table 63. History of exacerbation was measured in the past 12 months. Disease status was represented by markers of lung function (FEV in the table), inflammation (CRP in the table) and multivariable approach (BODE in the table). Clinical outcomes of interest were TTFE and AER in the 12 months of the follow-up.

Table 63 Summary of nutritional status markers and their relationship with the past, current and future clinical status

Nutritional status marker	Did the history of exacerbation influence the nutritional marker?	Was the current disease status associated with the nutritional marker?			Did the marker of nutritional status influence clinical outcomes?	
		FEV	CRP	BODE	TTFE	AER
Weight / BMI	No	No	Yes	No	No	No
FFM / FFMi	No	Yes	Yes [b]	Yes [c]	Yes	No
FM / FMi	No	Yes	Yes	No	No	No
Appetite score	No	Yes	No	Yes	Yes	Yes
Grip strength	No	No	Yes	No	No	No
Grip endurance	No	No	Yes	Yes	No	No
6MWT	Yes [a]	Yes [d]	No	Yes [d]	Yes[d]	Yes[d]

FEV – representing lung function markers (FEV1, FVC, FEV/FVC, TLCO)
CRP – representing inflammation (CRP, fibrinogen, IL-6)
BODE – representing COPD severity marker
[a] *frequent vs infrequent exacerbators*
[b] *women only*
[c] *men only*
[d] *6MWT stopped vs 6MWT not-stopped*

Presented summary table shows that nutritional markers were independent of medical history. At the same time, the majority of nutritional markers at baseline (13 out of 21 considered combinations) showed association with disease markers at baseline. However, only fat mass, appetite and grip endurance showed relevance on a cohort level, rather than for specific sub-groups. Considering association between baseline nutritional status with clinical outcomes in the follow-up, it was lean mass, appetite score and walk test that has shown relevance. Appetite was relevant for both clinical outcomes on a cohort level, while lean mass only appeared to be related to TTFE, and walk test showed relevance when focusing on discontinuation of the test. It appeared clear that lean mass, appetite and walk test have the greatest potential to show relevance to TTFE. Therefore, more detailed summary of associations between the selected markers and clinical outcomes, based on results from chapters 6,7 and 8 was presented in Table 64. All three selected nutritional markers showed a significant difference in TTFE between those with good and poor results. Each of the three nutritional markers indicated that 50% of those with poor results experienced they first exacerbation within a month, with results ranging from 2 weeks up to 2 months.

Table 64 Summary of relationships between appetite score (AS), fat-free mass index (FFMi), 6-minute walking test (6MWT) and time to first exacerbation (TTFE)

	Correlation to TTFE	TTFE - Log rank test	p-value
AS	r=0.251, p=0.005	AS≤28pts M=40 95%CI 23-57 AS>28pts M=77 95%CI 0-217	p=0.021
FFMi	Men - NS Women - NS	T1+T2 M=72 95%CI 43-100 T3 M=27 95%CI 16-38	p=0.013
6MWT	Men - NS Women Rho=0.378, p=0.004	6MWT<350m M= 39 95%CI 20-58 6MWT≥350m M= 170 95%CI 21-319	p=0.001
		6MWTui M= 75 95%CI 21-129 6MWTs M=36 95%CI 16-56	p=0.001

M-median, CI – confidence interval, NS – no statistical difference,
6MWTui – walk test uninterrupted, 6MWTs – walks test stopped
r=Pearson's correlation, rho-Spearman's' correlation

When the same markers were compared with exacerbation frequency in the follow-up, results were less consistent (Table 65). Appetite and walk tests differed between the groups, but there were wide standard deviations in each case.

Table 65 Summary of relationships between appetite score (AS), 6-minute walking test (6MWT) and exacerbation rate (AER)

	Correlation with AER	Frequent vs. infrequent exacerbators *
AS [pts]	Rho =-0.232 p=0.009	26.4±4.6pts vs 28.5±3.9pts p=0.001
6MWT [m]	Men Rho=-0.254 p=0.036 Women Rho=-0.274 p=0.039	268.1±105.2m Vs 329.8±109.5m p=0.002

M-median, CI – confidence interval, NS – no statistical difference,
*Mann-Whitney test

In summary, standard demographic and disease severity markers were in majority not associated with clinical outcomes. In contrary, nutritional markers, especially lean mass, appetite and walk test, independent of the exacerbation history, showed many associations with disease markers and relevance to clinical outcomes. History of exacerbations appeared to be a good indicator of TTFE amongst frequent exacerbators, while nutritional markers were showing relevance to TTFE in the whole cohort. Considering that each nutritional marker has identified a marginally different group of patients, combining all three markers together could provide a better estimation of the risk of exacerbation and time to next exacerbation.

Using a single nutritional marker as an indicator of clinical outcomes appeared to be useful in some patients, therefore the hypothesis was that the more nutritional domains are assessed as unfavourable, the higher the risk of poor clinical outcome measured with TTFE, independent of the disease severity or medical history. Unfavourable results in two domains were considered to carry a higher risk of poor outcome than in one domain, while a patient with three unfavourable results would be considered to have the highest risk of poor clinical outcomes. This hypothesis was tested using nutritional markers, which in previous chapters were shown to relate to TTFE – appetite score, 6-minute walk test and fat-free mass index. This analysis aimed to identify what is the simplest combination of nutritional information that can identify patients with high risk of exacerbation in the near future.

9.2 Methods

Based on the results from previous chapters, appetite, body composition and walk test were identified as domains showing relevance to TTFE in studied cohort. Following clinical relevance of AS, FFMi and 6MWT individually, a multicomponent approach was adapted and tested. A nutritional risk score (NRS) was created to represent number of nutritional components exhibiting poor results. As this required categorical approach, criteria for 'unfavourable' (poor) and 'good' results for each nutritional marker were chosen based on the highest relevance to the clinical outcomes (Table 66). Patients with the AS of 28 points or less (AS≤28points) were shown to have a shorter TTFE, than patients with AS>28 points (Chapter 7). The 6-minute walk test showed relevance to TTFE independent of the applied interpretation criteria. Therefore, two best criteria were selected and tested – stopping during the test (NRS_S) and distance of less than 350m (NRS_D) (Chapter 8). The last domain was based on body composition. Patients with the highest fat-free mass index (FFMi) defined by sex-specific tertiles, have shown shorter TTFE, than patients within the middle or in the lowest FFMi tertile (Chapter 6).

Table 66 Criteria applied for each domain of nutritional risk score based on clinical relevance: appetite score (AS), fat-free mass index (FFMi) and 6-minute walking test (6MWT)

	AS	FFMi	6MWT	
Unfavourable	≤28pts	T3	Stop	<350m
Good	>28pts	T1&T2	Non-stop	≥350m

T1, T2, T3 – sex-specific tertiles of FFMi, T1-the lowest values

The nutritional risk score was calculated as a total of points from the three domains – AS, FFMi and 6MWT. Each unfavourable result was assigned one point (Table 66). The risk score was therefore from zero to three points with the three points indicating the most unfavourable nutritional score, potentially related to the highest risk of exacerbation in the near future. When four components were analysed, score followed the same rule and risk score was then from zero to four points with four points indicating the most unfavourable nutritional score.

The analysis was performed in several steps. First, based on the results from previous chapters, three clinically most relevant nutritional markers were selected. Next, out of three selected markers (AS, FFMi and 6MWT) a two-component index was created using every possible pair of markers. As the walk test was considered to be informative in two different forms (stopping criteria or total distance walked) both forms were used, which resulted in five pairs of nutritional markers.

Next, three-component index was produced and it had two versions due to two forms of walking test component. Following that, most commonly used marker of exacerbation risk, history of exacerbations, was added to the three-component index to assess if by doing so exacerbation risk can be predicted any more accurately.

Lastly, simplification process was applied. As BIA is not available for home use to COPD patients, and currently is not part of standard equipment in GP practices or hospitals either it was replaced with simpler marker, BMI, which was closely related to results of FFMi. Replacing one marker with another was done with the assumption that the new marker will identify the same patients. To test that, a categorization of body weight was done following the same approach as for FFMi - sex-specific tertiles were generated, and patients in the lowest and medium tertile were assigned zero points in the NRS score, while those with the weight in the highest tertile were assigned one point. Then, nutritional risk score with weight (NRS_{WT}) instead of lean mass (NRS_{FFMI}) was calculated, and frequencies in all categories were compared. The 6-minute walk test, commonly used and validated in COPD patients, was considered not to be convenient to perform in clinical settings and would be most likely avoided (e.g. guessed rather than performed) by patients using the score themselves. Considering that results in this thesis showed that none other physical capability marker was related to clinical outcomes, and they required specialist equipment, it was proposed to replace physical capability component with an alternative component of high relevance to TTFE and high clinical utility, like the history of exacerbations. Therefore, following clinical pragmatism, nutritional exacerbation risk score including appetite, body weight and history of exacerbation was tested.

At each step, analysis included:

- Prevalence in each score and proportion of individuals who exacerbated within 30 days within each score
- Hazard ratio using Cox regression (unadjusted) to assess difference in risk of exacerbation within 30 days between different scores
- Graphical presentation of differences in TTFE between different scores using Kaplan Mayer plots
- Where, relevant, Venn diagrams were used to compare prevalence of results or median TTFE if index was created from more than two components
- During the analysis, in three and four component NERS, when results appeared indifferent between highest score groups, risk scores of 2 and 3 were combined into a score 2+.

- Additionally, for main three-component score, comparison of clinical markers was performed and using t-test tested significance of the difference for each clinical marker between patients with score 0 and score 2+.

9.3 Results

9.3.1 Two components model

The highest prevalence of poor clinical outcomes was seen in score 2 when using appetite and walking distance (52 patients), while the lowest prevalence in the score 2 (18 patients), was for lean and walk stopping pair (Table 67). All sets were statistically significant with increasing HR for increasing score (Table 68).

Table 67 Prevalence in two-domain exacerbation risk score and proportion of patients within each score, who exacerbated within 30 days from the assessment

NERS	FFMi + stop	FFMi + distance	FFMi + AS	AS + distance	AS + stop
Score 0	42	36	26	30	33
TTFE ≤ 30days [n]	10	3	5	7	8
Score 1	63	51	71	39	68
TTFE ≤ 30days [n]	29	31	30	13	21
Score 2	18	36	24	52	20
TTFE ≤ 30days [n]	9	12	11	26	18

FFMi – fat-free mass index: highest tertile or another tertile; Stop – 6-minute walk tests: stopped or uninterrupted; Distance – 6-minute walk tests: <350m or >350m; AS – appetite score: ≤28pts or >28pts; HR- hazard ratio, CI – confidence interval

Amongst each two-domain sets, approximately 20% of individuals with score 0 experienced exacerbation within 30 days from the assessment, except for the set FFMi+distance, where only 8% exacerbated within a month. From third to half of the patients with score 1 have exacerbated within a month, and a similar proportion of patients with score 2 had exacerbation within 30 days.

Table 68 Hazard ratio of time to first exacerbation for different nutrition exacerbation risk scored based on two domains (Cox regression)

NERS		FFMi + stop	FFMi + distance	FFMi + AS	AS + distance	AS + stop
Score 0 vs 1	HR	1.961	2.627	1.666	2.109	2.255
	95% CI	1.3 – 3.1	1.5-4.5	1.05-2.6	1.2-3.6	1.4 – 3.7
	p-value	0.003	<0.001	0.030	0.007	0.001
Score 0 vs 2	HR	3.092	3.822	2.806	2.520	2.641
	95% CI	1.7-5.6	2.0-7.3	1.5-5.1	1.5-4.3	1.5-4.5
	p-value	<0.001	<0.001	0.001	0.001	<0.001

FFMi – fat-free mass index: highest tertile or another tertile; Stop – 6-minute walk tests: stopped or uninterrupted; Distance – 6-minute walk tests: <350m or >350m; AS – appetite score: ≤28pts or >28pts; HR- hazard ratio, CI – confidence interval

9.3.2 Three components model

Prevalence of unfavourable results in each of the three domains of interest (body composition, walk test and appetite) (Figure 51) and a number of individuals with a score of 0, 1, 2 or 3 were calculated.

When stopping during the walk test was included as criteria for the nutritional risk score (NRS_S), there were only 10 patients with unfavourable results in all three domains, while 44 patients had a risk score of two, and 46 had a risk score of 1. A 70% of those with a risk score of 3 were categorised as frequent exacerbators in the follow-up. In patients with a risk score of 2, frequent exacerbators ranged from 63% to 80%, while amongst those with a risk score of 1 it ranged between 40% to 66%. Amongst 21 patients with the risk score of 0, only 14% (three patients) were classified as frequent exacerbators during the follow-up.

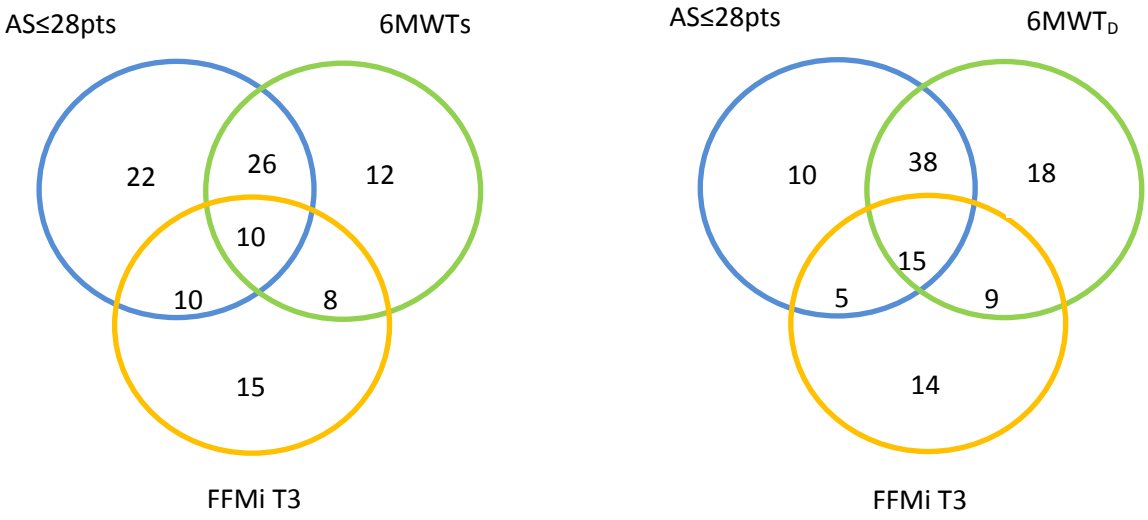


Figure 51 Prevalence (count) of unfavourable results for the three domains of nutritional risk score: poor appetite (AS≤28pts), high lean mass (FFMi) and poor walk test (stopping (6MWTs) or distance of less than 350m (6MWT$_D$))

The risk of exacerbation was compared between the patients with different scores (Table 69), showing that patients with good nutritional status (score 0) had significantly lower risk of exacerbation than patients with a score of 1, 2 or 3 (Figure 52). Considering the lack of significant difference in hazard ratio between the patients with a risk score of 2 and 3, those two groups were combined into a score of 2 or more (NRS$_S$ 2+) representing poor nutritional status (Figure 53).

When the distance of the walk test was included as the criteria for the risk score (NRS$_D$), there were only 15 patients with poor results in all three nutritional domains, while 51 patients had a score of 2. The risk of exacerbation was compared between the patients with different scores (Table 69), showing that patients with good nutritional status (score of 0) had significantly lower risk of exacerbation than patients with a score of 1, 2 or 3. Also in this case, because of lack of significant difference in hazard ratio between the patients with a score of 2 and 3, those two groups were combined into a score of 2 or more (NRS$_D$ 2+) (figure not shown). Other results did not differ between the score groups.

Prevalence of frequent exacerbators in NRS$_D$ groups was similar to the prevalence of frequent exacerbators in NRS$_S$ groups (data not shown). Considering high concordance between results of NRS$_S$ and NRS$_D$, the decision was made to focus analysis on the NRS$_S$. Stopping criteria was chosen over distance criteria because it was considered to be easier and more applicable in clinical practice, than the need for measuring the distance.

Table 69 Hazard ratio (HR) of time to first exacerbation for different nutritional exacerbation risk score (NERS) (Cox regression)

	$NERS_S$			$NERS_D$		
	p-value	HR	95.0% CI	p-value	HR	95.0% CI
0 vs 1	0.020	2.043	1.120 - 3.728	0.010	2.470	1244 - 4.901
0 vs 2	<0.001	3.344	1.811 - 6.172	<0.001	3.583	1.822 - 7.047
0 vs 3	0.001	4.116	1.812 - 9.351	<0.001	4.761	2.112 - 10.735
1 vs 2	0.031	1.636	1.045 - 2.561	NS	X	X
1 vs 3	0.051	2.014	0.997 - 4.070	0.042	1.928	1.024 - 3.630
2 vs 3	NS	X	X	NS	X	X
0 vs 2+	<0.001	3.470	1.909 - 6.309	<0.001	3.791	1.954 - 7.354

$NERS_S$ – score calculated based on stopping criteria in walking test domain
$NERS_D$ – score calculated based on distance criteria in walking test domain
CI – confidence interval

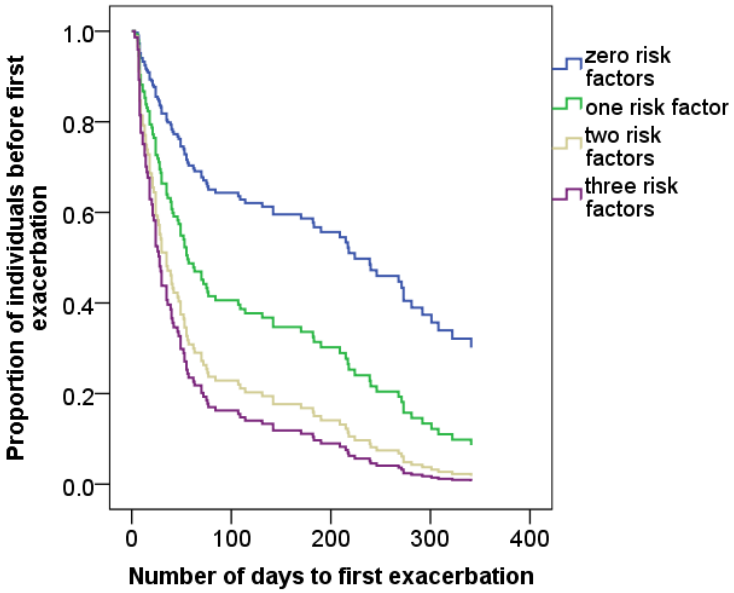


Figure 52 Time to first exacerbation in patients with different nutritional exacerbation risk score (walking domain categorised by stopping criteria)

Multicomponent analysis

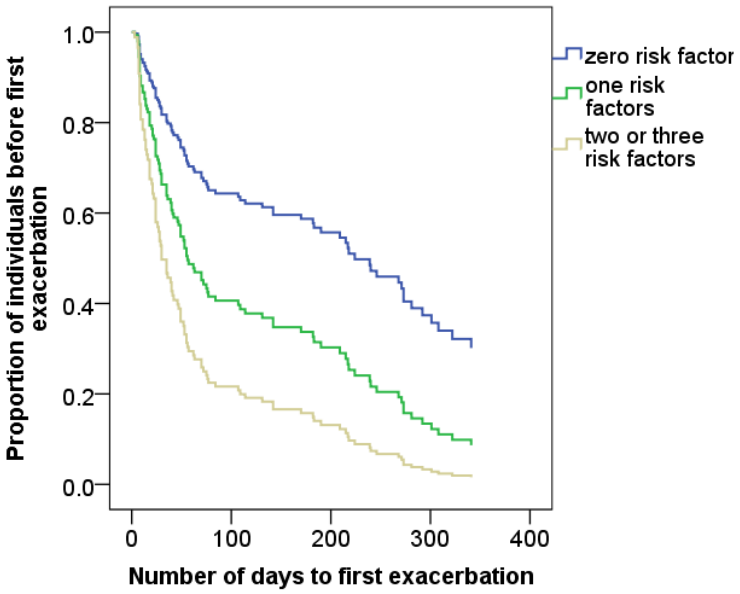


Figure 53 Time to first exacerbation in patients with different nutritional exacerbation risk score (NRS of 2 and 3 combined; walking domain categorised by stopping criteria)

The median TTFE was compared between the patients with different scores (Figure 54). Median TTFE in those with good nutritional status (score of 0) was 10-fold longer compared with those with poor nutritional status (score of 2 or 3). For 50% of patients with the score of 2 or 3, the first

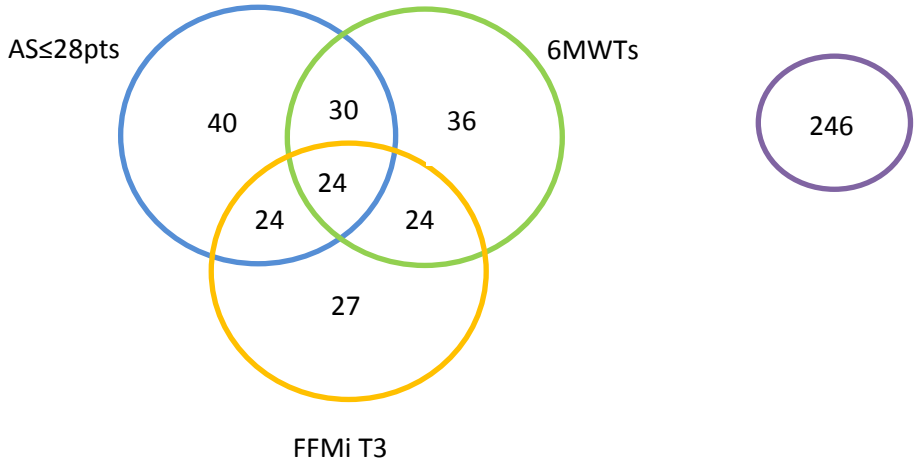


Figure 54 Time to first exacerbation (median, days) for patients with poor nutritional status measured with different markers, or with good nutritional status (purple circle);

AS – appetite score≤28pts; 6MWTs – 6-minute walks test stopping; FFMi T3 – fat-free mass index, exacerbation was developed within a month from the assessment.

Patients with worse nutritional status (the higher score) had significantly lower lung function (FEV1) and higher BMI (Table 70).

Table 70 Comparison of disease markers in patients with low and high nutritional risk score (NRS)

	NRS 0 (n=21)	NRS 2+ (n=54)	p-value*
Age [y]	71.5±7.8	65.9±8.6	NS
HAE [AE/y]	2.6±1.7	3.6±2.9	NS
FEV [L]	1.3±.5	1.1±4.3	NS
FEV [%]	50.9±13.6	43.2±15.7	0.040
TLCO [%]	70.6±19.4	56.4±14.0	0.045
BMI [kg/m^2]	24.5±2.9	29.7±6.3	<0.001
CAT [pts]	14.2±6.4	17.4±7.8	NS
CRP [mg/L]	6.1±6.3	9.3±11.7	NS
Fibrinogen [g/L]	4.7±0.9	4.8±1.0	NS
TTFE [days]	218.3±130.9	65.9±80.6	<0.001
AER [AE/y]	1.6±1.7	3.8±2.8	<0.001

*t-test; HAE – history of exacerbations; TTFE – time to first exacerbation; AER – exacerbation rate

The majority of patients with good nutritional status (score 0) exacerbated later, than within first 30 days. 1/3 of those with a score of 1 have exacerbated within 30 days, while 1/2 of those with score 2+ exacerbated within a month from the assessment (Table 71).

When compared two-components risk score with the three-components risk score, the three-component risk score was more accurate, especially when to compare the proportion of patients with good nutritional status (score 0) and exacerbation within 30 days (5% for three-component risk score and an average of 20% for two-component risk scores).

Table 71 Number of patients with first exacerbation within 30 days or more in different nutritional exacerbation risk score (NERS) groups

$NERS_S$	First exacerbation	
	≤ 30days	>30days
zero risk factors (n=21)	2	19
one risk factor (n=46)	16	30
two or three risk factors (n=54)	28	26

9.3.3 Nutritional risk score versus standard risk assessment

In clinical practice, the risk of exacerbations is estimated by a number of exacerbations in the past (history of exacerbations HAE), therefore the nutritional risk score was compared against standard practice. Results showed that patients with a history of frequent exacerbations had 87% higher hazard ratio than infrequent exacerbators (p=0.002, 95%CI 1.3 – 2.8). Hazard ratio for HAE was no higher than the one of the NRS, which suggests that high HAE is a significant risk factor. However, HAE was not able to predict the risk of an exacerbation within 30 days. Only 27 out of 49 patients who exacerbated within 30 days from the assessment were classified as frequent exacerbators in the past. Also, less than half of patients with HAE>2 had exacerbated within 30 days (27 of 62).

All scores had significantly higher risk of exacerbation than patients with the score of zero (Table 72), but having two, three or four unfavourable results in the score did not show a great difference in hazard ratio.

Table 72 Hazard ratio (HR) of time to first exacerbation for different nutrition exacerbation risk score including history of exacerbation as fourth domain (Cox regression)

$NERS_{S+HAE}$	HR	p-value	95% CI
Score 0 vs 1	2.323	0.040	1.039 - 5.195
Score 0 vs 2	4.813	<0.001	2.179 - 10.629
Score 0 vs 3	5.984	<0.001	2.635 - 13.588
Score 0 vs 4	5.394	0.001	1.911 - 15.224

HR- hazard ratio, CI – confidence interval

There was a visible difference in TTFE in patients with different risk scores when using four-components risk score (Figure 55). Median TTFE for those with score 0 was 308 days (95% CI 197-419 days), 77 days for the score 1 (95%CI 8-145 days), 41 days for the score 2 (95%CI 25-56 days), 13 days for the score 3 (95%CI 0-26 days) and 35 days for those with the score 4 (95%CI 7-63 days), with significant differences between the scores (log rank p<0.001). However, median TTFE was not consistently increasing with the score increase. Patients with poor results in all four components had longer median TTFE than patients with the score 3, which was probably related to smaller sample size in the score 4 than in the score 3.

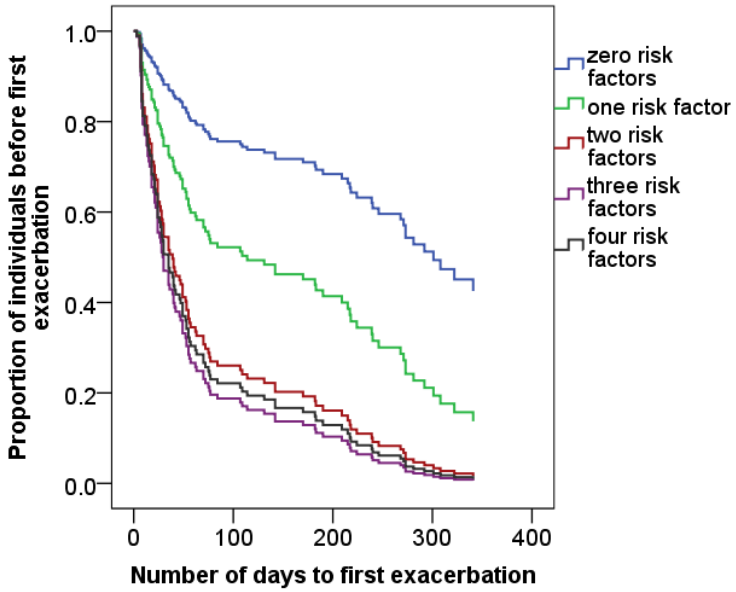


Figure 55 Time to first exacerbation in patients with different nutrition risk score (score components: appetite, walk test, lean mass, history of exacerbations)

9.3.4 Nutritional risk score - simplification for clinical utility

Various markers showed high correlation with FFMi (data not shown). Total body weight had the strongest relationship with FFMi ($r=0.858$, $p<0.001$, $n=124$). Except for single cases, the prevalence in all score groups was very similar between the NRS_{WT} and NRS_{FFMI} (Table 73). The differences in the assigned score were more likely in women (8 misclassified) than in men (4 misclassified).

Table 73 Proportion (count) of patients in different nutrition exacerbation risk score groups (NERS) based on fat-free mass index ($NERS_{FFMI}$) or body weight ($NERS_{WT}$)

Risk score		$NERS_{WT}$				Total
		0	1	2	3	
$NERS_{FFMI}$	0	20	1	0	0	21
	1	2	42	2	0	46
	2	0	2	38	4	44
	3	0	0	3	7	10
Total		22	45	43	11	121

Cox regression was used to estimate the risk of exacerbation in each score category. Patients with a score of 1 and 2+ had significantly higher hazard ratio than patients with good nutritional status (Table 74).

Table 74 Hazard ratio (HR) of time to first exacerbation for different nutrition risk score including appetite, body weight and walk test as components (Cox regression)

$NERS_{WT}$	HR	p-value	95% CI
Score 0 vs 1	2.417	0.004	1.328-4.400
Score 0 vs 2+	3.388	<0.001	1.871-6.133

HR- hazard ratio, CI – confidence interval

There was a visible difference in the TTFE in patients with different risk scores (Figure 56). Median TTFE for those with the score 0 was 246 days (95% CI 186-306), when it was 49 days for the score 1 (95%CI 31-67) and 30 days for those with the score 2+ (95%CI 16-44), with significant differences between the scores (log rank p<0.001).

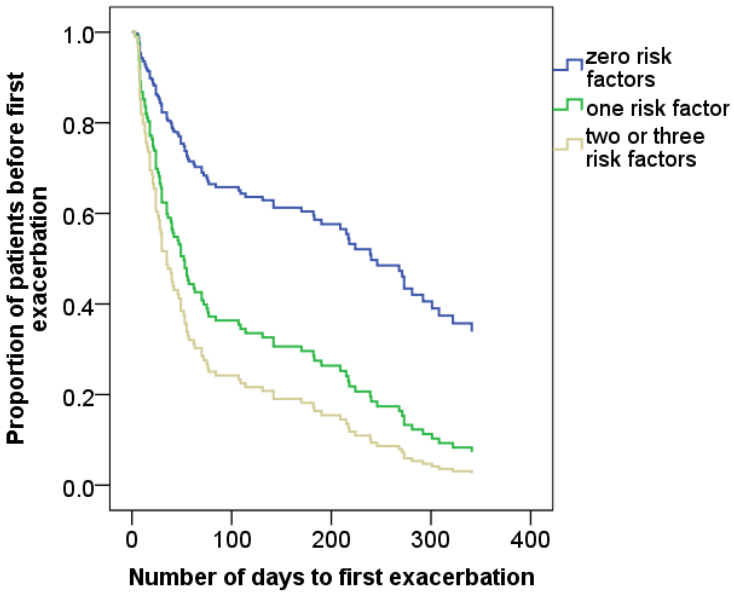


Figure 56 Time to first exacerbation among patients with different nutrition exacerbation risk scores (domains: appetite, body weight, walk tests)

Only 3 out of 23 patients with good nutritional status (score 0), had exacerbated within 30 days. Third of patients with the score 1 exacerbated in the first month, while half of those with score 2+ exacerbated in the first month. In summary, a nutritional risk score based on appetite score, body weight and walk test showed good relevance with clinical outcomes and potential to help identify patients at high risk of exacerbation in the next month.

There was a significant difference in the risk of exacerbation between patients with the score 0 compared to both scores 1 or 2+ (Table 75).

Table 75 Hazard ratio (HR) of time to first exacerbation for different nutrition risk scores including appetite, weight and history of exacerbations (Cox regression)

$NRS_{WT+AS+HAE}$	HR	p-value	95% CI
Score 0 vs 1	2.220	0.015	1.165-4.29
Score 0 vs 2+	3.578	<0.001	1.883-6.797

HR- hazard ratio, CI – confidence interval

There was a visible difference in TTFE between patients with different risk scores (Figure 57). Median TTFE for those with the score 0 was 268 days (95% CI 202-334), when it was 53 days for those with the score 1 (95%CI 17-89) and 30 days for those with the score 2+ (95%CI 9-51), with significant differences between the scores (log rank p<0.001).

Only 3 patients, out of 20 with risk score 0 based on appetite, body weight and HAE, had exacerbation within 30 days. Almost half of patients with the score 1 or more, exacerbated in the first month.

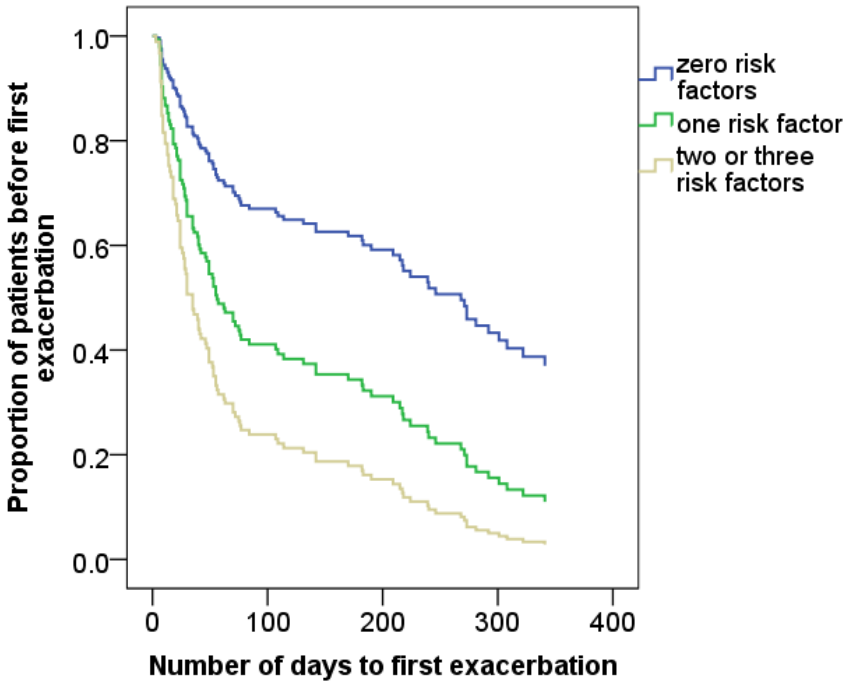


Figure 57 Time to first exacerbation among patients with different nutrition exacerbation risk scores (domains: appetite, body weight, history of exacerbations)

9.4 Discussion

Currently, COPD management is restricted to ameliorating respiratory symptoms and treating exacerbations, once developed. To move disease management forward, there is a need to identify patients at risk of exacerbation in the future, to then introduce protective and preventative interventions in order to avert exacerbation. The aim of this chapter was to explore the potential of nutritional status to identify COPD patients at high risk of exacerbation in the future. As shown in the previous chapters, demographic and respiratory markers were not helpful in identifying patients with exacerbation in the next month. Even history of exacerbations in the past month, however relevant to overall risk of exacerbation, was not specific enough to differentiate between patients who have and have not exacerbated in the following month. Therefore, based on the markers of nutritional status, of which most have shown to be independent of the medical history, but associated with the disease status at baseline, multicomponent approach was proposed as more informative than the single nutritional marker. Based on relevance with disease status at baseline and clinical outcomes in the follow-up, three nutritional markers have been selected as potential components of a risk score.

This chapter has revealed three main findings: a multicomponent approach provides better assessment of risk of exacerbation than single component; the risk score using multiple nutritional components has shown the potential to identify patients with risk of exacerbation in the next month, but is more accurate in identifying patients with low exacerbation risk, than with high exacerbation risk. Also, risk score based on the three (appetite, lean mass, walk test or history of exacerbations) was more accurate than risk score based on any two domains, while score using all nutritional domains and exacerbation history (four domains) showed no advantage over any three components score. Multiple variations of the score were tested, showing that appetite score was important component of any multicomponent approach, based on the comparison between each two component model. The simplest and clinically most applicable score was based on appetite score, body weight and history of exacerbations, and was able to identify patients unlikely to exacerbate within next month.

Standard practice is to use history of exacerbations as an indicator of exacerbation risk, so NRS was compared with standard approach. History of exacerbations as a single marker was no better predictor of exacerbation risk, than nutrition risk score. Less than half of patients with HAE>2 exacerbated within 30 days from enrolment, and half of those who exacerbated within 30 days had HAE>2. When the history of exacerbations was combined with the nutritional risk score, it statistically increased hazard ratio for each risk score. However, the scores of 2, 3 and 4 showed

very similar hazard ratio, suggesting that combining nutritional risk score with HAE does not increase the predictive value of the nutritional score.

The need for treatment based on phenotypic variations in COPD has already been recognised however no phenotype-specific treatments or management programs are available. The supposition was that patients with the same disease phenotype would have similar underlying pathomechanism and response to treatment. Focus on personalised medicine and disease phenotypes is essential to improve treatment efficiency and patients' quality of life but requires approach aiming at a limited number of disease subsets. Oversimplification has led to including all patients with irreversible airflow limitation in one COPD group, regardless the whole spectrum of pathways through which patients develop with COPD. However, over-phenotyping the COPD population, when focusing on the detailed underlying mechanism of disease development and progression, was shown to be a challenge. For that reason, the aim of this analysis was to identify nutritional markers related to shorter TTFE, to support evaluation of COPD phenotypes and explore new marker of exacerbation risk, going beyond phenotypes of frequent and infrequent exacerbators based on HAE only.

All three nutritional domains used in the multicomponent risk score could be used to identify nutritionally compromised patients. By combining those domains into a risk score, further multidimensional phenotypes were generated. Patients who had low appetite were overweight or obese and who needed to stop during a 6-minute walk, were shown to be at highest risk of exacerbation in the future. This nutritional compromised phenotype was in line with the history of exacerbations, as the majority of patients with unfavourable results in all three nutritional domains had more than two exacerbations in the past year.

The risk score was derived from several components, therefore same score could be assigned to individuals with different phenotype and underlying pathomechanism. An overweight patient with a high history of exacerbations, walking without interruption and with high appetite, or patient with normal weight, infrequent exacerbations, but stopping during the walk test and having a poor appetite – both individuals would be assigned risk score of two. Those two patients could have similar TTFE, but due to different mechanisms. Therefore, nutritional risk score could be considered more as an index. Also, nutritional risk score should not be considered as a marker of malnutrition, which previous nutritional screening tools were (e.g. MUST score). The higher the risk score, the more nutritional domains with unfavourable results, however, because of high lean mass categorised as an unfavourable result, the high score did not represent poor nutritional status. Proposed risk score could be considered as markers of nutritionally unfavourable nutritional status, which relates to increased risk of exacerbation in the future. Therefore,

patients with score 0 could be identified as favourable nutritional phenotype. Considering that appetite questionnaire, body weight and a number of exacerbations in the past 12 months could be recorded by patients' without approaching GPs, this risk score has the potential to be used as a monitoring tool for patients, by patients. In clinical practice, it could be easily used by doctors and nurses, with questionnaires filled by patients' while waiting for the visit in GP practice.

There was a number of COPD phenotypes recently published [70]. The majority of them focused on respiratory symptoms and markers, while only some included nutritional element, and most commonly, it was limited to BMI. In 2012 three distinct phenotypes were described suggesting that mild COPD has low mortality risk, while severe and moderate airflow obstruction was related to high mortality, along with low BMI in one phenotype and obesity in the other phenotype [327]. Recently, some new nutritional phenotypes were identified, like sarcopenic obesity, semi-starvation, muscle atrophy or cachexia in COPD [66, 188], which describe differences in body composition and related change in physical capability between patients, but not necessarily explores the difference in disease pathomechanism, progression or prevention of exacerbations. However, none of the phenotypes mentioned above has been explored for its ability to identify individuals at risk of worse respiratory outcomes, exacerbation risk in the following months, as most phenotypes focused on mortality risk.

The nutritional risk score is currently the first outcome index that has a potential to predict TTFE in the studied cohort, and as the only index used in COPD, includes appetite marker. The novel score presents a new dimension of assessment in COPD – patient based and patient focused. In contrary to previous indices, NRS could possibly discriminate patients with high and low risk of exacerbation in the near future. The NRS has limitations, as identifies patients' at low risk much better than patients' at high risk of exacerbation, but this could still aid in clinical decision-making. Currently proposed indices, like BODE or DOSE [76, 268], focus on mortality and survival length. As much as this is informative on a population scale, this has not much application in everyday practice for an individual. To manage patients' disease there is a need to understand what are the short-term risks, are those patients going to exacerbate in next month or are they at risk of having more than 2 exacerbations in the next year. This could be of great clinical relevance, if in the future, actions could be defined to modify the outcome, hence identification of the risk of exacerbation in the next month could be actioned upon with relevant, targeted treatment or intervention. That would equip doctors with tools and interventions to prevent exacerbations and potentially improve patients' outcomes. Being able to identify patients who are most likely to exacerbate in the next 30-90 days or identifying those who are more likely to have more than 2 or 4 exacerbations in the following year is the first step to improved management.

In summary, the aim of this chapter was to compare the single and multicomponent approach when using nutritional markers as indicators of poor clinical outcomes measured with the risk of an exacerbation within the next month. The nutritional multicomponent risk score showed some potential to identify patients with risk of exacerbation in the near future. Various components were considered, but in all scenarios appetite score was the core of the risk score, showing the importance of this marker in predicting clinical outcomes. Nutrition risk score was more precise in identifying frequent exacerbators than the history of exacerbations and, by including straightforward and inexpensive assessment methods, has the potential to be easily implemented in clinical practice, if proven its value in larger and more varied COPD cohort.

9.5 Limitations

Obtained results demonstrate only the first step, proof of concept analysis, and a possibility that a nutritional multicomponent approach has a value in predicting TTFE. This analysis was inherently limited by the method by which it was performed, because the data for this analysis was obtained from a study set up for a different purpose. If one was to develop this multicomponent score in an independent study, additional steps for the analysis would be required. Those steps were not performed in this analysis, because of a number of limitations, the most important being the cohort size and lack of high sensitivity of selected cut-points for each score component. The biomedical statistician team at University of Southampton were consulted on this topic, the dataset in its current form was not deemed suitable for more detailed analysis of the multicomponent score. Kaiser-Meyer-Olkin Measure of Sampling Adequacy was below 0.500 suggesting that factor analysis was likely to be inappropriate, which was likely caused by sample size being less than the recommended 150.

If the cohort size was determined to sufficiently power nutritional analysis, creating a risk score would include following steps:

1. Determining the most relevant components based on relevance to TTFE, irrespective of disease markers (regression and time-to-event analysis);
2. Identifying cut-points for each component that have high sensitivity to distinguish individuals with different TTFE (ROC analysis);
3. Determining if each component should be scored in a binary or multiple scores format, i.e. would two cut points (hence three categories) for AS be more accurate than one cut-off in distinction between individuals with different TTFE;
4. Weighing the value of each component based on its relevance and proportion of the variance it explains;

5. Testing multicomponent score and its ability to discriminate between individuals with different TTFE;
6. Validating the score in an independent cohort to explore score validity.

The score would require verification both in a similar group of patients and in groups of various phenotypes. This evaluation would be necessary to test validity of this score across all COPD patients. If the relationship between the NRS and outcomes was demonstrated in various COPD cohorts, the next step should explore the modifiability of each component and the extent to which change (improvement) in one or more components could influence TTFE. Only then, the future work could move onto exploring interventions focused on the NRS components, which would be most beneficial and cost effective in order to prevent exacerbations in patients with increased exacerbation risk.

10 Summary, discussion and future works

Chronic obstructive pulmonary disease is a life-changing, incurable disease, affecting more than a million people in the UK. For decades, clinicians and scientists have been trying to find ways to improve patient's quality of life and efficiency of treatments. However, the mechanisms of the disease development and progression are not fully understood. Current disease management is focused on symptom relief and exacerbation recovery, with elements of general health improvement through pulmonary rehabilitation. Treatments to prevent exacerbations prior to their onset are mostly none-specific (use if infection is known to be circulating in the community), limited, and exposed to new challenges like pathogens rapidly developing resistance to antibiotics. Treatments available to prevent exacerbation appear to be limited mainly due to lack of tools to identify a pre-exacerbation state.

Exacerbations cause a high burden to health, deteriorate the quality of life, increase acute healthcare cost and increase the risk of death [67, 265, 268, 328, 329], hence there is a need to identify those who are at greatest risk. To date, respiratory markers used to identify the risk of exacerbation have not proved clinically helpful, and patient's own history of exacerbations has previously been the most accurate indicator of overall exacerbations risk [38]. However, even history of exacerbations is not able to identify patients who are likely to exacerbate within next month, limiting the ability to intervene to prevent the development of the exacerbation.

It has been recognised for many years that COPD patients are made up of many endotypes and phenotypes [58, 59, 61, 70, 228, 327, 330, 331], therefore the need of individualised therapy programs and personalised medicine appears to be especially relevant. Various previous studies have attempted to characterise these phenotypes by focusing on a single domain [54, 331], often basing this on observable differences (i.e. pink puffer and blue bloater), rather than their relationship to clinical outcomes. They could be referred to as descriptive phenotypes. In order to make a difference to the disease management and, ultimately, the patient's lives, there is a need for outcomes-driven phenotypes. This is challenging, as there are no published results identifying multidimensional phenotypes relating to TTFE.

When phenotyping has been based on airway disease, these have focused on airflow limitation, dyspnoea and pulmonary hypertension [34, 56, 291]. Little attention has been given to the non-respiratory aspects of COPD. If nutritional aspects were considered, it has focused on low BMI and cachexia or sarcopenia, which have been recognised as unfavourable phenotypes in COPD [61, 66].

There is a need to go beyond respiratory symptoms and exacerbation history, to gain a better understanding of COPD clinical progression and to aid clinical management. Poor nutritional status has been shown to play an important role in many diseases, such as cancer, tuberculosis and kidney diseases [1, 332, 333]. Based on the supposition that better nutritional status improves resilience to infections, the aim of this thesis was to test if nutritional markers could identify nutritional phenotypes with different risk of COPD exacerbation in the near future and to explore if any nutritional markers or their combination can indicate a pre-exacerbation state.

Based on the literature review, it was initially assumed that relationship between nutritional status and COPD exists and change in FFMi could be used to monitor disease progression or status. From various plausible pathways linking nutrition and COPD, this thesis focused on the hypothesis that for any given disease status those with the worse nutritional status will present worse clinical outcomes and disease progression. Results obtained with this thesis suggest that relationship between the nutritional status and disease status is complex and focusing on FFMi may not be helpful for certain patients phenotypes.

Systematic review of the literature has exposed methodological inconsistency in functional and structural assessments of the lean mass, which led to evaluation of body composition and grip tests measurement techniques. Comparison between various body composition techniques (BIA, DXA, D2O, BIS) has shown that fat-free mass differs insignificantly between the methods, but provides results always in the same rank order.

This thesis aimed to explore the relationship between nutritional markers and exacerbations. Results were obtained in three nutritional domains – body composition, appetite, and physical capacity. The relevance of a single nutritional marker was then compared with the multicomponent approach, looking into relevance with TTFE. Appetite has shown to be the important component, which when combined with other nutritional markers or history of exacerbations, helped to identify patients at high risk of an exacerbation within a month. Each nutritional component considered in this thesis and relevance with clinical outcomes are discussed below.

Body composition

Following the direction suggested by ERS [61], body composition was tested and relationship with TTFE was analysed. Results showed that:

- this cohort presented with high prevalence of overweight and obesity, and only small proportion of individuals with visible malnutrition;
- low lean mass did not relate to shorter TTFE;

- high level of lean mass was related to shorter TTFE, but this was not see in relation to fat mass;
- relationship between high lean mass and shorter TTFE was not reflected in the AER results;
- prevalence of lean depletion in this cohort varied depending on criteria used for lean depletion identification, which carries a high risk of misclassification;
- prevalence of low amount of lean tissue and high amount of fat tissue was low in this cohort and therefore limited the estimation of the effect of sarcopenic obesity on clinical outcomes;
- the methodology of body composition assessment and interpretation in COPD cohorts is heterogeneous and difficult to combine for a comparison or meta-analysis with other studies.

Results of body composition in this cohort were highly unexpected. Previous studies have shown either worse clinical outcomes (mortality, hospitalisation) in patients with a low amount of lean tissue [65, 67, 135, 150, 185, 187, 270, 272], or lack of relationship between body composition and clinical outcomes [136, 267]. In this cohort, the direction of the relationship was the opposite, with shorter TTFE among those with the highest amount of lean mass. Moreover, relationship with TTFE was not reflected in the AER, which could suggest false positive findings. It should be noted, that previously published studies were not looking at TTFE, but at mortality or risk of hospitalisation, which potentially could have influenced the relationship, however, it is rather unlikely to be the main cause of differing results.

Previous studies have suggested that low amount of lean tissue was related to higher mortality or hospitalisation, but the results of this thesis have suggested the opposite, which begs the question what was different about studied cohort in comparison to previously studied patients. Studied COPD cohorts show many similarities: patients were enrolled based on the same or very similar criteria for COPD and lung function obstruction; majority enrolled patients were in a stable state (unless specifically focusing on exacerbations), even though it was defined by different number of weeks free of exacerbation; had various proportion of men and women but often alike.

The first major distinction between previously studied COPD cohorts and AERIS cohort is a disease activity measured by frequency of exacerbations. The AERIS cohort enrolled only patients with at least one exacerbation in the past year, hence more active disease. Other studies did not include such criteria, which resulted in enrolment of many patients with the less active disease. This was evident in the ECLIPSE cohort, where almost 60% of patients at baseline had no exacerbations in

the past year [62]. Such simple difference within inclusion criteria could cause biased enrolment and be related to major differences in the underlying pathomechanisms.

Another relevant difference is the prevalence of patients with evident malnutrition (i.e. low BMI) and proportion of individuals with overweight and obesity. In the AERIS cohort, there were only 9 patients (7%) with BMI<20kg/m^2, while 29% patients at baseline had BMI>30kg/m^2. Previously, the majority of studies exploring nutritional component in COPD had a higher proportion of malnourished patients [67, 150]. Studies published in 2016 were including over 20% of underweight patients [152, 157] or even restricted cohort to underweight patients only [158], suggesting BMI bias in nutritional studies. Lack of obvious malnutrition should not be considered as an indication of a good nutritional status.

The above suggests, along with the difference in disease activity, that this cohort was represented mostly by patients who would not immediately rise nutritional concerns based on the outlook, but presented with higher disease activity, while other studies had significant proportion of visibly malnourished patients with lower disease activity. It is worth noting that, this cohort included only a few patients classified as 'sarcopenic obese', as there is a high likelihood that these patients should be considered as a separate phenotype, where a low amount of lean and high amount of fat would present different relationship with TTFE. In this phenotype, a higher amount of fat per unit of lean would be expected to relate to worse clinical outcomes. This, in turn, raises the question what is the difference in the pathomechanism of poor resilience between visibly malnourished, normal to overweight and sarcopenic obese patients from a nutritional perspective.

In the Introduction to this thesis, there were different potential mechanisms of poor nutritional status discussed. It could be assumed, that those hypothetical pathomechanisms of lean loss differ between phenotypes. Patients with higher disease activity would be expected to have higher inflammation level and higher oxidative stress, which would support increased protein degradation through raised activity of a UPS system. Also, use of glucocorticoids around exacerbation would stimulate activity of the UPS, while loss of appetite at the same time could decrease intakes of nutrients like vitamin E, which could lead to decrease in UPS inhibition (longer UPS activity), or vitamin C and zinc which limiting antioxidative activity [234, 237, 334]. Demonstrated change in appetite around exacerbation supports the assumption of inadequate nutritional intake around exacerbation, which in order to satisfy the demands, would increase catabolic processes to counteract inflammation and oxidative stress. This would be in line with the theory of oxidative burst and increased nutritional needs to counterbalance generated oxidative particles [112, 113]

Irrespective of the true pathomechanism of this process, the results in this thesis appear, at first, to be physiologically unlikely. One potential explanation is that that high lean could have been a proxy for the high amount of fat tissue, and fat tissue would be considered to have a pro-inflammatory activity. However, results in this study have not shown the relationship of the high amount of fat tissue with clinical outcomes, or even with inflammation levels. The second theory is that the studied cohort in this thesis had higher BMIs than the majority of previous COPD studies. It is possible that higher body mass, which in this cohort also meant higher lean mass, was related to increased cardiac risk, the risk of diabetes or metabolic syndrome [335]. Therefore, a higher amount of lean was indicating a higher risk of co-morbidities, and mechanisms leading to those comorbidities were indirectly influencing susceptibility to exacerbation.

The theoretical model cannot be confirmed or refuted based on the result of this thesis, since the activity of UPS, the level of nutrients and oxidative stress level were not analysed here. Some of those markers have been collected in the AERIS cohort, which in the future could enable additional analysis and further exploration of mechanisms underlying wasting and potentially, decreased resilience to exacerbations.

Results at this stage emphasise the risk of nutritional advice or intervention, because of the variability of body composition phenotypes in COPD and potentially specific nutritional needs and risks in each group. While the patients similar to those in the studied cohort, with worse outcomes related to high lean mass, could potentially benefit from weight loss programs, this would have a detrimental effect on sarcopenic patients. Until clear differentiation between body composition phenotypes and relationship with short and long-term clinical outcomes are not established, recommending interventions to change body weight should be advised with caution. In addition, recommendation for increased food intake or nutritional supplementation to increase body weight and lean mass would raise a concern, if the risk of poor clinical outcomes in patients with a high amount of lean would be confirmed in other cohorts. Therefore, future research regarding body composition in COPD requires:

- evaluation and standardisation of body composition measurement and interpretation protocols based on relevance to short- and long-term clinical outcomes;
- large longitudinal epidemiological observations of COPD with various disease activity, severity and nutritional status to verify different phenotypes and establish criteria for high and low resilient phenotype;
- implementation of body composition monitoring programs in COPD clinics in order to establish the prevalence of different nutritional phenotypes, once criteria are established and validated.

Further work would depend on results of those initial steps. Overall, there is a need for further exploration of the understanding of natural history and pathophysiology of changes in body composition in COPD population and identifying cost-effective and clinically applicable markers that could be used for population screening.

Appetite

After body composition results had suggested direction of relationship to be opposite to assumed (high lean mass indicated shorter TTFE), other nutritional markers were assessed as an alternative. Change in body weight, hence composition, is most likely caused by a change in appetite, however other aspects can also play important role (e.g. increased catabolism, cancer). Therefore, appetite questionnaire was used to explore the changes in the appetite around exacerbations and the relationship between the appetite in stable disease and the clinical outcomes. Appetite was measured with simple, 8-questions questionnaire and results showed that:

- appetite questionnaire was easy and quick to use by patients and had very high response rate;
- patients with low appetite (below 28 points, 70% maximum score) had a shorter TTFE and higher AER in the follow-up;
- appetite in stable COPD was not related to the inflammation level or frequency of previous exacerbations;
- poor appetite was not related to lower BMI, suggesting that low appetite was not a permanent state, but temporary change prior to exacerbation;
- appetite score was as good predictor of exacerbation, as the history of exacerbations;
- use of a single or combination of questions, less than the full 8, was not related to clinical outcome;
- change in appetite around exacerbation was evident in many patients, but this was not related to change in inflammation status, exacerbation severity or standard respiratory markers. In addition, some patients had higher appetite score at exacerbation than before.

Presented results are not comparable with other studies, as this novel approach of looking at appetite in stable COPD was not applied previously. There are limited data on appetite change at exacerbation, but where they have been reported, they are in line with findings in this thesis [304, 336]. Loss of appetite is anecdotally a common problem at exacerbation. For a long time it was considered as a consequence of exacerbation and increased inflammation, however presented

results suggest that appetite changes before the changes in inflammation level. This may be because commonly used systemic inflammation markers, CRP and fibrinogen, may be insensitive or not representative of localised inflammation, which could be influencing appetite regulation. Therefore, there is a need to explore appetite regulation mechanism in COPD and identify pathways that cause appetite loss. Only then, there is a chance for development of interventions restoring appetite, assuming that this would address the problem of resilience loss. Potentially, appetite regulation is linked with major exacerbation development mechanism, and understanding change in appetite could help understand how exacerbations develop from a systemic perspective.

Previously, research focused on the amount of lean, especially on patients with lean depletion, and appetite loss was considered as one of the causes if weight loss and wasting. However, there is a link between body composition and appetite on a metabolic level through adipose tissue. This is a highly active tissue that secretes hormone-like factors such as leptin and adiponectin [337], which could be one of the potential paths that link nutritional status and exacerbation resilience. Adipose tissue secretes appetite-regulating hormones, but also cytokines and chemokines and, therefore, regulates inflammation [337]. Recently, various epithelial cell types have been shown to secrete leptin, which along with the universal distribution of leptin receptor, reflects the variety of biological effects it has [337, 338]. Moreover, leptin expression was identified in lungs (bronchial epithelial cells and alveolar macrophages) in smokers compared with never smoker [338]. Structurally, leptin is a cytokine, and it up-regulates inflammatory immune response by macrophage phagocytosis and secretion of inflammatory cytokines, such as IL-1 and TNF [339]. It has been shown, that leptin level increases at exacerbation, and returns to normal at resolution, which could have twofold effect – decreasing the appetite at exacerbation and further increasing inflammation during the acute exacerbation [43]. This, together with an inflammatory effect of leptin, gives a strong message that adipose tissue (and leptin) could play an important role in exacerbation resilience and shows an interesting alternative to current focus on lean mass.

Currently, evidence on appetite in stable COPD and around exacerbation is scarce, therefore no recommendations or interventions regarding appetite have been published. Potentially, interventions for those with a poor appetite should focus on the combination of restoring appetite, through modification of appetite regulatory pathways, as well as directed nutrients supplementation to compensate appetite loss. However, the current understanding of nutritional needs of patients with COPD is poor. The first principle that needs to be established is whether COPD patients have a different nutrient requirement compared to the healthy population, or, do they differ between the phenotypes. If so, what are the nutrients that play a crucial role in maintaining or improving exacerbation resilience?

Identifying one or more nutrients that, if supplemented, could maintain resilience in COPD regardless of appetite, will require extensive explorations. Nutrition supplement clinical trials are notoriously challenging, expensive and time-consuming. Therefore, future research should be based on mechanistically determined pathways of action as a sound foundation for clinical trials. Although direct evidence is not available for COPD patients, data from other inflammatory conditions may give surrogate hints of the nutrients that may play a role in exacerbations resilience. One of the micronutrients of interest is zinc, as decreased level have been shown to decrease endothelial cell integrity and vascular barrier function, leading to increased exposure to inhaled agents and toxins [340]. Also, decreased zinc was recognised to have anti-apoptotic activity and regulates monocytes activation, all together creating conditions potentially conducive to chest infections [114]. The second micronutrient that may play a role in exacerbations resilience is pyridoxine (PLP). Increased inflammation can cause a decrease in PLP level, which in turn decreases the production of anti-inflammatory agents, such as IL-2. The poor intake would also effect macronutrients such as amino acids. Poor appetite causes decreased level of taurine, which is involved in clearance after the oxidative burst, and decreased levels lead to higher oxidative damage [112, 113].

Therefore, next steps for research in the role of appetite in exacerbation resilience would consider:

- analysis of appetite change in large multi-phenotype COPD population using simple tools like CNAQ questionnaire;
- evaluation of CNAQ interpretation criteria for identification of low resilience phenotype on a large population;
- assessment of change in inflammation and appetite regulating markers (leptin) in periods of increased risk of appetite change (a pre-exacerbation phase) identified using questionnaires.

Considering initial stage of research in understanding appetite changes and its role in COPD, there are many areas that require exploration, and proposed steps are only a few of many. However, considering valuable results obtained in this thesis, suggests that appetite is a great potential to aid understanding of mechanisms regulating resilience to exacerbations. Overall, there is a need for understanding the natural history and pathophysiology of changes in appetite in COPD population and identifying cost-effective and clinically applicable markers, which could be used for population screening and disease monitoring.

Physical capacity

Limitation in physical capacity in COPD patients is common and relevant from a clinical perspective, as determines worse clinical outcome [265, 268, 270, 273, 310] and this effect was shown to be independent of lung function impairment [341]. However, the cause of physical limitations in COPD are not fully understood, with the potential role of inactivity and deconditioning, hypoxia, drug therapy and systemic inflammation [248] playing an important role. Emerging evidence supports the role of nutritional status in physical capacity [180]. In this study, physical capacity was measured using three physical capacity tests – 6-minute walk test, grip strength, and grip endurance. Results showed that:

- the methodology of physical capacity assessment, especially grip tests, is heterogeneous, and there is a need for standardised operating protocols including interpretation criteria for capacity tests other than 6MWT, which already has guidelines published;
- only the 6-minute walk test, but not grip strength or grip endurance, was related to TTFE;
- stopping during walk test was as strongly related to TTFE as criteria based on distance, hence test discontinuation was considered to be the simplest criteria of physical capability;
- having poor results in one test was not an indication of poor results in any other test, confirming that used tests measure different aspects of physical capability and they are not related to clinical outcomes to the same extent.

Walking tests are widely used and show a good relationship with long-term clinical outcomes in COPD studies [139, 269, 273, 342-346], however, there is no evidence of relationship to TTFE in COPD. In this thesis, the focus was on TTFE demonstrating a novel use for a well-known test. Most previous studies used distance to discriminate good and poor results, with one other study using stopping during walk test as a marker of poor results [311]. Using test discontinuation as criteria requires attention to the cause of stopping, which in this cohort was shown to be either breathlessness, pain or fatigue. Grip tests were shown to have little value in predicting TTFE and AER and considering equipment and training cost, they were concluded to be of a lesser value in estimating exacerbation resilience in wider use.

Walking tests are a simple test that measures a complex output, therefore it would be difficult to identify one treatment or therapy to improve its results. Patients stopping due to pain could require pain management strategies, while some of the pain could be related to muscle weakness and fatigue. Hence, patients stopping due to different causes could be considered to represent different nutritional phenotypes, and they would require different interventions.

Supplementation was already used for patients undergoing physical training [347, 348], but increasing post-exercise dietary protein and carbohydrate intake was shown not to be essential for response to training similar to those in healthy [348]. However, based on a meta-analysis of 12 studies [91], an overall improvement of nutritional status through regular supplementation was shown to have a positive effect on functional outcomes.

Considering the relevance of walking test in measuring COPD exacerbation resilience, the next steps would include:

- evaluation of the relevance of walking test discontinuation in a large, multi-phenotype COPD population;
- exploration of the relevance of different distance walking criteria in a multi-phenotype COPD population;
- exploration of natural history and pathophysiology of muscle dysfunction with consideration of different disease phenotypes.

These are only walking-test relevant explorations, while physical capacity and muscle dysfunction research have still many more questions to address, including the role of nutrients on muscle dysfunction mechanism, the extent to which inflammation and oxidative stress stimulates muscle dysfunction. Research in these areas has already begun, however, consideration of different COPD nutritional phenotypes have not been included in many physical capacity studies, which could potentially aid better understanding of this matter.

Multicomponent approach

Single nutritional marker approach has shown that nutritional markers are not associated with the past medical history, yet relate to markers of disease status at baseline and future clinical outcomes. Results have shown that poor appetite, a higher amount of lean tissue and poor physical capability, measured with slow walking or stopping during the walk test, were related to the TTFE and potentially marking a pre-exacerbation phase. Further, a novel approach was tested, to explore if the multicomponent approach would show stronger or more sensitive relationship with the TTFE. Body composition, appetite, and physical capability were combined into a two-component and three-component risk score, and tested against TTFE.

To categorise the risk of poor clinical outcomes, an unfavourable result in each nutritional component was given one point. The combination of any two or more nutritional markers with unfavourable results, were shown to be predictive of reduced TTFE with a higher identified risk than by a single component approach. Poor nutritional status identified by two or more components have shown significantly shorter TTFE and a higher risk of an exacerbation within

following 30 days. A pragmatic approach was then taken to find the most easily reproducible combination to potentially apply to clinical practice. The appetite questionnaire was shown to be simple to understand and easy to complete by patients. Walking tests are relatively easy to perform, however, require time, space and motivation, therefore, it was assumed to be less likely to be voluntarily carried out by patients and clinicians on a regular basis. Similarly, body composition was considered to be challenging in clinical practice, as the availability of equipment is limited. Therefore, two other highly relevant markers were selected as an alternative to walking test and body composition. Body weight and history of exacerbation were selected due to their clinical applicability. The accuracy of the simplified risk score, using appetite, body weight and history of exacerbation, was close to that one using the originally selected measurements (appetite, lean mass and walk test) in identifying patients at high risk of exacerbation. The highest accuracy of the nutritional risk was in identifying patients who were unlikely to exacerbate in the next month and appetite score was a important part of all multicomponent approaches, suggesting its high importance in identifying patients at high risk of exacerbation or even a pre-exacerbation state.

Using multicomponent approach including nutritional components has shown to be more helpful in identifying patients at risk of exacerbation in the following month, than currently used history of exacerbations. Results have shown that: patients with a higher frequency of exacerbations in the past were likely to have a higher frequency of exacerbations in the follow-up but not all patients with a high history of exacerbation have exacerbated within the first month of the follow-up.

Proposed alterations to the multicomponent nutritional risk score were considered to potentially improve the utility of it. In clinical practice walking test is a time-consuming test, which requires space, motivation and assistance during the test, therefore may not be seen as easy to implement on a regular basis. If the nutritional risk score was to be used by patients themselves at home for disease monitoring, then some patients could find it too exhausting or would lack the motivation to repeat it monthly. Therefore, to avoid a situation where domains of risk score are estimated, rather than measured, walking test was considered to potentially be too demanding. However, if used in research settings, using walk test could be more feasible. Further research in this area could explore stopping causes in more details, as well as explore potential methods of results improvement.

In this cohort, history of exacerbations was obtained from the patients, but also crosschecked with medical records, suggesting some discrepancies between reported and recorded numbers of exacerbations. Accurate recall of exacerbations rate has limitations, as some patients may not

recognise mild exacerbations, long lasting exacerbations could be considered as multiple exacerbations and differentiation between two and three exacerbations in the past 12 months (criteria for exacerbation frequency) could be difficult for some patients to report accurately. If medical records were used to report exacerbations (e.g. GP records) as a source, there is a risk that mild exacerbations were not reported to the medical team, or that patients supplied with emergency medications have used those and did not contact their clinician about some infections. Therefore, seemingly simple information could be inaccurate in some circumstances. However, if the value of the NRS score was validated and proven on a large COPD cohort, potentially both patients and clinicians would see the value in the more detailed monitoring of exacerbation frequency. Use of simple exacerbations diary could be helpful in obtaining accurate information.

It is worth recognising that a risk score of 2 was not a single phenotype because it can be made up of patients with any combination of explored three markers. Pathomechanism and underlying cause of the exacerbation predisposition could be different amongst this patient group. Therefore, NRS should be seen as a clinical COPD indicator, while the combination of domains included in the score could be considered to represent different nutritional phenotypes. In theory, NRS identifies eight potentially different patient phenotypes.

Clinically, NRS could be interpreted into a traffic light system, however that is only if score verification in wider and various cohorts shows similar predictive value. Patients with good nutritional status (risk score 0) would be considered to have a low risk of exacerbation, they could be assigned 'green' light. One-third of patients with score 1 have exacerbated in the next month, therefore they could be assigned a 'yellow' light. Half of those with poor nutritional status identified by two or more components (score 2+) have exacerbated within a month, therefore they could be assigned a 'red' light. However simple this approach appears to be, presented results are only the beginning of the exploration and there are many steps to validate it and explore potential treatment options and targets within each colour. Considering that appetite has shown high relevance to clinical outcomes both on an individual and combined level, it could be first tested if a single approach focusing on appetite in a larger cohort would have greater use than multicomponent approach.

Each risk score component with a predictive power requires further investigation and better understanding. Why some patients have a poor appetite? What is the mechanism suppressing their appetite? Can it be altered, if so, how? Would it be possible to avoid effects of poor appetite through nutritional supplementation? Why would lean mass predispose to worse clinical outcomes? Is high lean mass a surrogate of another marker? Is it high total body weight that has higher importance? Why low lean mass was previously shown to indicate worse outcomes, while

in this cohort it was high lean mass? Does each nutritional phenotype have different underlying mechanisms leading to exacerbation? What are those mechanisms? Can the process leading to exacerbation be altered? These questions require further research as most cannot be confidently answered currently.

Given this novel findings in identifying patients at high risk of exacerbation in the next month, which potentially also marks the pre-exacerbation phase, the next natural step for the future work is verification of its validity in different cohorts and explorations that go beyond simple relationships. In addition, research should focus on exploring each NRS components and answering component-specific questions as discussed earlier. First, there is a need to repeat this analysis in appropriately powered cohort to determine best cut-points and weighting system for each score component. Analysis in this thesis, due to certain limitations, used simplified approach with cliniclally determined cut-offs and with binary scoring of equal weights for each component. If results of appropriately powered cohort were to show similar findings to one in this thesis, further steps would require validation of the risk score across different COPD cohorts (retrospective data from COPD patients or prospective cohorts). This should consist of similar patients as in this cohort, but also of patients presenting different phenotypes, to evaluate the applicability of NRS in different COPD phenotypes. As the risk score could potentially help in both monitoring and disease management, the score should be tested for its scalability and applicability in different settings and populations. Next, the relationship between the change in one or more domains of the NRS and its effect on the clinical outcomes should be explored. If improvement (or decrease) in one NRS domain could improve (or deteriorate) clinical outcomes, that could lead to explorations of individualised treatments or therapies. The identification of the therapies or interventions should be for two distinct purposes, acute and chronic management. Firstly, the acute care of patients identified in the pre-exacerbation phase, where aim would be to use treatments to prevent the exacerbation in those with identified high risk. Secondly, those domains that have placed them at risk could be augmented to improve their long-term disease management. Simultaneously, by decreasing the prevalence of exacerbations, healthcare costs are likely to reduce as the potential interventions are likely to cost significantly less than the exacerbations management, especially those that require hospitalisation. Overall, by identifying patients at risk of exacerbation and by limiting the number of exacerbations that would fully develop, disease management and patients' experience could be significantly improved.

Limitations of the use of nutritional phenotypes in COPD

Development of a new method of assessment of disease severity or activity is a first step on the application pathway. Presented results are only proof of concept analysis, as use of appropriate statistical approach was limited by insufficient sample size and lack of statistical significance of applied cut-points for each component. The potential use of nutritional phenotypes to identify patients with decreased exacerbation resilience, if proven its validity in larger cohorts, has to be considered beneficial for patients or the health care providers. This has to be balanced with the cost of implementation, staff involvement and the need for clear and conclusive evidence in order to encourage clinicians and health professionals to use nutritional tools in the management of a respiratory condition. Therefore, two major applicability limitations for the nutritional phenotypes identified through the NRS score are cost effectiveness of the score and acknowledging nutrition as a valid component of the clinical care.

Hospitalizations account for over half of the cost of COPD care in the UK [349]. Evidence of health care cost in COPD patients with different nutritional status is scarce. In 2007 Collins et al. demonstrated the lowest cost for patients with BMI within the obesity range and a 2-3-fold increase in those with BMI below 20kg/m^2 [350]. To allow comparison of the cost of any clinical approach, a standardised method for calculating the cost effectiveness is required. The most commonly used method in health economics currently is the Incremental Cost Effectiveness Ratio (ICER) [351]. This method considers the cost of an approach while offsetting the cost of inaction or the current accepted best practice. This cost difference is then given a value depending on the change in the quality of life for the patients as measured using quality-adjusted life years (QALY). The incremental cost of including nutritional phenotypes in the disease management, would require consideration of the cost of training, clinical assessment, and potential interventions. This upfront cost of implementation is likely to lead to a significant reduction of a total cost of COPD care. As an example, the training and implementation of the NRS score proposed in this thesis has a potential to decrease the cost of exacerbation management through identification of a pre-exacerbation phase, as long as interventions directed at this time effect in reduction of the AER in high-risk patients. Given the strong evidence that exacerbations lead to deterioration of the quality of life [28, 71] then the resulting improved quality of life, due to this novel nutritionally based assessment, would lead to a favourable ICER value.

NICE deny the existence of a threshold for ICER values in recommending treatments in the NHS, but studies have shown that only 8% of interventions with ICERs greater than £30’000/QALY are accepted [352]. Further research into the cost of implementing the NRS score and the cost of potential interventions that prevent COPD exacerbations are needed to allow any approximation

of this ICER to be calculated, but based on evidence of cost-effectiveness in other nutritional interventions [105], this ICER is likely to be highly cost-effective.

The second challenge for the introduction of the nutritional component into a daily clinical practice, is related to resistance to and undervalue of nutritional assessments in general clinical practice. Except for oncologists and gastroenterologists, nutritional status assessment is often perceived by non-nutritionists as a second category assessment, with assumed low clinical relevance. The personal experience of the author is that respiratory clinicians often refuse to acknowledge the connection between nutritional status and lung disease, with the exception of health professionals who have a personal interest in nutrition. This negative attitude towards nutrition can be changed if strong and consistent evidence is generated proving the relevance of nutritional status in COPD. The nutritional assessment has to be performed to the best standards, which requires performing the same measurements in the same way. There is an illusion of standardisation, as there are operating procedures available, but there is a wide variety of those: local, study-specific, national or published by scientific societies. There is a need to agree internationally a methodology for every commonly performed measurement (e.g. anthropometry, BIA) including positioning of the measurement, equipment, and equations used for potential calculations. Currently, the selection is often biased by equipment availability. Even if one was looking for guidance or advice on method selection, there is no single institution that would take an international responsibility for managing and developing a coherent approach to nutritional assessment.

Once measurements are accomplished in the same way, irrespective of the measurer, then standardised data presentation is needed to allow comparison and combining of results from different cohorts and countries. Currently, review of nutrition status data in COPD studies revealed freedom in data presentation.

Having internationally agreed methodology on how to perform measurements, also requires consensus on data interpretation. Currently, this applies to height, weight, and BMI, where WHO reference ranges are used for adults and centile charts for children. Therefore, overweight in one study means the same as overweight in another study. Unfortunately, this does not apply to other nutritional diagnosis as lean depletion or poor appetite could be defined using various criteria. The same person in one study could be classified as lean depleted, while in another, would not. Interpretation of FFMi is a good example of the challenge, as a number of available cut-offs and reference ranges is large. Many of them were defined based on specific cohorts and should not be used interchangeably, irrespective of the population or condition. Currently, criteria for lean

depletion are used randomly and are arbitrarily chosen by the researcher, determining the prevalence of lean depletion by chance through selected cut-off.

There is a long way for nutrition science to achieve standardisation across assessment techniques, but only by defining those needs there is a chance to improve nutrition data quality leading to better understanding of human nutrition in various conditions. Lack of consensus and standardisation on so many levels makes it difficult to translate research results into clinical practice. Even though nutritional measurements have potential to aid assessment and better management of COPD patients, which has been recognised by both American and European Thoracic Societies [61, 353], currently published results often lack benefit for patients. Encouraging clinicians to consider nutritional component in COPD management beyond BMI is one of the challenges and is most likely to be successful if nutritional evidence is strong and coherent

Conclusions

This thesis was set to determine if poor nutritional status measured with body composition, appetite, or physical capability predisposes COPD patients to worse clinical outcomes. Results have shown that nutritional markers are independent of the previous history of exacerbations, but they associated with various markers of disease status at baseline and with clinical outcomes in the future. Analysis has demonstrated, in contrary to previously published results, that loss of lean mass was not marking worse clinical outcomes, while the loss of appetite, as expected, was related to worse outcomes.

Patients with poor nutritional status, identified either by a single marker or by a multicomponent approach, have shown to have a higher risk of exacerbations in the near future, than patients with good nutritional status. In addition, those with unfavourable results in more nutritional domains have shown to have worse clinical outcomes, than those with only one nutritional component compromised. The multicomponent approach appears to have greater relevance to TTFE in the following 30 days than a single nutritional marker or than a history of exacerbation. In addition, appetite seemed to be the important element of the risk, suggesting its importance in the identification of individuals with high exacerbation risk and potential to mark a pre-exacerbation status.

This thesis provided a novel and important findings and evidence for the relevance of nutritional status in COPD and offered a multicomponent approach that, if further studied, has the potential to improve patients' health and quality of life. Given this is the first time that poor appetite was linked with the time to first exacerbation and exacerbation risk and that assessment of nutritional status has potential to mark a pre-exacerbation state in COPD patients, it is an exciting development in future clinical management.

Appendix A Systematic review - COPD cohorts included in the review

Table 76 Characteristic of the cohorts and studies included in the systematic review

Reference	Cohort	Inclusion criteria	Exclusion criteria	Number of participants (M)	Age	Follow-up	FEV_1		Fev_1/FVC	Mortality	
							L	%pred.		Nr	%
Ansari 2012 [265]	SS, UK	COPD	current exacerbation, antibiotic therapy or recent hospitalisation	188 (93)	72.5	no	-	45.1±22.1 (M); 53.2±20.6 (W)	-	-	-
Benedik 2011 [136]	SS, Slovenia	AE hospitalisation, GOLD II-IV	die during hospitalization	108 (81)	71 ± 10	0.5 y	0.94±.046	-	-	-	-
Budweiser 2008 [184]	SS, Germany	SAE, current NPPV due to hypercapnic CRF at least 3 month	obesity-hypoventilation syndrome, BMI>34, pulmonary infection or apparent oedema	93(65)	65.5 ± 8.0	no	-	33.9±12.1	-	-	-
Coleta 2008 [267]	SS, Brazil	SAE, GOLD criteria, LTOT for at least three month	AE or hospitalisation in 6 weeks prior	78 (43)	66.0 ± 8.9	1 y	-	40.7±16.1	-	12	15.4
Cortopassi 2011 [186]	SS, US	GOLD II-III, >40yoa, >10pack-years, chest x-ray	AE in 2 months prior, myocardial infraction, uncontrolled	18 (18)	64.3 ± 9.7	no	1.51±0.73	44.8±20.4	43.6±13.5	-	-

Appendix

Reference	Cohort	Inclusion criteria	Exclusion criteria	Number of participants (M)	Age	Follow-up	FEV_1		Fev_1/FVC	Mortality	
							L	%pred.		Nr	%
			hypertension, angina, oedema								
Eisner 2007 [132]	FLOW cohort, US (company members)	health care utilization for COPD in last 12 months	cancer, language barrier	355 (143)	58 ± 6.2	no	1.71±0.77	57.9±22.6	0.6±0.16	-	-
Faganello, 2010 [268]	SS, Brazil	>40yoa, smoking history>10 pack-years, post. FEV/FVC<70%, GOLD I-IV,	history of asthma, lung cancer, unstable COPD, exacerbation or hospital admission within six weeks	120 (85)	64.8 ± 9.5	1 y	-	60.9±25.2	-	-	-
Gelamo Pelegrino 2009 [264]	SS, Brazil	SAE, FEV/FVC<70%+X-rays, AE within preceding 3 months	unstable, water retention, cardiovascular diseases, osteoarticular diseases	68 (49)	64.3 ± 9.2	no	-	62.9±27.8	-	-	-
Giron 2009. [67]	SS, Spain	at admission for AE, FEV<80% & FEV/FVC<0.7, >10pack-year, GOLD II-III	asthma, end stage renal disease, sleep apnoea, cystic fibrosis	78 (78)	71±10	no	1.05±0.38	42±15	-	-	-

Reference	Cohort	Inclusion criteria	Exclusion criteria	Number of participants (M)	Age	Follow-up	FEV_1		Fev_1/FVC	Mortality	
							L	%pred.		Nr	%
Hallin 2011 [180]	SS, Sweden	FEV1<60%, smokers or ex-smokers, GOLD II-III	other diseases that could interfere with exercise: ischemic coronary disease and musculoskeletal problems	49 (14)	64	no	-	31	-	-	-
Heijdra 2003 [137]	SS, US	SAE, FEV<55%, >55yoa,	chest or lung surgery, ventilatory dependency, malignancy,	68 (36)	66 ± 8	no	0.97 ±0.29	38 ± 11	48 ±10	-	-
Hitzl 2010 [65]	SS, Germany	SAE, CHRF due to COPD or severe RTD, HMV for at least 3 months	obesity-hypoventilation syndrome, BMI>34, pulmonary infection or apparent oedema	93 (65)	65.5 ± 8.0	4 y	-	33.9±12.1	-	53	40.5* (COPD &RTD)
Marino 2010[269]	SS, Brazil	SAE, GOLD II-IV, >50yoa, smokers or non-smokers,	AE within 2 months prior, home oxygen, Sp)2<85% at rest, physical training	26 (16)	70.3 ± 6.7	no	-	46.5±1.3	39.5±1.8	-	-
Kurpad 2006 [270]	SS, India	at admission for AE, FEV<80% & FEV/FVC<0.7, >20pack-year	asthma, cardiac failure, chronic liver disease, chronic kidney	25 (25)	57.8 ± 7.2	no	-	55.1±20.9	-	-	-

Reference	Cohort	Inclusion criteria	Exclusion criteria	Number of participants (M)	Age	Follow-up	FEV_1		Fev_1/FVC	Mortality	
							L	%pred.		Nr	%
			disease, fluid overload status								
Mehrotra 2010 [271]	Health ABC, US (a sub-cohort)	ATS criteria, black and white	life-threatening condition, participation in another study	268 (154)	73.2	6.1 y	-	57.2±15.9 S; 65.4±18.4 NS	-	83	31
Sabino 2010 [139]	SS, Brazil	SAE, GOLD III,	AE within last 3 months, current smokers, LTOT	32 (23)	64	no	-	-	-	-	-
Schols 2005 [66]	SS, Netherlands	SAE, at admission to PR	nutritional intervention, intensive exercise training, unstable, type 1 diabetes, cardiovascular diseases, thyroid disease, IBD	412 (318)	64 ± 9	2-5 y	-	39±15	-	190	46
Soler-Cataluña 2005 [272].	SS, Spain	SAE, >20 pack-years, GOLD criteria	>15% reversibility in spirometry, asthma, bronchiolitis, cystic fibrosis, heart failure, renal failure,	96 (96)	69 ± 9	3 y	1.10±0.54	44.2±18.5	-	24	25

Reference	Cohort	Inclusion criteria	Exclusion criteria	Number of participants (M)	Age	Follow-up	FEV_1		Fev_1/FVC	Mortality	
							L	%pred.		Nr	%
			systemic steroid use, exacerbation within 2 months preceding enrolment								
van Wetering, 2008 [273]	INTERCOM cohort, Netherlands	SAE, Wmax less than 70% predicted	prior rehabilitation, comorbidities precluding the exercise	180 (132)	67 ± 9	no	-	67±9 G2; 43±5 G3	-	-	-
Vermeeren 2006 [178]	COSMIC cohort, Netherlands	40-75yoa, current or ex-smoker >10pack years, at least 2 AE in the last 12 months with steroids or antibiotics	respiratory disease other than COPD, regular oxygen therapy, alcohol, solvent or drug abuse evidence	389 (217)	63 ± 7	no	-	-	-	-	-
Vestbo 2006 [150]	Copenhagen City Heart Study (a sub-cohort)	FEV1/FVC<0.7 or presence of chronic mucus hyper secretion	self-reported asthma	1898 (974)	62.5	7 y	G1-2.40; G2-1.59; G3-0.94; G4-0.58;	G1-97; G2-67; G3-42; G4-25;	-	683 (59 COPD related causes)	60% men (COPD - related causes 49%)
Vilaro 2010 [179]	SS, Spain	Group 1)SAE Group 2) at admission to hospital for AE	Pneumonia, pulmonary embolism, asthma, ventilatory	121(111)	63 ± 11	N/A	-	-	-	-	-

Appendix

Reference	Cohort	Inclusion criteria	Exclusion criteria	Number of participants (M)	Age	Follow-up	FEV_1		Fev_1/FVC	Mortality	
							L	%pred.		Nr	%
			support, oedema, 1) AE in last 3 months 2) ICU admission,								
Waschki 2011 [274]	SS, Germany	SAE, GOLD I-IV,	AE within 2 months, acute heart failure	170 (128)	64	4 y	-	58.8 ±21.1 S; 41.4±21.9 NS	-	26	15.4

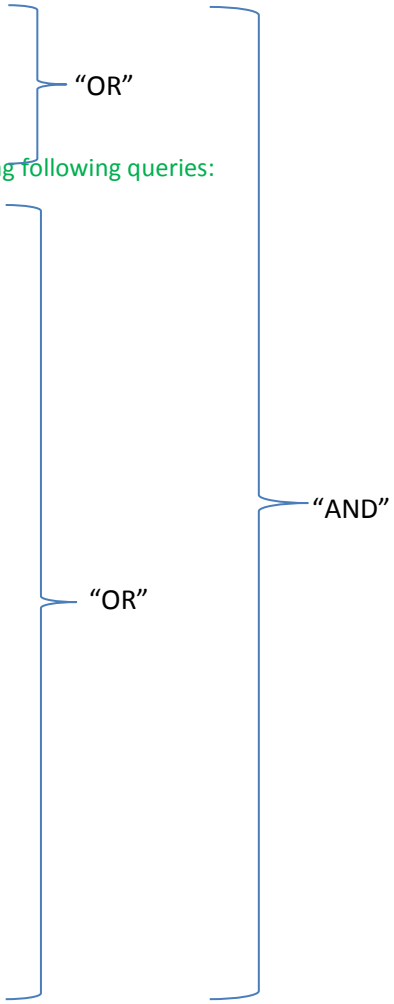
SS – study specific; SAE – stable at enrolment; ICU – intensive care unit; G2, G3, – GOLD, stages, PR- pulmonary rehab

EX – exacerbator, NONEX – non-exacerbator; D- depleted, ND – non-depleted; M-men, W-women; S – survivals, NS – non-survivals; T – total,

Appendix B Search terms used in systematic review

1. Search terms chosen for the systematic review:
    2.1. Selecting articles on COPD using following queries:
        - COPD
        - COAD
        - chronic obstructive *ADJ2* disease
        - emphysema
        - bronchitis

    2.2. Selecting articles on body composition using following queries:
        - body composition
        - fat free mass
        - fat-free mass
        - FFM
        - fat free mass index
        - fat-free mass index
        - FFMI
        - lean body mass
        - body lean mass
        - muscle mass
        - bioelectrical impedance
        - bio-electrical impedance
        - BIA
        - handgrip strength
        - grip strength
        - handgrip endurance
        - grip endurance
        - mid-upper arm muscle circumference
        - mid upper arm muscle circumference
        - MUAMC
        - mid-upper arm circumference
        - mid upper arm circumference
        - MUAC
        - skinfold



Appendix C Review of FFMi in COPD – Web of Science only

To explore the difference in body composition between healthy and COPD patients a discrete review of Web of Science database was performed. The aim was to identify studies reporting FFMi for COPD patients separately for each gender and identify cohort reporting data in both COPD and healthy population. The time period was chosen between 1990 and time of review (2014), as the concept of FFMi was first described in 1990. From an initial 94 articles identified, 14 studies provided data on mean and standard deviation (SD) values for FFMi in male COPD patients and of these, 11 also provided results for female COPD patients. Using the mean and SD values for FFMi, a cumulative normal distribution was produced with Excel software in order to demonstrate the differences in distribution of FFMi between COPD and healthy, men and women. This modelling was used further for additional analysis, which is beyond the scope of this section.

Mean and standard deviation of FFMi from COPD patients (moderate and severe COPD patients [344]) and healthy controls in a similar population [137] were used and a simulation of the cumulative normal distribution of FFMi was performed (Figure 58). There was a visible difference in FFMi distribution between healthy and COPD, and as expected, men had visibly higher lean mass than women. Results in COPD patients were overlapping, for man and women and severe patients showed lower lean mass than moderate group of patients.

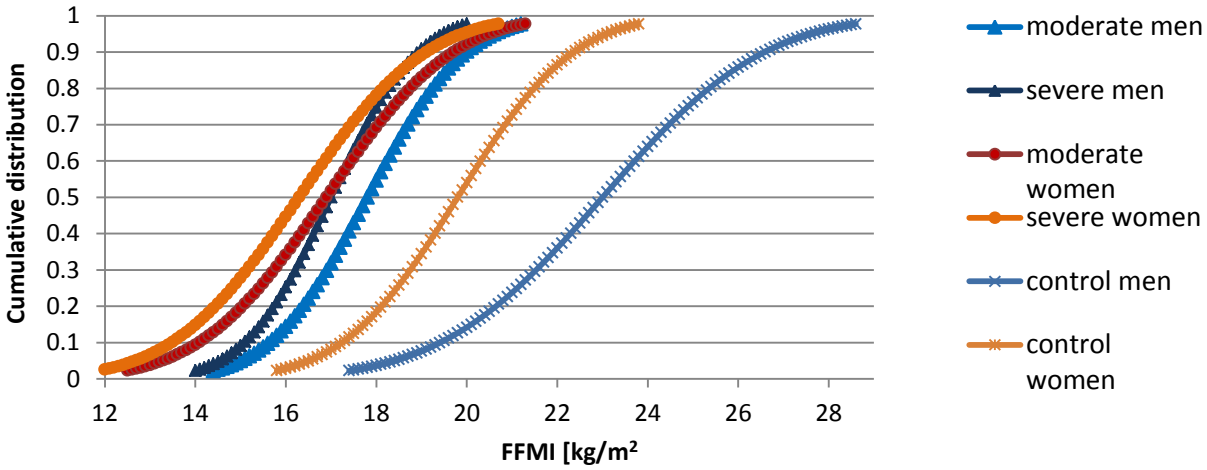


Figure 58 Fat-free mass index (FFMi) distribution in moderate and severe COPD patients and healthy controls – data modelling

Appendix D Criteria for sarcopenia

Table 77 Definitions of sarcopenia across groups looking for consensus

Working group		Gait speed*		Grip strength		Muscle mass	
ESPEN SIG		≤ 0.8m/s	&	-	&	a percentage of muscle mass 2SD below the mean measured in young adults of the same sex and ethnic background	
IWGS		≤ 1.0m/s	&	-	&	ALM / Ht^2 Men ≤7.23 kg/m^2 Women ≤5.67 kg/m^2	
EWGSOP	Sarcopenia	≤ 0.8m/s	or	Men <30 kg Women<20 kg	&	ALM / Ht^2 Men ≤7.23 kg/m^2 Women ≤5.67 kg/m^2	
	Severe sarcopenia	≤ 0.8m/s	&	Men <30 kg Women<20 kg	&	ALM / Ht^2 Men ≤7.23 kg/m^2 Women ≤5.67 kg/m^2	
FNIHSP	slowness & weakness & low lean mass	≤ 0.8m/s	&	Men <26 kg Women<16 kg	&	ALM / BMI Men <0.789 Women <0.512	ALM Men <19.75kg Women<15.02kg
	Weakness & low lean mass	-	-	Men <26 kg Women<16 kg	&	ALM / BMI Men <0.789 Women<0.512	ALM Men <19.75kg Women<15.02kg

** Gait speed in 4-m walking test; ALM – appendicular lean mass as a sum of lean mass from both arms and legs (based on DXA); Table based on [80, 82-84]*

Appendix EAERIS study additional information

Table 78 Frequency of repeated measurements in the AERIS cohort (tests included in this document)

Test	Enrolment	Follow-up (months)				Exacerbation
		3	6	9	12	
General information						
Demographics	✓					
Medical history	✓					
Medication review	✓					✓
Smoking history	✓					
Smoking status	✓	✓	✓	✓	✓	✓
Physical examination	✓	✓	✓	✓	✓	✓
Nutritional markers						
Anthropometry	✓	✓	✓	✓	✓	✓*
Appetite score	✓	✓	✓	✓	✓	✓
Body composition	✓	✓	✓	✓	✓	
Grip tests **Strength** **Endurance**	✓	✓	✓	✓	✓	
6 Minute walk test	✓		✓		✓	
Nutritional biochemistry	✓		✓		✓	✓
COPD markers						
Spirometry	✓	✓	✓	✓	✓	✓
Single breath (TLCO)	✓		✓		✓	
Blood - Inflammation	✓	✓	✓	✓	✓	✓

*only height and weight

Table 79 Standard Operating Procedures (SOP) for nutrition status assessment used in the AERIS study

Measurement	SOP number
Height	SCBR/GEN/V4/006, June 2012
Weight	SCBR/GEN/V5/007, February 2013
MUAC	SCBR/GEN/V3/118, June 2013
TSF	SCBR/GEN/V2/108, May 2012
MFAC	SCBR/GEN/V3/118, June 2013
Waist circumference	SCBR/GEN/V3/118
Body composition	WTCRF-BRU/GEN/V2/117, March 2012
Grip tests	WTCRF-BRU/GEN/V1/161, August 2011
6MWT	WTCRF-BRU/GEN/V1/145, January 2011,

Appendix F Appetite and body composition markers

Table 80 Comparison of body composition markers in low and high appetite score (AS) groups (t-test)

	Women			Men		
	n	low AS (n=35) vs high AS[z] (n=23)	p-value	n	low AS (n=33) vs high AS[z](n=34)	p-value
MUAC [cm]	57	29.9±4.9 vs. 31.3±6.1	NS	67	30.1±3.5 vs 31.1±3.4	NS
MUAMC [cm]	57	23.4±3.5 vs. 23.8±3.2	NS	67	25.0±3.1 vs 25.8±2.8	NS
MUAMA [cm^2]	55	44.4±13.7 vs. 46.0±12.6	NS	67	50.5±12.8 vs 53.7±11.4	NS
FFM [kg]	56	40.6±7.1 vs. 41.2±8.0	NS	66	57.8±11.1 vs 50.8±9.2	NS
FFMi [kg/m^2]	56	15.7±2.1 vs. 15.9±2.5	NS	66	19.2±2.6 vs 20.1±2.8	NS
FFM [%]	56	58.2±8.0 vs. 58.5±6.3	NS	66	71.7±5.5 vs 70.5±4.5	NS
Imp50 [kHz]	56	588.5±86.5 vs. 573.4±83.7	NS	66	493.7±76.2 vs 472.7±71.5	NS
Waist [cm]	57	98.5±16.3 vs. 98.3±16.6	NS	65	105.5±13.8 vs 108.3±13.7	NS
FM [kg]	55	29.0±10.1 vs. 30.5±11.5	NS	66	23.4±7.6 vs 26.0±2.8	NS
FMI [kg/m^2]	55	11.2±3.9 vs. 11.8±4.3	NS	66	7.8±2.4 vs 8.6±2.8	NS
FM [%]	55	39.7±7.5 vs. 41.5±6.3	NS	66	28.5±5.4 vs 19.5±4.5	NS
TSF mm]	55	21.0±7.5 vs. 19.7±7.6	NS	67	16.2±8.9 vs 16.8±7.7	NS
6MWT [s]	56	264.1±93.2 vs. 311.7±99.0	0.079	67	277.6±123.6 vs 336.9±115.0	0.046
Grip endurance [s]	51	40.2±19.8 vs. 62.9±57.8	0.051	64	41.87±22.1 vs 56.1±55.1	NS
Max grip [N]	48	180.9±61.5 vs. 181.7±50.4	NS	61	303.9±77.7 vs 278.7±76.6	NS

[z]Low AS≤28pts, NS – not significant

Appendix G Evaluation of grip tests quality

One of the conclusions of the systematic review was the great variability of grip test methods, inconsistent hand tested (left vs right, dominant vs non-dominant), number of repeats of the test and calculation of results (average, sum, selection of one value). In addition, a horizontal audit of the data collected in the epidemiological longitudinal project run by the Southampton COPD group (AERIS) had shown that some of the obtained results were improbable. To maintain high quality of grip test data and to support the research team, a review of the local SOP for grip strength and grip endurance was performed. The aim of this evaluation was to determine criteria for inclusion/rejection of the grip test results to be used at the time of the measurement during the study visits.

According to the SOP and equipment available in the Southampton COPD group, grip strength and endurance tests were performed using MIE analyser, which has no previously validated or internationally approved measurement protocols. Lack of quality control criteria urged to evaluate initial results and verify their repeatability and reproducibility. For that purpose, measurements performed on a pilot group of 37 subjects from the longitudinal cohort (AERIS) during the first 5-months of the follow-up, were assessed and reviewed to establish test inclusion criteria. The main purpose of this process was to understand what are potential causes of poor quality results (non-repeatable and non-reproducible results) and what the risk of obtaining poor quality results is.

This review process of grip strength results was performed in two steps. The first phase included visual analysis of graphs representing each test (sample graph presented in Figure 59). Next, comparison of different tests with similar insufficiencies (delayed moment of grip, the total test time of less than required five seconds) was performed to confirm which errors are causing poor quality results. Based on this analysis, an initial quality control steps were established: test needs to have rapid start (steep beginning on the graph); maximum grip strength has to be reached within 0.6 seconds from the test start; test need to last no less than 4 seconds and be as stable as possible. To facilitate quality control process during the AERIS study visits, a decision tree was created (Figure 60).

This evaluation process was performed to improve the quality of measurements and obtained results, however, did not include any formal data analysis. This was part of a data management process, as during horizontal audit of results obtained of the first weeks of the study some improbable results were identified. Therefore, once quality assurance criteria were defined,

results obtained in the study up to the review process were assessed posthoc, and poor quality tests were discarded.

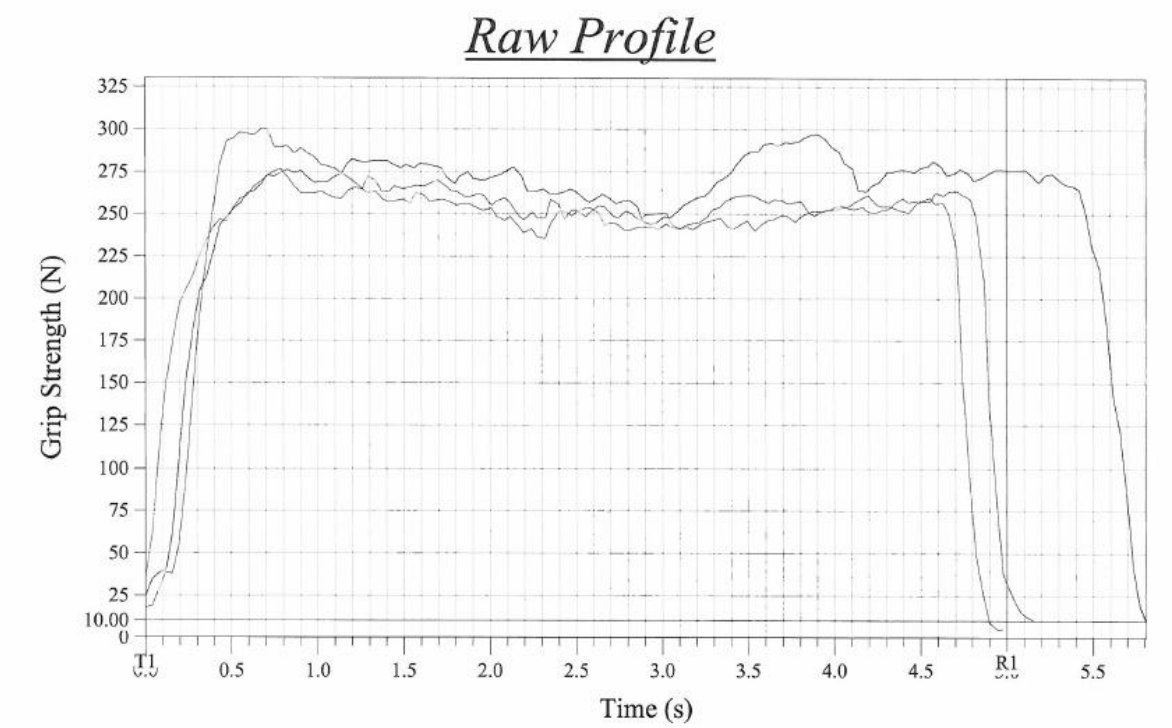


Figure 59 Sample graph representing three replicates of a grip strength test

Evaluation of grip strength data raised another question. Each grip strength test was performed in three replicates. Initially, an arbitrary decision was made that a mean of the three values should be used as a final value for analysis, however, there was no evidence of the benefit of such approach over using a single highest value out of the three replicates. The assumption was that small variability between the three replicates would suggest using all three values and calculating the mean as a way of minimising the noise. However, considerable variability between replicates from the same visit would indicate that quality control process cannot minimise the personal variance in the results, and the single highest value should be used as the final result. This evaluation was scheduled to be performed at the end of the study, using all grip strength results. Unfortunately, format the data were provided in the final database approved for the analysis disabled such analysis. Due to initial arrangements with the study sponsor, database generated from data entered on the study site was then arranged and approved by the sponsor and final dataset contained only the mean of the three measurements, disabling assessment of replicates variability. Therefore, in accordance with the initial decision, an average of all good quality replicates (results which did not meet the set up quality standards were discarded before the data entry to the sponsors system) was used in the analysis for the AERIS study.

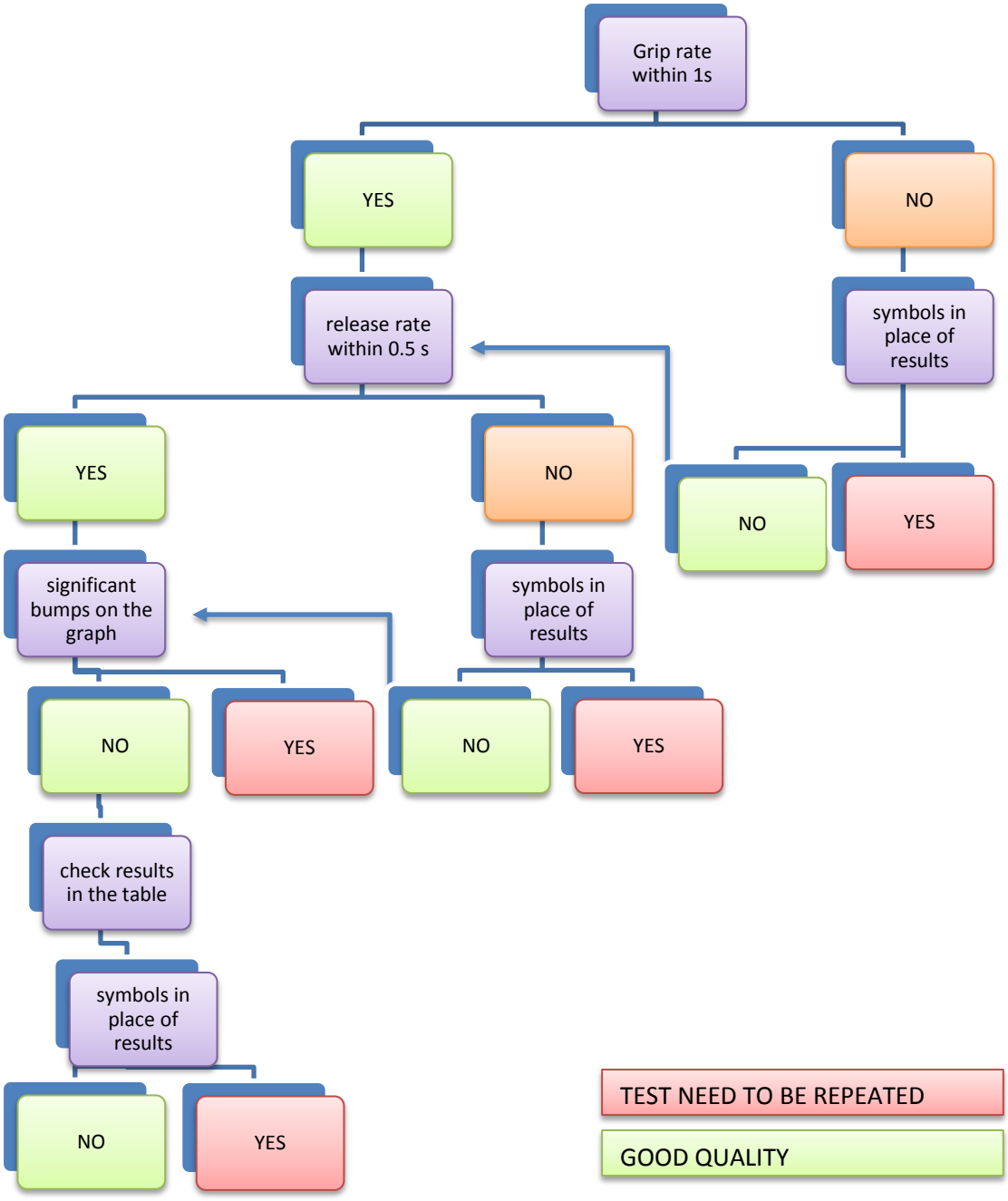


Figure 60 Decision tree for grip strength test using MIE - Quality Control steps

Appendix H Details of scores: CAT, BODE, MUST

Table 81 Scoring of the COPD assessment test (CAT) score

Most positive answer	Range of scoring						Most negative answer	Score
I never cough	0	1	2	3	4	5	I cough all the time	
In have no phlegm (mucus) in my chest at all	0	1	2	3	4	5	My chest is completely full of phlegm (mucus)	
My chest does not feel tight at all	0	1	2	3	4	5	My chest feels very tight	
When I walk up a hill or one flight of stairs I am not breathless	0	1	2	3	4	5	When I walk up a hill or one flight of stairs I am very breathless	
I am not limited doing any activities at home	0	1	2	3	4	5	I am very limited doing activities at home	
I am confident leaving my home despite my lung condition	0	1	2	3	4	5	I am not at all confident leaving my home because of my lung condition	
I sleep soundly	0	1	2	3	4	5	I don't sleep soundly because of my lung condition	
I have lots of energy	0	1	2	3	4	5	I have no energy at all	
Total score								

Table 82 BODE scoring system

Variable	Points				
	1	2	3	4	Score
Body Mass index [kg/m^2]	<21	≥21			
FEV1 [% predicted]	>65	50-64	36-49	≤35	
modified medical research council dyspnoea scale score	0-1	2	3	4	
6-minute walk test [m]	≥350	250-349	150-249	≤149	
Total					

Table 83 Modified medical research council dyspnoea scale score

Modified MRC score	Description
0	Breathless only with strenuous exercise
1	2 Short of breath when hurrying on the level or up a slight hill
2	Slower than most people of the same age on a level surface or Have to stop when walking at my own pace on the level
3	4 Stop for breath walking 100 meters or After a walking few minutes at my own pace on the level
4	Too breathless to leave the house.

Appendix

'Malnutrition Universal Screening Tool'

BAPEN
www.bapen.org.uk

MAG
Malnutrition Advisory Group
A Standing Committee of BAPEN

BAPEN is registered charity number 1022907 www.bapen.org.uk

'MUST'

'MUST' is a five-step screening tool to identify **adults,** who are malnourished, at risk of malnutrition (undernutrition), or obese. It also includes management guidelines which can be used to develop a care plan.

It is for use in hospitals, community and other care settings and can be used by all care workers.

This guide contains:

- A flow chart showing the 5 steps to use for screening and management
- BMI chart
- Weight loss tables
- Alternative measurements when BMI cannot be obtained by measuring weight and height.

The 5 'MUST' Steps

Step 1

Measure height and weight to get a BMI score using chart provided. *If unable to obtain height and weight, use the alternative procedures shown in this guide.*

Step 2

Note percentage unplanned weight loss and score using tables provided.

Step 3

Establish acute disease effect and score.

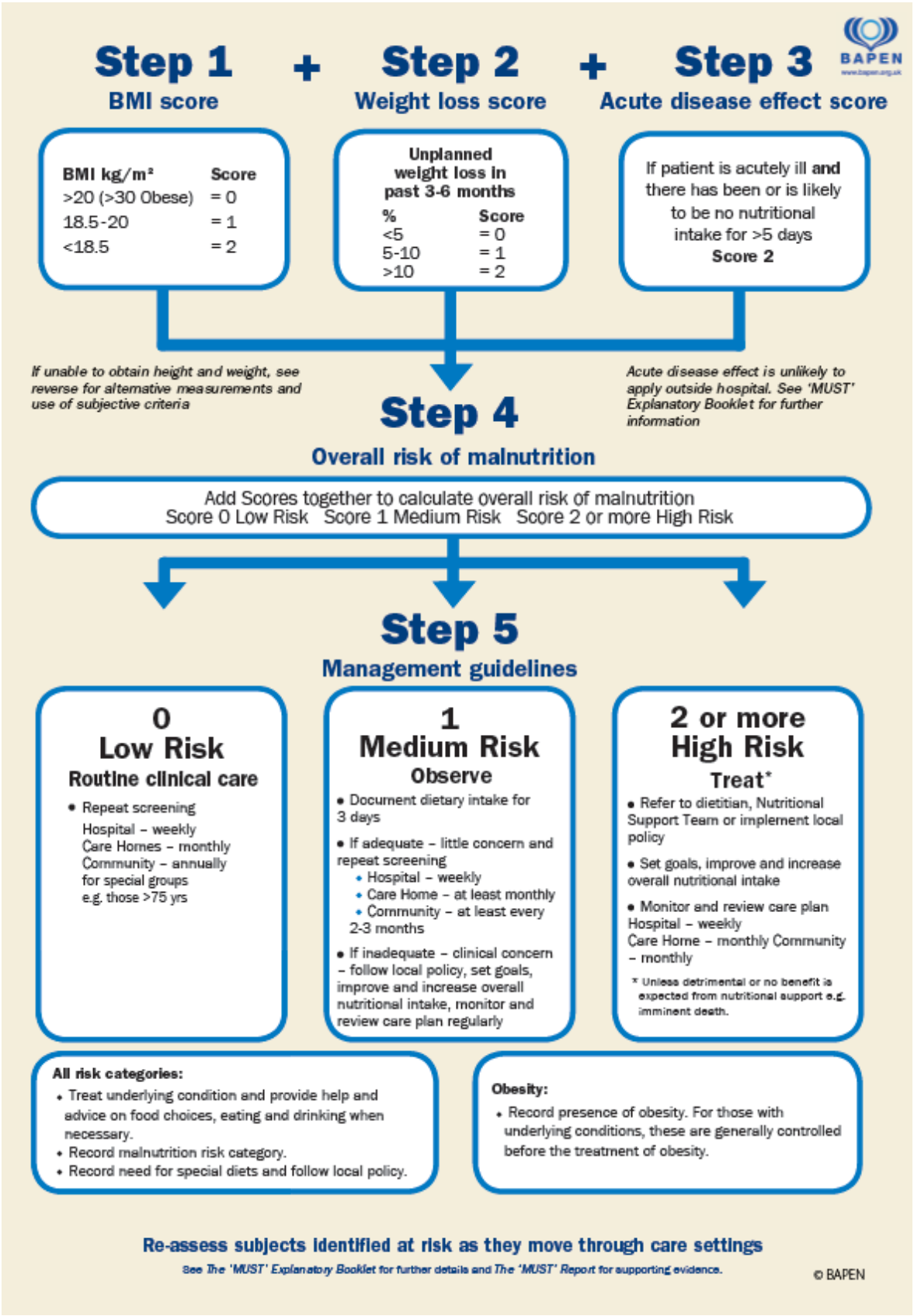
Step 4

Add scores from steps 1, 2 and 3 together to obtain overall risk of malnutrition.

Step 5

Use management guidelines and/or local policy to develop care plan.

Please refer to *The 'MUST' Explanatory Booklet* for more information when weight and height cannot be measured, and when screening patient groups in which extra care in interpretation is needed (e.g. those with fluid disturbances, plaster casts, amputations, critical illness and pregnant or lactating women). The booklet can also be used for training. See *The 'MUST' Report* for supporting evidence. Please note that 'MUST' has not been designed to detect deficiencies or excessive intakes of vitamins and minerals and is of **use only in adults.**


Step 1
BMI score
+
Step 2
Weight loss score
+
Step 3
Acute disease effect score
BAPEN
BMI kg/m²
Score
>20 (>30 Obese) = 0
18.5-20 = 1
<18.5 = 2
Unplanned weight loss in past 3-6 months
%
Score
<5 = 0
5-10 = 1
>10 = 2
If patient is acutely ill and there has been or is likely to be no nutritional intake for >5 days
Score 2
If unable to obtain height and weight, see reverse for alternative measurements and use of subjective criteria
Acute disease effect is unlikely to apply outside hospital. See 'MUST' Explanatory Booklet for further information
Step 4
Overall risk of malnutrition
Add Scores together to calculate overall risk of malnutrition
Score 0 Low Risk Score 1 Medium Risk Score 2 or more High Risk
Step 5
Management guidelines
0
Low Risk
Routine clinical care
• Repeat screening
Hospital – weekly
Care Homes – monthly
Community – annually
for special groups
e.g. those >75 yrs
1
Medium Risk
Observe
• Document dietary intake for 3 days
• If adequate – little concern and repeat screening
• Hospital – weekly
• Care Home – at least monthly
• Community – at least every 2-3 months
• If inadequate – clinical concern – follow local policy, set goals, improve and increase overall nutritional intake, monitor and review care plan regularly
2 or more
High Risk
Treat*
• Refer to dietitian, Nutritional Support Team or implement local policy
• Set goals, improve and increase overall nutritional intake
• Monitor and review care plan
Hospital – weekly
Care Home – monthly Community – monthly
* Unless detrimental or no benefit is expected from nutritional support e.g. imminent death.
All risk categories:
• Treat underlying condition and provide help and advice on food choices, eating and drinking when necessary.
• Record malnutrition risk category.
• Record need for special diets and follow local policy.
Obesity:
• Record presence of obesity. For those with underlying conditions, these are generally controlled before the treatment of obesity.
Re-assess subjects identified at risk as they move through care settings
See The 'MUST' Explanatory Booklet for further details and The 'MUST' Report for supporting evidence.
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Appendix

Alternative measurements and considerations

BAPEN
www.bapen.org.uk

Step 1: BMI (body mass index)

If height cannot be measured

- Use recently documented or self-reported height (if reliable and realistic).
- If the subject does not know or is unable to report their height, use one of the alternative measurements to estimate height (ulna, knee height or demispan).

Step 2: Recent unplanned weight loss

If recent weight loss cannot be calculated, use self-reported weight loss (if reliable and realistic).

Subjective criteria

If height, weight or BMI cannot be obtained, the following criteria which relate to them can assist your professional judgement of the subject's nutritional risk category. Please note, these criteria should be used collectively not separately as alternatives to steps 1 and 2 of 'MUST' and are not designed to assign a score. Mid upper arm circumference (MUAC) may be used to estimate BMI category in order to support your overall impression of the subject's nutritional risk.

1. BMI

- Clinical impression – thin, acceptable weight, overweight. Obvious wasting (very thin) and obesity (very overweight) can also be noted.

2. Unplanned weight loss

- Clothes and/or jewellery have become loose fitting (weight loss).
- History of decreased food intake, reduced appetite or swallowing problems over 3-6 months and underlying disease or psycho-social/physical disabilities likely to cause weight loss.

3. Acute disease effect

- Acutely ill and no nutritional intake or likelihood of no intake for more than 5 days.

Further details on taking alternative measurements, special circumstances and subjective criteria can be found in *The 'MUST' Explanatory Booklet*. A copy can be downloaded at www.bapen.org.uk or purchased from the BAPEN office. The full evidence-base for 'MUST' is contained in *The 'MUST' Report* and is also available for purchase from the BAPEN office.

BAPEN Office, Secure Hold Business Centre, Studley Road, Redditch, Worcs, B98 7LG. Tel: 01527 457 850. Fax: 01527 458 718. bapen@sovereignconference.co.uk BAPEN is registered charity number 1023927. www.bapen.org.uk

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'MUST' is supported by the British Dietetic Association, the Royal College of Nursing and the Registered Nursing Home Association.

Appendix I Comparison of body composition assessment techniques

Table 84 Summary of advantages and limitation of body structure assessment techniques

	Advantages	Inconveniences & limitations	Obtained information
BMI	Low equipment cost Portable Non-invasive and convenient for the patient Training for measurer short and straightforward Low inter-observer variation (if properly trained)	Lack of internationally approved protocols Common low-quality measurements No information on lean and fat Potential higher intra-observer variation (especially between centres and protocols)	Body size and proportion No information on body composition
Skinfolds	Low equipment cost Portable Non-invasive and convenient for the patient Training for measurer short and straightforward	Multiple internationally standardised protocols Measurements require more practice (skinfolds) Potential higher inter- and intra-observer variation (especially between centres and protocols) Limited interpretation of results	Indication of regional fatness
Circumferences	Low equipment cost Portable Non-invasive and convenient for the patient Training for measurer short and straightforward Low inter-observer variation	Lack of internationally standardised protocols Potential higher intra-observer variation (especially between centres and protocols) Limited interpretation of results	Body size Identifying type of obesity No information on body composition
BIA	Relative low equipment cost (single cost) Portable Non-invasive and convenient for the patient Training for measurer short and straightforward Very low inter- and intra-observer variation Reference ranges included in software* Can be used in individuals with chronic lung diseases Measurements can be performed including whole body or selected body parts	Limited use in individuals with metal implants Limited use in individuals with abnormal hydration status Not for use in individuals with pacemakers Results influenced by hydration status Proprietary equations determine FFM	Body composition (FM, FFM, TBW) Body physical properties (resistance, impedance, phase angle) General or regional body composition

Appendix

	Advantages	Inconveniences & limitations	Obtained information
DXA	Fast Convenient for the patient Reference ranges included in software*	Inaccurate for obese individual (anterior-posterior thickness >23cm) Radiation exposure (low dose) Limited use in individuals with metal implants Limited use in individuals with abnormal hydration status Limited use in individuals with chronic lung diseases which disables staying in supine position for extended period High equipment cost Trained specialists to perform measurements Not portable	Body composition (FM, FFM, TBW, bone density) General or regional body composition
D20	Medium equipment cost Acceptable in all age groups Safe convenient Gold standard for TBW	Delayed results Requires laboratory analysis Limited portability Trained specialists to perform measurements Technical method limitations (repeated measurements within several hours, limited food and drink consumption during assessment)	Body composition (TBW) Can be used to estimate FFM and FM

Reference ranges and interpretation has number of limitations, which are discussed further in this chapter

Appendix J Comparison study - approval

NHS
Health Research Authority
NRES Committee London - Bromley
Level 3, Block B
Whitefriars
Lewins Mead
Bristol
BS1 2NT

Telephone: 01173421390
Fax:01173420445

12 August 2015

Dr T.M. Wilkinson
Associate Professor in Respiratory Medicine
University of Southampton
Tremona road, MP 218, C level, Southampton General Hospital
SO16 6YD

Dear Dr Wilkinson

Study title:	**A comparison between BIA, BIS, D2O, pQCT and DXA along with grip test to assess level of agreement between different body composition markers in COPD patients**
REC reference:	**15/LO/1233**
IRAS project ID:	**181442**

Thank you for your letter of 7th August 2015, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Vice Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager, Miss Georgina Castledine, nrescommittee.london-bromley@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
GP/consultant information sheets or letters [GP_letter]	1	20 May 2015
Letters of invitation to participant [Patient_invitaition_letter]		
Non-validated questionnaire [Q1_compliance_restrictions]	version 1.0	20 May 2015
Non-validated questionnaire [Q1_compliance_restrictions]	1.0	20 May 2015
Other [BIA operating procedure]	v2	19 March 2012
Other [Grip tests operating procedure]	v1	08 August 2011
Other [DXA scan operating procedure]	v1	09 August 2011
Other [BIS operating procedure]	v1	14 January 2014
Other [pQCT instruction]	1.0	02 July 2015
Other [D2O draft operating procedure]	1.0	26 May 2015
Other [Response to PO]		07 August 2015
Participant consent form [Consent_form]	1.1	24 July 2015
Participant information sheet (PIS) [PIS_V1.0]	2.0	24 July 2015
REC Application Form [REC_Form_26062015]		26 June 2015
Research protocol or project proposal [Study protocol]	1.0	27 May 2015
Summary CV for Chief Investigator (CI) [T.Wilkinson 2 page cv]		
Summary CV for student [M.Wojtas_CV]	version 1.0	20 May 2015

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from NRES. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

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With the Committee's best wishes for the success of this project.

Yours sincerely

Ms Carol Jones
Chair

Email: nrescommittee.london-bromley@nhs.net

Enclosures: "After ethical review – guidance for researchers" *[SL-AR2]*

Copy to: *Jennifer Peach, R&D Department, University Hospital Southampton NHS Foundation Trust*

Appendix K Body composition and COPD – detailed results – AERIS study

Body composition can be measured by a number of surrogate markers (arm measurements, waist measurements) and use of FFMi and FMi as markers of lean and fat was a chosen pragmatic approach. However, other anthropometry markers were compared with disease marker as well, for more complete picture of the relationship.

When continuous anthropometry data were used, history of exacerbations was not related to anthropometry markers for either men, or women and patients' disease perception (CAT score) did not correlate with any anthropometry markers in both sexes (data not shown). The majority of lean and fat markers were correlated with lung function measured by FEV/FVC in both men and women, while other lung function markers were related to selected anthropometry and body composition markers (e.g. FEV% was correlated to triceps skinfold and impedance at 50kHz in women only) (Table 85, Table 86).

There was no difference in anthropometry markers between men with different COPD severity, but there was a significant difference in anthropometry markers between women with more and less severe COPD (Appendix KTable 87). Anthropometry markers were similar in those with 2 or less exacerbations compared with those with more than two exacerbations in the year before the study. Anthropometry markers did not differ between BODE score groups either for men, or women (data not shown).

Only four patients in this cohort had MUAC below 23cm, therefore the use of such interpretation criteria was futile. Similarly, increased risk of metabolic complications (waist circumference) was identified in 17 patients (13% cohort), while 41 were identified to have substantially increased risk of metabolic complications. Thirteen percent of a cohort represents a small sub-group and not sufficient to categorise patients into two distinct groups. However, 41 patients accounted for 1/3 of the AERIS cohort, which enables analysis of differences in clinical outcomes between the two groups (low and high risk). A log-rank test for a relationship with TTFE showed no difference between normal and high WC (data not shown). As none of the previously published criteria for waist circumference and arm measurements were related to clinical outcomes, therefore, sex-specific tertiles were used to identify patients with the lowest values (T1) and the highest values (T3) for all anthropometry markers, but none showed a relationship with TTFE (data not shown).

Anthropometric measurements were used also to compare with AER, but there was no difference in AER between WC or MUAC groups. Similar to the analysis of TTFE, sex-specific tertiles were

used to identify patients with the lowest values (T1) and the highest values (T3) for all anthropometry markers, but none showed a relationship with AER (data not shown). When anthropometry markers of individuals from three categories of exacerbation frequency were compared (≤2AE/y, 2-4AE/y, >4AE/y) there was a significant difference in MUAMC in women (data not shown) with the lowest MUAMC amongst those who had more than four exacerbations.

Table 85 Correlations between body composition markers and lung function and inflammation markers at baseline in men (n=68)

		FEV1 [L]	FEV [%]	FVC [L]	FEV/FVC	TLCO [%]	Imp50 [kHz]	CRP1 [mg/L]	Fibrinogen [g/L]
BMI [kg/m^2]	R	0.238	0.238	-0.035	0.397**	0.349*	-0.619**	0.270*	0.045
	P	0.051	0.051	0.777	0.001	0.032	<0.001	0.026	0.732
AC [cm]	R	0.343**	0.235	0.181	0.329**	0.291	-0.499**	0.257*	0.057
	P	0.004	0.054	0.141	0.006	0.077	<0.001	0.034	0.663
MUAMC [cm]	R	0.293*	0.160	0.129	0.267*	0.158	-0.377**	0.096	0.073
	P	0.015	0.192	0.295	0.028	0.345	0.002	0.438	0.578
MUAMA [cm]	R	0.294*	0.161	0.122	0.276*	0.170	-0.371**	0.096	0.073
	P	0.015	0.188	0.320	0.023	0.306	0.002	0.438	0.578
TSF [cm]	R	0.127	0.136	0.097	0.138	0.328*	-0.243*	0.215	0.001
	P	0.303	0.270	0.430	0.261	0.044	0.048	0.079	0.992
WC [cm]	R	0.163	0.107	-0.007	0.285*	0.244	-0.518**	0.233	0.128
	P	0.191	0.393	0.953	0.021	0.139	<0.001	0.059	0.340
FM [kg]	R	0.169	0.151	0.016	0.272*	0.212	-0.326**	0.218	0.054
	P	0.170	0.223	0.899	0.026	0.202	0.007	0.076	0.687
FM [%]	R	-0.047	0.067	-0.198	0.125	0.088	0.129	0.189	0.129
	P	0.704	0.590	0.107	0.312	0.599	0.298	0.125	0.330
FMi [kg/m^2]	R	0.088	0.148	-0.136	0.280*	0.232	-0.291*	0.257*	0.082
	P	0.478	0.231	0.272	0.022	0.160	0.017	0.036	0.537
FFM [kg]	R	0.401**	0.206	0.315**	0.332**	0.305	-0.722**	0.163	-0.007
	P	0.001	0.095	0.009	0.006	0.062	<0.001	0.187	0.959
FFMi [kg/m^2]	R	0.326**	0.262*	0.077	0.428**	0.394*	-0.829**	0.215	-0.032
	P	0.007	0.032	0.537	<0.001	0.014	<0.001	0.081	0.810
FFM [%]	R	0.031	-0.086	0.196	-0.144	-0.096	-0.125	-0.198	-0.146
	P	0.803	0.490	0.113	0.244	0.565	0.315	0.109	0.270
Imp 50 [kHz]	R	-0.237	-0.227	-0.068	-0.322**	-0.337*	x	-0.163	0.080
	P	0.053	0.065	0.583	0.008	0.038	x	0.187	0.546

Table 86 Correlations between body composition markers and lung function and inflammation markers at baseline in women (n=59)

		FEV1 [L]	FEV [%]	FVC [L]	FEV/FVC	TLCO [%]	Imp50 [kHz]	CRP[1] [mg/L]	Fibrinogen [g/L]
BMI [kg/m^2]	R	0.244	0.218	0.012	0.437**	0.184	-0.620**	0.266*	0.212
	P	0.062	0.096	0.929	0.001	0.359	<0.001	0.042	0.123
AC [cm]	R	0.279*	0.210	0.097	0.399**	0.194	-0.621**	0.188	0.130
	P	0.034	0.113	0.467	0.002	0.332	<0.001	0.158	0.353
MUAMC [cm]	R	0.064	0.017	0.040	0.133	0.169	-0.446**	0.073	0.050
	P	0.640	0.898	0.769	0.330	0.399	0.001	0.591	0.725
MUAMA [cm]	R	0.061	0.013	0.036	0.136	0.172	-0.438**	0.073	0.050
	P	0.653	0.922	0.794	0.319	0.390	0.001	0.591	0.725
TSF [cm]	R	0.417**	0.353**	0.212	0.482**	0.132	-0.475**	0.200	0.092
	P	0.001	0.008	0.117	<0.001	0.511	<0.001	0.140	0.519
WC [cm]	R	0.164	0.104	-0.031	0.359**	0.193	-0.528**	0.219	0.146
	P	0.218	0.437	0.815	0.006	0.335	<0.001	0.098	0.298
FM [kg]	R	0.190	0.159	-0.028	0.390**	0.153	-0.525**	0.319*	0.265
	P	0.162	0.241	0.837	0.003	0.456	<0.001	0.017	0.061
FM [%]	R	-0.063	0.040	-0.252	0.217	0.139	-0.146	0.304*	0.367**
	P	0.643	0.769	0.061	0.107	0.497	0.283	0.023	0.008
FMi [kg/m^2]	R	0.131	0.170	-0.112	0.391**	0.130	-0.481**	0.324*	0.301*
	P	0.337	0.211	0.412	0.003	0.526	<0.001	0.015	0.032
FFM [kg]	R	0.426**	0.205	0.354**	0.316*	0.239	-0.731**	0.209	-0.038
	P	0.001	0.127	0.007	0.017	0.240	<0.001	0.119	0.787
FFMi [kg/m^2]	R	0.421**	0.312*	0.271*	0.412**	0.245	-0.819**	0.271*	0.037
	P	0.001	0.018	0.041	0.001	0.227	<0.001	0.041	0.796
FFM [%]	R	0.048	-0.058	0.244	-0.270*	-0.141	0.173	-0.248	-0.297*
	P	0.722	0.669	0.067	0.042	0.493	0.198	0.063	0.033
Imp 50 [kHz]	R	-0.293*	-0.281*	-0.224	-0.270*	-0.250	X	-0.213	-0.015
	P	0.027	0.034	0.094	0.042	0.217	X	0.111	0.917

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

R – Pearson's correlation; P- p-value; [1] Results for Spearman's correlation

Table 87 The difference in body composition markers between GOLD classes in women (ANOVA with Bonferroni correction, n=25 GOLD 2, N= 25 GOLD 3, n=8 GOLD 4)

	difference between GOLD 2 and 4			Difference between gold 3 and 4	
	Sig.	Mean Difference	Std. Error	Mean Difference	Std. Error
BMI [kg/m2]	0.03	6.3*	2.4	6.0*	2.4
MUAC [cm]	0.01	6.3*	2.0	6.4*	2.0
MUAMC [cm]	0.06	2.4	1.3	3.3	1.3
MUAMA [cm]	0.07	9.3	5.2	12.1	5.2
TSF [cm]	0.00	10.3*	2.8	8.3*	2.8
WC [cm]	0.02	14.6	6.2	17.5*	6.2
FM [kg]	0.05	9.1	4.1	10.2	4.1
FM [%]	0.48	2.4	2.9	3.5	2.9
FMi [kg/m^2]	0.10	3.2	1.6	3.4	1.6
FFM [kg]	0.02	7.7*	2.8	7.8*	2.9
FFMi [kg/m^2]	0.01	2.7*	0.8	2.4*	0.8
FFM [%]	0.28	-4.0	2.9	-4.6	2.9
Imp 50 [kHz]	0.06	-81.0	33.0	-56.6	33.2

*p-value<0.05

Table 88 Pearson's correlations for nutritional markers (body composition, appetite and physical capability) at baseline

Correlations		Men				Women			
		AS [pts]	GE [sec]	GS [N]	6MWT [m]	AS [pts]	GE [sec]	GS [N]	6MWT [m]
BMI [kg/m^2]	R	0.183	0.080	-0.109	-0.071	0.184	-0.074	0.071	-0.262*
	P	0.138	0.527	0.404	0.563	0.167	0.601	0.626	0.049
AC [cm]	R	0.191	0.190	0.117	0.024	0.273*	0.043	0.196	-0.175
	P	0.122	0.130	0.371	0.843	0.040	0.762	0.177	0.194
MUAMC [cm]	R	0.080	0.193	0.125	0.018	0.260	0.052	0.136	-0.015
	P	0.517	0.124	0.337	0.885	0.055	0.721	0.356	0.911
MUAMA [cm]	R	0.073	0.189	0.136	0.023	0.241	0.061	0.146	-0.020
	P	0.555	0.131	0.296	0.853	0.076	0.673	0.322	0.883
TSF [cm]	R	0.163	0.035	0.006	0.013	0.116	0.146	0.284	0.055
	P	0.186	0.783	0.963	0.919	0.399	0.312	0.050	0.688
WC [cm]	R	0.162	0.106	-0.105	-0.123	0.182	-0.038	0.050	-0.332*
	P	0.197	0.410	0.431	0.325	0.175	0.791	0.731	0.012
FM [kg]	R	0.169	0.105	-0.143	-0.176	0.222	-0.030	0.003	-0.272*
	P	0.174	0.408	0.276	0.153	0.104	0.836	0.985	0.044
FM [%]	R	0.068	0.034	-0.395**	-0.347**	0.243	-0.054	-0.290*	-0.310*
	P	0.589	0.790	0.002	0.004	0.073	0.707	0.046	0.021
FMi [kg/m^2]	R	0.129	0.059	-0.250	-0.223	0.205	-0.082	-0.100	-0.313*
	P	0.300	0.646	0.054	0.069	0.133	0.569	0.498	0.020
FFM [kg]	R	0.216	0.142	0.280*	0.151	0.170	0.044	0.362*	-0.015
	P	0.081	0.264	0.030	0.222	0.210	0.758	0.011	0.914
FFMi [kg/m^2]	R	0.191	0.083	0.072	0.081	0.175	-0.060	0.254	-0.073
	P	0.125	0.513	0.586	0.515	0.198	0.676	0.079	0.591
FFM [%]	R	-0.068	-0.031	0.413**	0.315**	-0.172	0.045	0.160	0.347**
	P	0.586	0.808	0.001	0.009	0.206	0.756	0.272	0.009
Imp 50 [kHz]	R	-0.155	-0.057	-0.103	-0.227	-0.160	0.046	-0.229	-0.036
	P	0.214	0.655	0.433	0.064	0.239	0.749	0.114	0.792

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 89 Median time to first exacerbation for patients in different fat-free mass index tertiles

FFMi	Median TTFE [days]	95% Confidence Interval [days]	Log-rank	
T1 lowest	56	29 - 84	P=0.041	
T2 middle	77	14 -140		
T3 highest	27	16 - 38		P=0.012
T1 + T2	72	44 - 100		
T1 – the lowest tertile, T2 – the middle tertile, T3 – the highest tertile, T1+T2 – combined lowest and middle tertile				

Table 90 Median time to first exacerbation for patients' within tertiles of body impedance

Imp50	Median TTFE [days]	95% Confidence Interval [days]	Log-rank	
T1 lowest	35	22 - 47	P=0.039	
T2 middle	56	12-101		
T3 highest	76	12 – 140		P=0.019
T2 + T3	70	50 - 91		
T1 – the lowest tertile, T2 – the middle tertile, T3 – the highest tertile, T2+T3 – combined middle and the highest tertile				

Appendix L Physical capability – additional results – AERIS study

Table 91 Criteria for tertiles of physical capability test

	Grip strength	Grip endurance
Tertile 1	M≤251.87N W≤147.63N	M≤34.77s W≤30.72s
Tertile 3	M≥323.17N W≥210.33N	M≥58.80s W≥52.89s

Table 92 Hazard ratio for time to first exacerbation using various criteria for different physical capability tests

	Model 1		
	HR	95% CI	P-value
GS [N]	1.001	0.998-1.003	0.534
GS by 26kg(m) 16kg(W)	0.905	0.586-1.398	0.654
GE [sec]	0.997	0.991-1.003	0.299
GE T1 vs T3	1.315	0.807-2.145	0.272
6MWT [m]	0.998	0.996-1.000	0.029
6MWTs vs 6MWTui	1.896	1.279-2.812	0.001
6MWT by 350m	1.947	1.282 – 2.956	0.002

Results have shown that capability tests were not closely correlated to one another. The only 6MWT related to grip strength, but it was a weak relationship (Table 93).

Table 93 Pearson's correlations between physical capability tests

	n	Grip endurance	P-value	n	6minute walk test	P-value
Grip strength all [N]	104	X	NS	109	0.274	0.004
Grip strength men [N]	59	X	NS	61	0.238	0.064
Grip strength women [N]	45	X	NS	48	0.327	0.023
Grip endurance [s]				116	X	NS

To explore the relationship between each pair of capability tests, results were compared between low and high groups of another test. The 6MWT was significantly lower in those with low grip strength when compared to those with normal grip strength (Table 94).

Table 94 Difference in 6-minute walk test (6MWT) and grip endurance (GE) tests between patients with low (n=39) and normal (n=71) grip strength (GS)

All			Women		Men	
	low GS vs. normal GS[z]	p	low GS vs. normal GS[z]	p	low GS vs. normal GS[z]	p
6MWT [s]	268.6±92.5 vs 314.2±117.2	0.028	256.9±74.3 vs 298.3±94.3	NS	277.2±104.6 vs 327.2±133.0	NS
GE [s]	52.2 ± 66.1vs 48.6±24.3	NS	52.9±61.1 vs 48.0±30.1	NS	51.6±71.2 vs 49.1±19.1	NS

[z]Low GS≤26kg for men and 16kg for women, p- p-value for t-test

Grip strength was significantly different between men and women, therefore this analysis was repeated for each sex individually, but there was no difference in physical capability results between low and normal grip strength groups. 6MWT was also significantly lower in those with low grip endurance compared with high endurance (Table 95). However, neither grip strength nor grip endurance showed a difference between those who stopped during 6MWT when compared to those who did not stop (Table 96).

Table 95 Difference in 6-minute walk test (6MWT) and grip strength (GS) tests between patients with low and high grip endurance (GE)

	low GE vs. high GE [z]	p-value
6MWT [s]	273.5±108.9 vs 329.7±112.2	0.030
GS [N]	217.7±70.9 vs 256.4±86.7	0.045

[z]Low GE=lowest tertile, high GE=highest tertile, x – no significant correlation, NS – not significant

Table 96 Difference in grip endurance (GE) and grip strength (GS) between patients who stopped during 6-minute walk test (6MWT) compared to those who did not stop

	stopped vs. uninterrupted 6MWT	p-value
GE [s]	45.3±48.6 vs 52.0±36.5	NS
GS [N]	234.4±80.0 vs 250.0±93.5	NS

To understand the relationship between all three physical capability tests results of different physical capability tests were mapped together. A Venn diagram was used to explore a number of individuals with poor grip strength, poor grip endurance and poor walking test. Number of individuals with low GS (GS<26kg for men and GS<16kg for women, n=39), low GE (people with the lowest endurance, T1, n=39), and individuals who stopped during the 6MWT at baseline (n=58, Figure 61a) or those who had 6MWT<350m (n=80, Figure 61b) were presented. There was a similar number of individuals who had two or three poor results at baseline, while the highest number of people presented poor 6MWT results (both criteria) independent of low GE or low GS.

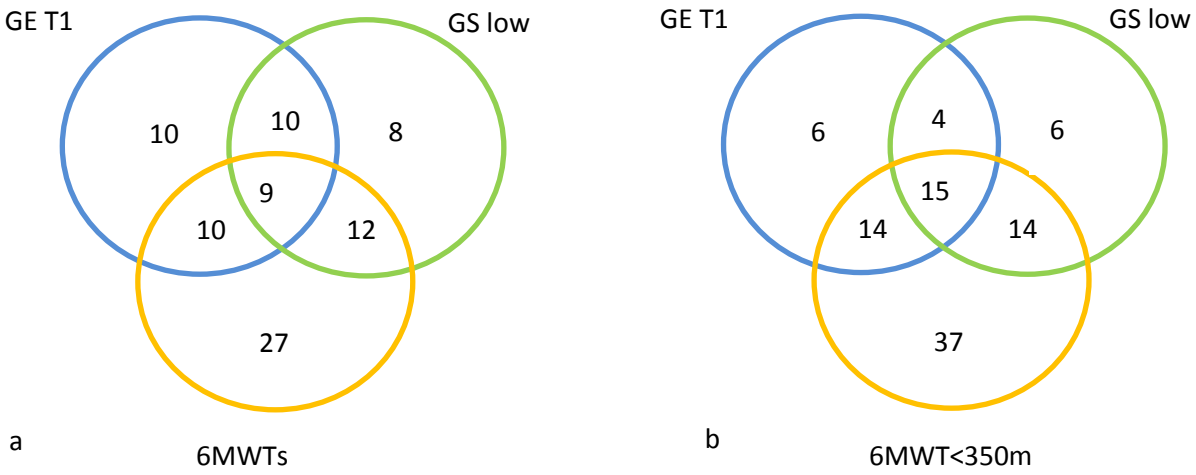

Figure 61 Venn diagram (number of patients) with low grip strength GS (M<26kg, W<16kg), low GE (lowest tertile), and those who stopped during 6-minute walk test (6MWT) at baseline (a) or those with 6MWT<350m (b)

In summary, having poor result in one test was not an indicator of a poor result in any other physical capability test.

Appendix M Publications related to the thesis

1) 10th International Symposium on body composition, 11-14 June 2014, Cascais Portugal

Consistency of lean mass depletion assessment methods in COPD studies

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Chronic obstructive pulmonary disease (COPD), a major cause of mortality, is associated with weight loss and change in body composition, especially lean mass loss. Recent studies show a protective effect of higher lean mass against exacerbations but identifying those most at risk of lean depletion remains a challenge, both in terms of the choice of measure of fat-free mass or muscle mass and the criteria used to define lean depletion. The aim of this review was to examine fat-free mass index (FFMi) cut-off values used in studies of COPD patients.

A systematic review (years 1993-2013) was performed to explore the basis of methods and cut-offs used to identify a lean deficit and clinical outcomes in COPD. Of 24 relevant articles 19 assessed body composition using bioelectrical impedance analysis (BIA) but only 14 reported FFMi using cut-offs derived from five primary sources.

Five different lean deficit cut-offs for men and two for women were identified based on population distributions observed in healthy or COPD subjects. Four different BIA devices, each with their own proprietary algorithms to derive FFM, were used and one study used DXA. Different processes were used to define low FFMi, including less than the 10th centile, FFMi values associated with a BMI of 18.5 kg/m^2, or the assumption that lean mass should be >67% of ideal body weight in men and 63% for women. These different approaches led to lean deficit cut-offs that ranged from 16.0 to 17.8kg/m^2, in men and 14.6 to 15.0kg/m^2 in women. Only two studies presenting cut-offs for different age groups recognised that FFMi declined with age.

There is no consistency between COPD studies that use FFMi cut-offs to identify those at risk from a lean deficit or explore the relationship with clinical outcomes. Cut-offs appear device and context specific, and miscategorisation could occur if applied universally. There is a need to better define the most appropriate criteria to identify those at risk of a lean deficit in clinical practice using a standardised approach that recognises differences in age, sex, and race.

2) Nutrition Society Winter meeting, 9-10 December 2014, London, UK

Variability of lean mass depletion in chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide. Skeletal muscle wasting is a clinically relevant systemic manifestation of COPD that predicts morbidity and mortality independently from the severity of lung function [341]. Loss of skeletal muscle mass is observed in about 20-40% of COPD patients [275] and this is associated with a 50% reduction in median survival [66]. Given the importance of recognising lean mass depletion in this population, it is key that clinicians have accurate and reproducible measures to identify skeletal muscle wasting. An index of fat-free mass (FFMi) is used to estimate the lean mass and can be measured by several techniques including bioelectrical impedance analysis (BIA). In the literature, there are multiple proposed cut-offs of FFMi to identify lean mass depletion. Our aim was to study the variability of FFMi in COPD patients and investigate the effect of applying the different proposed cut-off values for lean mass depletion.

We systematically searched the Web of Science database using broad search terms to identify studies from 1990 to 2014 with data on FFMi in COPD patients. The time period was chosen as the concept of FFMi was first described in 1990 [255]. From initial 94 articles identified, 14 studies provided data on mean and standard deviation (SD) values for FFMi in men COPD patients and of these, 11 also provided results for women. Using the mean and SD values for FFMi, a cumulative normal distribution was produced with Excel software in order to demonstrate the implications of using different cut-offs identified in the systematic review on the percentage of patients categorised as lean deplete.

12 of the 14 studies identified in the review used BIA as their method for determining fat-free mass. The remainder used DXA and skinfold thickness respectively. The mean FFMi for men COPD patients ranges from 16.8 kg/m^2 to 19.8 kg/m^2. Three different lean deficit cut-offs for men were identified in the review, 16 kg/m^2, 17 kg/m^2 and 17.4 kg/m^2. Similar variability was found in the women results, with the mean FFMi varying from 14.7 kg/m^2 to 18.2 kg/m^2. There were also 3 different lean deficit cut-offs (14 kg/m^2, 14.6 kg/m^2 and 15 kg/m^2). Further analysis showed when using an FFMi cut-off of 16 kg/m^2, the percentage of men COPD patients identified as lean deplete

varied from 4.6% to 36.9%. This increased from 19.6% to 65.5% when an FFMi cut-off of 17.4 kg/m^2 is used. A similar spread of data was seen in the women results.

This review and analysis highlight the complexity of diagnosing lean depletion accurately in the COPD population. The combined effect of the variability in FFMi across different COPD populations together with different cut-offs makes it extremely difficult to know with certainty what the true prevalence of lean depletion is. Further work needs to be done to establish an accurate method of diagnosing lean depletion in COPD patients, taking into account the patient population, sex and age.

3) Nutrition Society Winter meeting, 9-10 December 2014, London, UK

Do lean markers relate to exacerbation rate in chronic obstructive pulmonary disease? Preliminary results from AERIS study

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Previous studies suggested body composition might be related to COPD progression(1) however no definitive studies link lean markers (muscle mass or function) to exacerbation frequency(2). We aimed to investigate this relationship.

An initial cohort of 36 stable COPD subjects (GOLD 2-4) from the AERIS(3) study was analysed. Fat-free mass (FFM) was assessed by bioelectrical impedance and function with grip strength (GS), grip endurance (GE) and six-minute walk test (6MWT). FFM, the impedance at 50kHz (Imp50) and GS were adjusted for height and presented as indexes (i). The frequency of exacerbations was prospectively recorded for 12 months and adjusted for length of follow-up. A rate of >0.17 exacerbation/month (i.e. ≥2 exacerbations/year) indicated frequent exacerbators. Schols lean depletion cut-offs (FFMi <16 men, <15 women kg/m^2) were applied.

75% (27) subjects were defined as frequent exacerbators: 53% moderate, 88% severe and 100% very severe COPD subjects. COPD severity and exacerbation rates were similar between sexes (0.3 exacerbations/month). Women had significantly lower weight, FFM, FFMi, GS, and GSi but higher Imp50 and Imp50i than men (all $p<0.05$). In women alone, FEV_1% was significantly related to FFMi ($r=0.52$) and to 6MWT ($r=0.61$). FFMi, GS, GSi and GE were significantly related to 6MWT ($r=0.49$; 0.81; 0.72; 0.49 respectively). There were no statistically significant differences for FFM, FFMi, GE, GS, GSi, Imp50, Imp50i, or 6MWT between the frequent and infrequent exacerbator groups for either men or women. For men, there could be a trend towards frequent exacerbators for low 6MWT and high IMP50 values. Using Schols criteria 36% of subjects (2 men, 11 women) were lean deplete, and the exacerbation rates were similar in the 2 subgroups.

Our preliminary results do not provide evidence that FFMi, Imp50, GS or 6MWT are associated with a higher frequency of exacerbation, however, Imp50 and 6MWT in men may have predictive value. Lack of statistical significance may be due to the small sample size and a high proportion of

frequent exacerbators. Further analysis of the full AERIS cohort may yield more information on the predictive value of lean markers on exacerbation frequency and treatment response.

4) ERS International Congress, 26-30 September 2015, Amsterdam, Netherlands

Preliminary results of relationship between appetite and clinical presentation of COPD patients in AERIS study

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Patients with COPD experience weight loss with disease progression, which can partially be attributed to the loss of appetite most obviously around exacerbations. There is a need to better understand the role that differences in appetite may play in the resilience to infection. We sought to determine whether patients with poor appetite have subsequently more exacerbations and shorter time to first exacerbation (TTFE).

Appetite was measured with Council on Nutrition appetite questionnaire (CNAQ, 8 questions) in 127 moderate to severe stable COPD patients from AERIS study (NCT 01360398) at enrolment. Differences in exacerbation history over the previous 12 months, exacerbation rate over the 12 months follow-up, TTFE and TLCO, FEV1, FEV1/FVC were compared between those with/without a poor appetite (CNAQ score < 70%).

Those with a poor appetite by CNAQ score had a higher exacerbation rate (+29%**), shorter TTFE (median 40 days IQR 115 vs 77 days IQR 252; p=0.018) and lower FEV1(-18%*), FEV1/FVC (-16%*) and TLCO (-13%*) than those with a better appetite [*p<0.01, **p<0.05]. Higher exacerbation rate was still evident in those with a poor appetite after adjusting for the difference in the history of exacerbation. There was no difference in BMI between poor and better appetite groups, and TTFE was comparable across different BMI groups.

Identifying poor appetite using simple tools such as CNAQ during routine consultations of COPD patients should be considered as an additional risk predictor of COPD exacerbations.

5) 12th FENS European Nutrition Conference, Berlin 20-23 October, 2015

Prevalence of lean depletion in COPD using different impedance equations to predict body composition

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Bioelectrical impedance analysis (BIA) is a simple method to assess body composition, especially fat-free mass (FFM). It is commonly used in Chronic Obstructive Pulmonary Disease (COPD) studies to identify patients with lean depletion. It remains unclear if lean depletion relates to the worse clinical outcome, although this might be due to the variety of equations and cut-offs used.

We sought to investigate how using different FFM equations would identify different proportions of COPD patients as lean depleted, when using constant FFM index (FFMi) cut-offs for each sex. The scale of the effect was assessed on populations and at the individual level.

Body composition was measured in 124/127 moderate to severe stable COPD patients from AERIS study (NCT01360398) at enrolment using Bodystat QuadScan4000. Height, weight and raw BIA results (resistance, reactance) were used to calculate FFM using 7 different validated equations. The Schols FFMi cut-off (<15kg/m2 women, <16kg/m2 men) was applied to define lean depletion.

FFM calculated by different equations was consistently lower in women than in men, mean and associated SD ranging from 41.0±5.8kg to 45.4±8.3kg (FFMi 15.8±1.8kg/m2 to 17.5±2.7kg/m2) in women and 53.5±5.7kg to 59.6±10.1kg (FFMi17.7±1.4kg/m2 to 19.6±3.6kg/m2) in men.

The difference between the means was greater than 2kg in women in 10/21 pairwise comparisons of the FFM from each equation. This was also observed in 14/21 pairwise comparisons in men. The width of widest limits of agreement (mean difference +/-1.96SD) was 10.6kg and 19.5kg for women and men respectively. Prevalence of lean depletion ranged between 15.2%-22.4% (23%-39% women, 4%-18% men) depending on the equation used.

Different equations result in different proportions of patients identified as lean depleted leading to a risk of individuals being misclassified. There is a need to standardise approaches across

studies and clinical practice to allow guidance of nutritional care in response to true lean depletion.

Appendix N AERIS study protocol - publication

BMJ Open

Acute Exacerbation and Respiratory InfectionS in COPD (AERIS): protocol for a prospective, observational cohort study

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ABSTRACT

Introduction: The aetiology of acute exacerbations of chronic obstructive pulmonary disease (COPD) remains incompletely understood and strategies for treatment and prevention have not altered significantly for many years. Improved understanding of the role of respiratory pathogens in acute exacerbations of COPD (AECOPD) is required and the use of molecular microbiological techniques may lead to insights into host–pathogen interactions and the development of more targeted therapeutic approaches.

Methods and analyses: Acute Exacerbation and Respiratory InfectionS in COPD (AERIS) is a longitudinal epidemiological study to assess how changes in the COPD airway microbiome contribute to the incidence and severity of AECOPD. Patients with COPD aged 40–85 are followed monthly for 2 years, and reviewed within 72 h of onset of symptoms of AECOPD. Exacerbations are detected using daily electronic diary cards. Blood, sputum, nasopharyngeal and urine samples are collected at prespecified timepoints. Molecular diagnostic and typing techniques are used to describe the dynamics of airway infection during AECOPD and stable disease, and associations with clinical outcome. This study aims to refine the case definition of AECOPD to reflect the possible microbiological aetiology. AERIS will assess the impact of AECOPD on health-related quality of life and healthcare resource utilisation, and the possible interactions between nutritional status, infection and immune responses.

Ethics and dissemination: AERIS is conducted in accordance with the Declaration of Helsinki and Good Clinical Practice, and has been approved by the institutional ethics and review board. All participants must provide written informed consent. The results obtained will be disseminated at international medical conferences and in peer-reviewed publications.

Discussion: Few other studies have addressed the complexity of the microbiological and systemic components of COPD or employed real-time electronic tracking of symptoms to identify AECOPD and potential aetiological triggers.

Results: Results of AERIS will increase our understanding of the contribution of pathogens to AECOPD, potentially leading to new targeted therapeutic and preventative interventions.

Trial registration number: ClinicalTrials.gov NCT01360398.

Strengths and limitations of this study

- Conducted in a specialised hospital that has extensive experience in respiratory research.
- Comprehensive assessment of clinical status, microbiology, functional status, nutritional status, health-related quality of life and healthcare resource utilisation in individual patients in a single large cohort during stable chronic obstructive pulmonary disease (COPD) and acute exacerbations of COPD (AECOPD).
- AECOPD are proactively identified through patient-completed electronic diaries.
- Cohort retention is a key factor in the successful delivery of such a study and with in-depth sampling protocols, participant engagement, comfort and feedback are key factors in optimising cohort retention and comprehensive data collection.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is an inflammatory disease of the lung, characterised by progressive airflow limitation that is not fully reversible.[1] COPD is the most common chronic respiratory illness in older adults, affecting an estimated 210 million people worldwide.[2] This condition has a substantial impact on quality of life.[2] The Global Burden of Disease Study found COPD to be the third leading cause of death globally and the ninth leading cause of years of life lost due to premature mortality in 2010,[3] accounting for 3.7% of years lived with disability and 3.1% of disability-adjusted

life years worldwide.[4 5] COPD also imposes a substantial socioeconomic burden. In 2001, the total cost of COPD in Europe was reported to be €38.7 billion.[6]

Considerable progress has been made concerning the epidemiology, pathophysiology and clinical management of COPD in recent years. However, significant challenges remain. Improved understanding of acute exacerbations of COPD (AECOPD) is a key research priority. AECOPD are highly relevant clinically, being a major cause of COPD-related morbidity and mortality,[7–11] as well as accounting for a substantial proportion of the significant social, healthcare and economic burden of COPD.[6] It has been estimated that AECOPD account for approximately 70% of total healthcare costs associated with COPD.[12] Patients with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage II disease or greater experience one or two exacerbations annually. Exacerbation varies from patient to patient with severity of disease.[13] Various triggers for AECOPD have been identified[1]; however, up to 75% of all exacerbations are associated with the detection of bacterial and/or viral respiratory pathogens.[14 15] Exacerbations associated with detectable respiratory pathogens have been shown to have a more marked impact on lung function and longer duration of hospitalisation than exacerbations of non-infectious aetiology.[14]

With the introduction of new molecular sequencing techniques, the traditional belief that healthy lungs are sterile has been refuted. There is increasing evidence that the lower respiratory tract contains a diverse microbial flora that differs between health and disease.[16–20] The presence of potentially pathogenic microorganisms in the inflamed airways of patients with COPD is well documented, with up to 50% of patients with stable COPD showing evidence of lower airway bacterial colonisation using traditional culture techniques.[15 21 22] In patients with COPD, bacterial detection in lower airway derived samples is associated with increased airway inflammation, reduced lung function and more frequent exacerbations.[23–25] Acquisition of new pathogen strains also appears to be associated with an increased risk of AECOPD.[15 21 26] Estimates of the relative contribution of different pathogens to AECOPD vary. However, non-typeable *Haemophilus influenzae* appears to be the major bacterial pathogen associated with AECOPD, followed by *Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Pseudomonas aeruginosa*.[14 15] Respiratory viruses commonly associated with AECOPD are diverse and include human rhinoviruses, influenza and parainfluenza viruses, respiratory syncytial virus, coronavirus and adenovirus.[15]

Improved understanding of the role of infectious pathogens in AECOPD is required to better understand the pathophysiology of the disease and may lead to the development of more targeted strategies for treatment and prevention. This article describes the objectives and design of Acute Exacerbation and Respiratory InfectionS in COPD (AERIS), a prospective longitudinal epidemiological study initiated in the UK to assess the role of respiratory infection in AECOPD. Molecular diagnostic and typing techniques will be used to describe the dynamics of airway infection and its potential association with clinical outcome. The study will also assess the impact of AECOPD on health-related quality of life and healthcare resource utilisation, as well as the possible interaction between disease endotype and exacerbations.

OBJECTIVES

The primary objective of the AERIS study is to estimate the incidence of all-cause AECOPD and of AECOPD with sputum containing bacterial pathogens (overall and by species). Secondary study objectives are summarised in table 1.

METHODS

Study design

This is an ongoing, single-centre, prospective, observational cohort study based at University Hospital Southampton, UK.

Table 1 Overview of primary and secondary objectives of the AERIS study

Level	Objective
Primary	▸ To estimate the incidence rate of all-cause AECOPD
	▸ To estimate the incidence rate of AECOPD having sputum containing bacterial pathogens (overall and by species)
Secondary	▸ To describe the proportion of overall and specific bacterial pathogens detected in sputum by severity of AECOPD
	▸ To describe the proportion of overall and specific bacterial pathogens detected in sputum in stable COPD
	▸ To estimate the incidence rate of AECOPD having sputum containing viral pathogens (overall and by species)
	▸ To describe the proportion of overall and specific viral pathogens detected in sputum by severity of AECOPD
	▸ To estimate the time elapsed between consecutive AECOPD episodes
	▸ To assess the impact of all-cause AECOPD and stable COPD on health-related quality of life
	▸ To assess the impact on healthcare use: – Of all-cause AECOPD – Of AECOPD having sputum containing bacterial pathogens – Of AECOPD having sputum containing viral pathogens

AECOPD, acute exacerbations of chronic obstructive pulmonary disease; AERIS, Acute Exacerbation and Respiratory InfectionS in COPD.

Table 2 Study inclusion and exclusion criteria

Inclusion criteria	*Participants must satisfy ALL of the following criteria at study entry:* ▸ Participants who the investigator believes can and will comply with the requirements of the protocol ▸ Written informed consent obtained from the participant ▸ Male or female aged 40–85 years ▸ Confirmed diagnosis of COPD based on postbronchodilator spirometry[27] with FEV_1 ≤80% of predicted normal and FEV_1/FVC <0.7 ▸ Moderate, severe or very severe COPD, according to GOLD staging[27] ▸ History of ≥10 pack-years of cigarette smoking*† ▸ Documented history of ≥1 exacerbation requiring antibiotics and/or oral corticosteroids or hospitalisation in the previous 12 months‡
Exclusion criteria	▸ A confirmed diagnosis of asthma (as only cause of obstructive respiratory disorder), cystic fibrosis, pneumonia risk factors or other respiratory disorders (eg, tuberculosis, lung cancer, etc) ▸ History of lung surgery ▸ α-1 antitrypsin deficiency as underlying cause of COPD ▸ Moderate or severe COPD exacerbation not resolved at least 1 month prior to enrolment and less than 30 days following the last dose of oral corticosteroids§ ▸ Long-term corticosteroid or antibiotic therapy ▸ Use of any antibacterial, antiviral or respiratory investigational drug or vaccine within 30 days of the enrolment visit ▸ Evidence of alcohol or drug abuse ▸ Presence of other conditions that the principal investigator judges may interfere with the study findings ▸ Risk of non-compliance or inability to comply with the study procedures ▸ Women who are pregnant or lactating or are planning on becoming pregnant during the study

*Former smokers are defined as those who have stopped smoking for at least 6 months.
†Number of pack years=(number of cigarettes per day/20)×number of years smoked.
‡Participants with recent COPD exacerbations, in stable condition, and having stopped antibiotics, can be enrolled 1 month postexacerbation.
§Participants can be enrolled when their AECOPD or pneumonia has resolved.
AECOPD, acute exacerbations of chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease.

Study population

Male and female patients with COPD between the age of 40 and 85 years are eligible for study participation provided they meet the following inclusion criteria: (1) a confirmed diagnosis of COPD with postbronchodilator forced expiratory volume in 1 s (FEV_1) ≤80% of the predicted normal value and FEV_1/forced vital capacity (FVC) ≤0.7, consistent with GOLD stage II–IV disease[27]; (2) current or ex-smoker with smoking history ≥10 pack-years and (3) one or more documented exacerbations of COPD treated with antibiotics and/or steroids in the 12 months prior to enrolment (table 2). Participants with recent COPD exacerbations, in stable condition and having stopped antibiotics, can be enrolled 1-month postexacerbation. Exclusion criteria include other known respiratory conditions, such as asthma, as the only cause of the respiratory obstructive disorder, α-1 antitrypsin deficiency, cystic fibrosis, tuberculosis, lung cancer, history of lung surgery and other conditions imposing pneumonia risk. Participants on long-term corticosteroid or antibiotic therapy at the time of enrolment and those who received antibiotics and/or steroids in the month prior to the enrolment were also excluded.

Clinical data collection

Participants are seen for an enrolment visit and then monthly for 2 years. Regular review of medications and, when required, changes to medical therapy and active smoking cessation advice are performed according to standard clinical practice at each visit. In addition to these scheduled visits, all participants are seen in the clinic within 72 h (3 days) of onset of symptoms of AECOPD. AECOPD is defined as worsening of at least two major symptoms (dyspnoea, sputum volume and sputum purulence) or worsening of at least one major symptom and one minor symptom (wheeze, sore throat, cold (nasal discharge and/or nasal congestion), cough and fever (oral temperature >37.5°C) without other cause),[28] considered clinically relevant at the site. Exacerbations are identified by means of electronic diary cards that participants complete daily. The data recorded daily in the electronic diary cards include self-performed peak flow measurement (peak expiratory flow (PEF) and FEV_1), a series of morning questions to identify symptoms of exacerbations[29] and the EXAcerbations of Chronic Pulmonary Disease Tool V.1.0 (EXACT-PRO) at bedtime. Participants are also asked to record any changes to their usual treatment. Data on patient-reported symptoms based on morning questions and on PEF/FEV_1 are transmitted daily to the study clinic. Changes/worsening in these symptoms are monitored by the study staff and participants are contacted and invited to the clinic when an exacerbation is suspected.

Study procedures

In addition to the daily monitoring undertaken through the patient-completed electronic diary cards, a wide

Table 3 Overview of study assessments performed at the scheduled monthly visits and at exacerbation visits

Description	Frequency of assessment*
Clinical variables	
Physical examination	Monthly and within 72 h of onset of exacerbation
Anthropometrics and nutritional screening (MUST)†	Quarterly
Intercurrent comorbidities	Monthly and within 72 h of onset of exacerbation
Medical history/medical record review	Study entry and within 72 h of onset of exacerbation
Vaccination history	Annually
Current medication	Monthly
Smoking status	Monthly
Urine pregnancy test	Study entry, final visit and within 72 h of onset of exacerbation
Chest CT scan	Study entry and final visit
Chest X-ray	Within 72 h of onset of exacerbation
Lung function testing	
Body box	Study entry and final visit
TLCO‡	Every 6 months and within 72 h of onset of exacerbation
Spirometry	Monthly and within 72 h of onset of exacerbation
6 min walk test	Every 6 months
Questionnaires and patient-reported outcome instruments	
ATS-DLD-78A (risk factors, disease history and smoking history)	Study entry
Healthcare use§	Monthly and within 72 h of onset of exacerbation
mMRC¶	Every 6 months
CAT questionnaire**	Quarterly and within 72 h of onset of exacerbation
EQ-5D index and VAS††	Quarterly and within 72 h of onset of exacerbation
NEADL‡‡	Quarterly and within 72 h of onset of exacerbation
CNAQ§§	Quarterly and within 72 h of onset of exacerbation
Biological specimen collection	
Blood sampling	
For routine biochemistry	Study entry
For cell-mediated immune response	Quarterly and within 72 h of onset of exacerbation
For biomarkers, blood counts and haematology	Quarterly and within 72 h of onset of exacerbation
For RNA transcript profiling	Every 6 months and within 72 h of onset of exacerbation
For vitamins, antioxidants and nutrients (20 mL)	Every 6 months and within 72 h of onset of exacerbation
Nasopharyngeal swab sampling¶¶	Monthly and within 72 h of onset of exacerbation
Sputum sampling	Monthly and within 72 h of onset of exacerbation
Breath sampling***	Monthly and within 72 h of onset of exacerbation
Urine sampling†††	Monthly and within 72 h of onset of exacerbation

*In addition to study entry.
†Height, weight, mid-arm circumference, waist circumference, triceps skin-fold measurement, fat-free body mass.
‡TLCO: transfer factor.
§Healthcare use includes medication, vaccination, oxygen therapy, use of mechanical ventilation, pulmonary rehabilitation treatment, surgical intervention, outpatient visits (including GP visit contacts to COPD team), emergency room visits, hospitalisations and productivity loss (time missed from work or usual activities due to worsening of COPD symptoms).
¶mMRC: Medical Research Council Dyspnea Scale score.
**CAT: COPD Assessment Test.
††VAS: visual analogue scale.
‡‡NEADL: Nottingham Extended Activities of Daily Living Scale.
§§CNAQ: Council on Nutrition Appetite Questionnaire.
¶¶In all participants at study entry and in a subcohort of 30 participants during the first year.
***In a subcohort of approximately 80 participants.
†††In all participants at study entry and within 72 h of every exacerbation and in the subcohort of 30 participants providing nasopharyngeal swabs during the first year of the study.
COPD, chronic obstructive pulmonary disease; GP, general practitioner; MUST, Malnutrition Universal Screening Tool.

range of study procedures are performed at study entry, scheduled monthly visits and exacerbation visits (table 3).

Clinical assessments

Quantitative high-resolution CT scans are performed at enrolment and study conclusion to describe the degree of bronchiectasis and emphysema noted and to exclude other acute or evolving lung pathologies besides COPD and sequelae of COPD. A physical examination is performed at all visits. Medical history, smoking status and details of medication use are updated monthly. Influenza and pneumococcal vaccination status is updated annually.

Lung function testing is performed using spirometry, body plethysmography (lung volumes, body box) and single breath diffusion (gas transfer, transfer factor

(TLCO)) at specified visits. The following outcomes are recorded: spirometry, FEV_1, FVC, FEV_1/FVC ratio, FEV_1% predicted, mid-expiratory flow between 25% and 75% of the FVC (MEF25-75), single breath diffusion (TLCO and rate of carbon monoxide uptake (KCO) and body plethysmography (total lung capacity (TLC), residual volume (RV), vital capacity and RV/TLC). At the enrolment visit, participants are asked to refrain from using short-acting bronchodilators for at least 6 h and long-acting bronchodilators for at least 12 h before key procedures. Prior to the subsequent follow-up visits, participants may use their usual medication normally. Lung function measurements are performed under controlled conditions and in the sitting position as per standard practice.

Anthropometrics (including but not restricted to height, weight, waist and mid-arm circumference and triceps skin-fold circumference) are measured quarterly. Grip strength and fatigability are measured using standard techniques. Anthropometric data are used to compute the Malnutrition Universal Screening Tool (MUST) score.[30] Nutritional information (including planned/unplanned weight loss and history and changes in food intake patterns) is collected quarterly according to MUST guidelines.

A posteroanterior chest X-ray (and lateral if required) is performed at all exacerbation visits, as per standard clinical practice, in order to rule out pneumonia.

Questionnaires and patient-reported outcome instruments

Various outcomes are assessed quarterly and at exacerbation using a series of questionnaires and patient-reported outcome instruments, such as the COPD Assessment Test (CAT),[31] the Nottingham Extended Activities of Daily Living (NEADL) Scale,[32] the Council on Nutrition Appetite Questionnaire (CNAQ)[33] and the EQ-5D.[34] The five items included in the EQ-5D index are mobility, self-care, usual activities, pain/discomfort and anxiety/depression. The BODE index (Body-Mass Index, Degree of Airflow Obstruction, Level of Functional Dyspnoea, Exercise Capacity)[35] will also be calculated.

Healthcare use is recorded at all visits, including medication, vaccination, oxygen therapy, use of mechanical ventilation, pulmonary rehabilitation treatment, surgical intervention, outpatient visits (including general practitioner visits and telephone contacts to COPD team), emergency room visits, hospitalisations and productivity loss (time missed from work or usual activities due to worsening of COPD symptoms). Potential changes in disease management following an exacerbation (eg, change in medication use) are also recorded.

Biological specimen collection

A wide range of biological specimens are collected from study participants (table 3). Blood samples are collected from all patients at study entry, quarterly and at exacerbation. Sputum samples are obtained by spontaneous expectoration or induced by stimulation according to standard methods from all patients at study entry, monthly and at exacerbation. Nasopharyngeal swabs are collected from all patients at study entry and then from a subcohort of 30 patients at monthly follow-up visits and at exacerbation during the first year of follow-up. Urine samples are collected from all patients at study entry and at exacerbation and from the subcohort of 30 patients at monthly follow-up visits during the first year. Breath samples are collected from approximately 80 patients (including the subcohort of 30 patients providing nasopharyngeal swabs) at monthly follow-up visits and at exacerbation visits during the first year.

Blood samples are analysed for disease-related biomarkers, biochemistry, cell-mediated immune response, RNA profile and nutrients. Sputum samples are processed by traditional culture techniques and multiplex PCR analysis for identification of potential respiratory pathogens (including, but not limited to, non-typeable *H influenzae*, *M catarrhalis*, *S pneumoniae*, *P aeruginosa*, *Staphylococcus aureus*, respiratory syncytial virus, parainfluenza virus, rhinovirus, human metapneumovirus, influenza virus, adenovirus and coronavirus). Sputum samples may also be analysed for disease-related biomarkers. Nasopharyngeal swabs are processed by traditional culture techniques and multiplex PCR analysis for potential pathogen identification. Urine samples may be processed for disease-related biomarkers. Breath samples are analysed by the selected ion flow tube mass spectrometry for identification of volatile organic compounds that may be characteristic for AECOPD.

Laboratory assays are performed at the Public Health Laboratory of Public Health England at University Hospital Southampton Foundation National Health Service (NHS) Trust, GlaxoSmithKline (GSK) Vaccines central laboratory and other GSK Vaccines designated laboratories. The assays use standardised and validated procedures. Aliquots of all biological samples are processed (if applicable), frozen and stored for possible further disease-related testing. Culture isolates are also stored. Any additional laboratory tests will be performed at a GSK designated laboratory.

Sample size calculation

The sample size calculation was based on the primary study endpoint of incidence of all-cause AECOPD. Assuming that, on average, each participant is observed for a period of 18 months and that two episodes of AECOPD can be expected per participant per year, if 120 participants are followed, the number of total person-years would be around 180 and during this time around 360 exacerbation events would be detected. If the distribution of events per participant follows a Poisson distribution with no overdispersion, an overdispersion factor of 1.5, or an overdispersion of 2, the approximate values of the lower and upper bounds of the 95% CI around the point estimate of two events per participant per year would be 1.8–2.2, 1.7–2.3 and 1.7–

2.3, respectively. So a sample size of 120 participants should ensure sufficient precision in the estimation of the incidence rate of all-cause AECOPDs.

Additionally, in order to follow 120 participants effectively, given the fact that participants may be eligible but withdraw quite early in the study possibly due to the deterioration of the participant's health, the decision was taken to replace participants who withdrew during the first year of follow-up, and recruit additional participants.

We will construct a CONSORT diagram and capture where possible reasons for screen failure, dropouts and loss to follow-up.

Data analysis

The primary study endpoints are the occurrence of all-cause AECOPD and the occurrence of AECOPD having sputum containing bacterial pathogens as detected by culture (overall and by species). The proportion of participants at each visit for whom a sputum sample was obtained will be computed; overall and by the method the samples were obtained (spontaneous or induced). The proportion of sputum samples obtained at each visit and positive for specific bacterial pathogens (overall and by bacterial species) will also be calculated. The incidence rate of all-cause AECOPD and of AECOPD having sputum containing bacterial pathogens (overall and by bacterial species) will be calculated, with 95% CI. The 95% CI of the incidence rate will be computed using a model which accounts for repeated events, namely the generalised linear model assuming a negative binomial distribution for the response variable with logarithm as link function, and the logarithm of time for follow-up as an offset variable as a preliminary approach. Other flexible approaches to statistical analysis may also be used. In addition, the same model with covariates (eg, smoking status at enrolment, number of moderate/severe exacerbations reported in the 12 months prior to enrolment, presence of respiratory pathogenic bacteria detected at the exacerbation visit and at previous visits) will be applied. Incidence rates will also be calculated for moderate AECOPD and for severe AECOPD.

DISSEMINATION

All participants must provide written informed consent to participate.

AERIS is being conducted in a specialised hospital that has extensive experience in respiratory research. AECOPD are proactively identified through patient-completed electronic diaries. After confirmation by phone, symptoms of an exacerbation trigger a clinic visit within 72 h of symptom onset to enable comparisons of samples from same patient in stable COPD and during AECOPD. Although this is an intensive study with prolonged follow-up, patients are expected to benefit from the improved access to expert care.

The results obtained will be disseminated by presentations at international medical conferences and peer-reviewed publications. Reporting will be in accordance with STROBE guidance.

DISCUSSION

The AERIS study has been initiated to comprehensively assess the role of infectious pathogens in AECOPD in a well-characterised cohort of patients. The study aims to explore the dynamics of airway infection and its possible contribution to AECOPD, as well as the potential role of chronic colonisation in stable disease. The overall objective of the study will aim at refining the case definition of AECOPD to reflect the possible microbiological aetiology of exacerbations. This is of note, since there is currently no commonly agreed definition of AECOPD and no current case definition includes a microbiological endpoint. The impact of AECOPD on health-related quality of life and healthcare use will be assessed in order to provide a complete picture of disease burden. The interaction between airway infection and systemic manifestations of COPD and nutritional status will also be assessed in detail for the first time. Biological specimens collected during the study may also be used for further disease-related testing, including molecular typing to describe and compare selected biomarkers in AECOPD and stable COPD, to explore cell-mediated immune response to specific bacterial antigens, and to develop non-invasive bacterial diagnostic methods.

To our knowledge, few other studies have employed real-time electronic tracking of symptoms to identify AECOPD and potential aetiological triggers. This is important since available data suggest that up to 50% of exacerbations may not be reported to healthcare providers and consequently exacerbation rates are lower in studies employing event-based criteria to define AECOPD.[36] Due to the close daily monitoring of symptoms to identify AECOPD, we anticipate that the exacerbation rate in this study will be higher than previously reported. This close monitoring and early therapeutic intervention at exacerbation may also impact on estimates of the overall burden of disease.

A number of other epidemiological studies have been initiated in recent years to further characterise our understanding of the natural history of AECOPD. However, it is important to recognise that most of these studies have not included molecular microbiological assessments. Recent large observational studies focusing on biomarker discovery have involved close phenotyping of patients with COPD, but have not studied the aetiology of exacerbations in depth.[37–39] In another study, potentially pathogenic bacterial strains were identified using molecular typing techniques, although viruses as potential airway pathogens were not investigated.[21] More recently, the prevalence and load of airway bacteria in stable and exacerbated AECOPD have been assessed in paired samples from 52 patients participating in the London COPD cohort study using modern

molecular techniques.[19] Airway bacterial prevalence and load was found to increase significantly during AECOPD, with quantitative molecular techniques proving more discriminatory than culture. However, assessment was limited to only the three most commonly detected airway bacteria (*H influenzae*, *S pneumoniae* and *M catarrhalis*). However, other potential pathogens and the overall respiratory microbiome may also contribute and have not yet been studied in detail.[17 18 20 40 41] In AERIS, samples will be acquired during AECOPD and stable disease and analysed for a wide range of potentially pathogenic bacteria and viruses using advanced PCR-based techniques as well as traditional culture-based methods.

A major strength of the AERIS study design is the comprehensive assessment of clinical status, microbiology, functional status, nutritional status, health-related quality of life and healthcare resource utilisation in individual patients in a single large cohort during stable COPD and AECOPD. The selection of participants with a history of at least a single exacerbation enriches the cohort to some degree and ensures an adequate number of exacerbations are sampled. It is accepted that some aspects of the analysis may not be generalisable to the subgroup of patients who never exacerbate. The analyses proposed in this study will generate epidemiological data to complement that derived from existing COPD cohorts and further explore determinants of COPD and the contribution of bacterial and viral pathogens to AECOPD, as well as to provide some understanding of the limitations of existing data. As exacerbation visits are triggered by patient diary data, accurate and timely diary completion is essential. All participants in this study receive diary training at enrolment and support is available from the study team at all times to promote accurate and complete diary keeping. Cohort retention is a key factor in the successful delivery of such a study and with in-depth sampling protocols, participant engagement, comfort and feedback are key factors in optimising cohort retention and comprehensive data collection.

Identification of novel approaches for the prevention of AECOPD is an important research goal. Long-acting β-agonists (LABA) and long-acting antimuscarinic bronchodilators remain the cornerstone of treatment for patients with COPD.[42] Combinations of LABA and inhaled corticosteroids are also used in patients with more severe disease and/or frequent exacerbations. Long-term treatment with macrolide antibiotics and pulsed quinolone therapy may be considered for exacerbation prevention.[7 43 44] However, concerns exist about the potential for development of antimicrobial resistance during long-term antibiotic therapy. Numerous other approaches are under investigation for the prevention of AECOPD, including anti-inflammatory drugs, immunomodulatory agents, immunotherapy, antioxidants and non-pharmacological strategies. Vaccination is another potential approach meriting investigation for reducing AECOPD risk. However, optimal strategies targeting key respiratory pathogens are not yet available to the clinician.

In conclusion, there have been considerable advances in our understanding of the epidemiology, pathophysiology and clinical management of COPD in recent years. However, there remains a genuine need to further explore the aetiology and pathogenesis of AECOPD. It is anticipated that results of this epidemiological study will increase our understanding of the contribution of bacterial and viral pathogens to AECOPD, the natural history of these events in association with the timing of symptoms and physiological changes, and will offer new direction for research into targeted therapeutic and preventative interventions.

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Contributors SB closely involved in all steps of the study and specifically wrote substantial parts of the protocol. CC was responsible for this observational study and was closely involved in the design of study. VK and AB closely involved in the conduct of this study. AT was involved in writing of microbiology parts of the protocol. EA was responsible for writing of statistical analysis plan and definition of statistical outcomes. SMV closely involved in the discussion on design and follow-up of the study. J-MD closely involved in all discussions. WRB closely involved in all discussions of design of this study. SC closely involved in all steps of the study. TW involved in all steps of the study. All authors provided intellectual input into the development of this manuscript, and have critically reviewed and approved the final version of the manuscript.

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Competing interests SB, VK, AB, AT, SC and TW received an institutional grant from GSK group of companies to conduct this study. SB reports

receiving grants, advisory board fees and assistance in attending conferences from GSK. He has also received fees for lecturing, advisory boards and teaching from Novartis, AstraZeneca and Boehringer Ingelheim. SC has also received project grant support, conference funding attendance and advisory board payments from Pfizer, GSK and Novartis (all payments were made to employing institution). TW has also received travel expenses, fees for advisory boards GSK related to this study, fees for advisory boards from Pfizer and AstraZeneca, reimbursement for travel and conference attendance from Boehringer Ingelheim, consultancy fees from Almirall, and financial support from Novartis and Retroscreen (travel expenses, consultancy and project support costs). CC, EA, SMV, J-MD and WRB are employed by GSK; CC, EA, J-MD and WRB also report GSK stock options.

Ethics approval The AERIS (Acute Exacerbation and Respiratory InfectionS in COPD) study is conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and has been approved by the relevant institutional ethics and review board and the Southampton Ethics Board.

Provenance and peer review Not commissioned; externally peer reviewed.

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Appendix O Data management plan

Data Management Plan

Storage and use of non-RDE data gathered in Epi-Hip 001

Name of project	**Epi-Hip 001**
Document characteristic	**Data management plan for non-RDE data collected within the Epi-Hip 001 and used to create subproject databases.**
Date of creation	**15/05/2013**
Date of modification	**18/12/2013;**
Version	**2.0**
Authors	**Lindsay Welch, Malwina Wojtas**

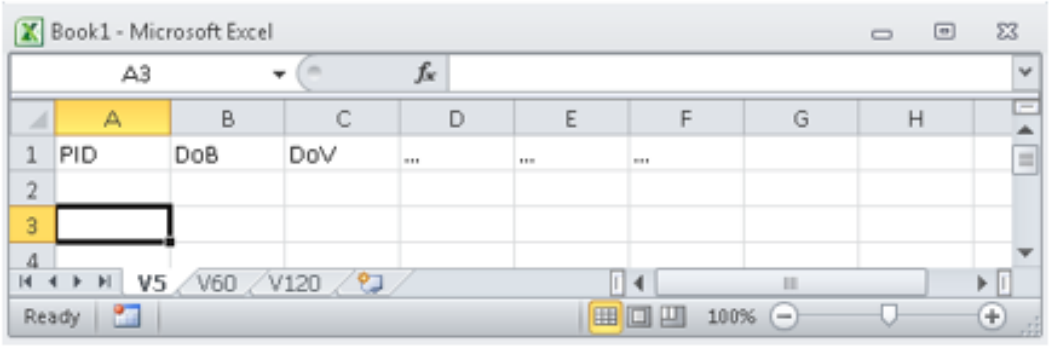
The data management plan has been created to provide a standardised understanding of the non-RDE nutrition databases, their content and collection, as well as to avoid unnecessary duplication e.g. re-collecting or re-working of data. This document is targeted at all PhD and MD students who are working on the Epi-Hip study and use the non-RDE nutrition data for their projects present and in the future or any other researchers using EPI-HIP 001 non-RDE data.

Information about data & data format

The non-RDE nutrition database consists only of "raw data" which means no calculated or estimated values are included, only values from Case Report Form (CRF) or other source document or test. Datasets with calculated values should be kept as a separate file in another folder and differentiated by name.

The data will be stored in a form of Microsoft Excel sheets in as much standardised form as possible. It is acknowledged, that different results require different organisation of database, however each dataset should be organised in the most similar way. The same data may be presented in different order for statistical purposes, but such files should not be kept with the original dataset. A separate folder named "Reorganised Dataset" should be created and files stored there should be named accordingly to the content "Reorganised_data_*NameOfDataset*", and must not be kept as an original copy of such dataset.

Data should be organised in a way that relates to Sponsor's format and coding system. Each column should refer to another variable and each row should contain results of subsequent patients. Such format secures compilation of different sheets between the databases. Data should be presented as in the sample below:



- Each raw represents another patient
- Each column represents another variable
- The first columns should always be the same:
  - 1) Patient Identification (PID) ,
  - 2) Data of Birth (DoB)
  - 3) Date of Visit (DoV).
- Consecutive visits are organised in separate spreadsheets with the same coding system:
  - V5 = Enrolment visit
  - V60 = Visit 6
  - V120 = Visit 12
  - V180 = Visit 18
  - V240 = Visit 24
  - V300 = Exacerbation (all exacerbations, identified by date)
- The same coding system should be used to name variables – all variable should be coded as shortly as possible adding visit code at the end preceded by symbol "_" , for example "FEV1_V60" for forced expiratory volume in one second on visit 6.
- All codes do not contain spaces (replaced by symbol "_" if needed) to allow easy and safe transfer between different software (ex. Excel to SPSS)
- Dataset files has to be named in the same format including key word (CT, nutrients, body_comp, etc.) followed by the date in format YYYY.MM.DD and version ex. CT_2014.01.07_V1.0
- Every time a significant change to the database is implemented e.g. change in layout, a new version is saved to the destination folder with version numbers increasing by one whole integer each time i.e. from version 1.0 to version 2.0. If minor changes are made, e.g. additional fields are added, and then the subsequent version is increased by 0.1 whole integers i.e. from version 1.0 to version 1.1.

Information about Quality Assurance of the data

As the study data is entered manually, a data checking process against the source documents is planed according to specific instructions. The quality assurance plan is described in the file

Nutrition database specification

Characteristic of nutrition data gathered in Epi-Hip 001 collected in the Nutrition Database

Name of project	**Epi-Hip 001 (AERIS)**
Document characteristic	**Specification of the Nutrition Database created within the Epi-Hip 001.**
Date of creation	**10/06/2013**
Date of modification	**18/12/2013, 22/02/2016**
Version	**3.0**
Authors	**Malwina Wojtas**

The Nutrition Database (ND) has been created to collect the nutrition information from Epi-Hip 001 study, which were not collected in the study RDE. The Nutrition Database consists of two files. The "NutritionDB" file includes selected anthropometric measurements, body composition and physical capacity test. Nutritional biochemistry results are in a separate dataset called "VitaminsDB". This document refers to NutritionDB only. VitaminsDB is described in a separate document.

Source data documentation

The Nutrition dataset consists of:

- anthropometric measurements not included in the RDE – source document is an anthropometric worksheet from patient CRF;
- body composition results not included in the RDE – source document is a BodyStat printout from body composition assessment performed with QuadScan 4000. If not available, some results are available on the anthropometric worksheet;
- grip test (strength and endurance) results not included in the RDE – source document is anthropometric worksheet and MIA software printout (if printout not available should be obtained from the software);
- Council on Nutrition Appetite Questionnaire detailed answers – source document is CNAQ form from regular visits.

'Database quality assurance plan', written by Ms Malwina Wojtas. This document was prepared after consultation with Dr Steve Wootton of the Nutritional Biomedical Research Centre (NBRC) and Chris Blackwell, Senior Quality Assurance Lead in Southampton Centre for Biomedical Research (SCBR).

Policies for access, sharing and re-use

The data will be available for GSK Biologicals to validate at their request. Data from non-RDE databases can be transferred to GSK, if requested and agreed, after signing Data Transfer Agreement (DTA) prepared for each database separately. All DTAs have to be signed by EPI-HIP PI Dr Tom Wilkinson and be recorded in the study site file.

Long-term storage and data management

The databases are stored on UHS NHS Foundation Trust Servers. These file servers, which include shared drives and personal (H drives) are backed up in real time. IT have two data centres on site – our main server room in trust HQ and a back-up server room behind the neurology department. As changes are made, they are saved to the servers in both locations; therefore if the main file servers should fail, the back- up servers will automatically back up. In addition, daily back-ups are taken from the servers and stored off site.

Data entry – general rules

The Nutrition dataset has been created by members of BRC in Nutrition. To standardise data entry process following rules have been implemented:

1) Only "raw data" are included in the database. No calculated values are included in the database, only values available from CRF. The mean of repeated measurements can be considered as a raw data.
2) Values which can be calculated based on measurements relationship, should be excluded with indication why such value is not included and how it should be calculated. This should be specified in the DMP.
3) Any value should be entered to a measurement precision
    a. values measured up to one decimal place should be entered up to one decimal place;
    b. if measurement gives result up to second decimal place, then two decimal places should be entered;
    c. if entered value is a mean of two or more results it should be entered according to rules 3.a and 3.b
4) If value is not available it should be noted, no empty spaces should be left:
    a. If a measurement has not been performed enter **NA** (not available);
    b. If a measurement has not been performed due to visit replacement (exacerbation visit) enter **EXAC**
    c. If a measurement has unbelievable result (example: height of 32 cm or waist circumference of 14 cm) it should be noted that there was a result, but is incorrect – enter **IR** (irrational result)
5) All values are entered in standardised metrics. Metrics are obtained according to Standard Operating Procedures.
6) In the dataset results are organised in the same order (anthropometric measurements, body composition, grip tests). Any other order can be created but has to be saved as a "Reorganised" file (see information in paragraph "Data format").
7) Events are coded as :
    - Enrolment = 5
    - Month 3 = 30
    - Month 6 = 60
    - Month 9 = 90
    - Month 12 = 120
    - Month 15 = 150
    - Month 18 = 180
    - Month 21 = 210
    - Month 24 = 240

Body composition - data entry protocol EPI HIP 001 study

In the EPI HIP 001 study body composition was measured on every three monthly visits (Enrolment, Month 3, 6, 9 up to 24) but not on exacerbation visits. The aim of this protocol is to standardise data entry procedure and clarify define coding system. This instruction was used to enter results from all regular visits for all the subjects from the year one and two beginning from enrolment visit.

Subjects: 001 – 152
Visit: regular M0, M3, M6… M24
Data: Body composition and MFA
Source documents: Bodystat printout and anthropometry worksheet
File name: Nutrition_Y1 and Nutrition_Y2

- PID – Patient ID (form 001 to 152)
- DOV – Date of visit in format DDMMMYYYY
- Each raw represents another patient
- Each column represents another variable
- Results from each visit should be entered into a separate tab
- No empty cells should be left

- Values has to be recorded with the measurement precision. If value is measured to one decimal place it should be recorded to one decimal place (even if recorded value is a mean).
- Missing data : NA" for "Not applicable and "TNP" for Test Not Performed"

Table 1 Variables included in Nutritional database

	Variable	Decimal places	unit	Full name	Source document
1	IMP5	0	kHz	Impedance at 5 kHz	Bodystat printout (sample file:  Sample bodystat printout - with com)
2	IMP100	0	kHz	Impedance at 100 kHz	
3	IMP200	0	kHz	Impedance at 200 kHz	
4	RESIST	0	ohm	Resistance	
5	REACT	1	ohm	Reactance	
6	PHA	1	degrees	Phase angle	
7	BFM	1	kg	Body fat mass	
8	DLW	1	kg	Dry lean weight	
9	TBW	2	L	Total body water	
10	ECW	1	L	Extracellular water	
11	ICW	1	L	Intracellular water	
12	BCM	1	Kg	Body cell mass	
13	TSW	1	L	Third space water	
14	MFA	1	cm	Mid forearm circumference	Anthropometry worksheet

While entering variables 1 – 13 check if height and weight were entered properly into the Bodystat machine (compare to mean value on anthropometric sheet)
Variable 14 is on the anthropometric worksheet only. The value that should be entered is a mean of 3 measurements.

Data entry of CNAQ questionnaire in EPI HIP 001 study

Data specification

In the EPI HIP 001 study CNAQ questionnaire is used on every three monthly visits for two years and at every exacerbation visit.

Subjects: 001 – 152
Visit: regular M0, M3, M6 … M24 & exacerbations
Source document: CNAQ questionnaire
File name: CNAQ_Y1 & CNAQ_Y2

Table 2 Example of CNAQ file set up

	A	B	C	D	E	F	G	H	I	J	K
1	PID	DOV	Event	CNAQ_1	CNAQ_2	CNAQ_3	CNAQ_4	CNAQ_5	CNAQ_6	CNAQ_7	CNAQ_8
2											

- PID – Patient ID (form 001 to 152)
- DOV – Date of visit in format DDMMMYYYY
- Each raw represents another patient
- Each column represents another variable
- Results from each visit should be entered into a separate tab
- No empty cells should be left
- CNAQ_1 – CNAQ_8 – answers to questions 1 to 8

Coding system for CNAQ questionnaire

Answers in the questionnaire are given in a form of "a", "b",… to "e". The value entered into the database is 1, 2, 3, 4 or 5. For details see table below. All the values are a single number with no decimal places.

Codes	1	2	3	4	5
CNAQ answer	A	B	C	D	E

Data qualification

The only correctly filled questionnaire can be entered i.e. single answer to every of 8 questions.
Questionnaire should be discarded and all question fields should be given "NA" (not available) if:

- One or more questions do not have answer
- One or more questions have more than one answer chosen
- One of the pages is missing and the only answer to four questions is available

In any case of discarding there should be a detailed note made in the tab "excluded questionnaires" where full explanation is given (what is the reason, because of which question was test discarded, etc.)

.

If the questionnaire is not included in the CRF, "DM" (data missing) should be entered in the database, after confirming in the visit note that the form was not filled.

Description of variables related to grip tests (strength and endurance)

Variables representing results of grip strength and grip endurance tests collected for the NPS cohort are presented in table 1. Additionally two variables – maximum grip strength in strength test and scored time (fatigability) in endurance test are available through RDE.

Table 3 Grip test results collected for NPS cohort

Grip strength test		
HGR	N/s	Hand Grip Rate
HFR	N/s	Hand Fatigue Rate
HF	%	Hand Fatigue
HRR	N/s	Hand Release Rate
Fatigue (Endurance test)		
HGmE	N	Hang Grip max Endurance
Targ	N	Target value
Elap	s	Elapsed time
TOT	%	Time on target

To facilitate decision process and reasoning of possible exclusion process explanation of each variable and its derivation is needed. Sample graph is provided (Fig. 1) and description of highlighted points (1 – 5).

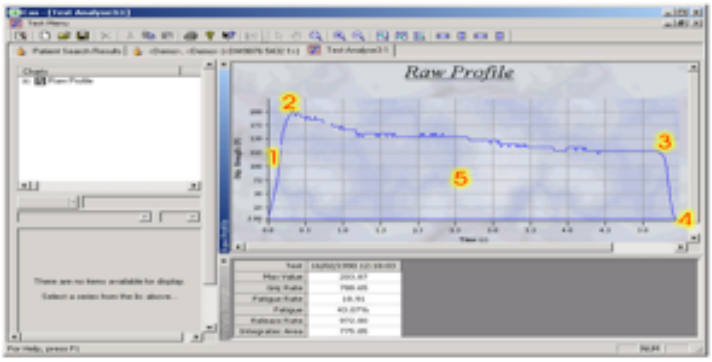


Figure 1 Sample graph of grip strength test

Maximum Value:	This is the peak force or moment achieved during the test (2)
Grip Rate:	The average value of the slope (1) excluding the first and last 10% of the curve. This value changes depending on joint pain and spacticity.
Fatigue Rate:	The average of the curve between the maximum value (2) and the end value (3). Fatigue rate should always be greater than zero if maximum contraction has been achieved. A flat curve would indicate either pain limitation or cheating.
Fatigue:	The drop in maximum grip (2-3) over the maximum grip (3) expressed as a percentage.
Release Rate:	The average value of the slope (3 to 4) excluding the first and last 10 percent of the curve. This value changes depending on joint stiffness and spacticity.
Integrated Area:	This is the total area under the curve (5), which is the measure of the work done.

Figure 2. Description of variables provided as a result of grip strength test

Regarding utility of listed variables following changes are suggested:

1) Endurance test
   a. Target value can be derived from the maximum strength in endurance test and can be definitely excluded from data entry
   b. Elapsed time presents total time test was performed for and it could be excluded*(*this value can be calculated using 'scored time' and 'time on target'*)
   c. Time on target presents precision of grip and is calculated based on scored time and elapsed time and it could be excluded * (*this value can be calculated based on 'scored time' and 'elapsed time'*)

* One of the values, 'elapsed time' or 'time on target', should be entered, as the calculations can be made only if of the values is available. Regarding the fact that for some tests original electronic source file are not available (only printouts in the folder) it is more likely to have endurance value printed, rather than time on target. Therefore it would be more reasonable to include elapsed time in the database.

2) Grip strength test
   a. Even though 'Fatigue Rate' is derived from the same variables as 'Fatigue', software does not provide values represented as '2' and '3' on the Figure 1, which are required to perform calculation of 'Fatigue'. From the analysis perspective 'Fatigue' provides more comparable value (percentage of drop in strength of grip) than 'Fatigue rate' (Newtons/second) and may be more useful. If any of the values is excluded, then it should rather be 'Fatigue rate'.

The new table of variables would include list presented in Table 2.

Table 4 New set of variables to be included in nutrition database

Grip strength test		
HGR	N/s	Hand Grip Rate
HF	%	Hand Fatigue
HRR	N/s	Hand Release Rate
Endurance test		
HGmE	N	Hang Grip max Endurance
Elap	s	Elapsed time

Data dictionary

All abbreviations and coding system used in the database are described in a sheet named "codes" in each file as well as in Table 1 below. If new variables are created based on row data, an appendix to the DMA should be created with an explanation of each new variable. The explanation of calculated variables should have the same format as data dictionary with two additional columns – one with used equation and second with rationales of the calculation (source of the equation).

Table 5. Data included in the nutrition database- DATA DICTIONARY

	Variable	Decimal places	Unit	Meaning	Lower limit	Upper limit	Source
Patient	PID	0	-	Patient id number	001	152	Subject card from enrolment visit
	DOB	0	-	Date of birth	01011929 (DDMMYYYY)	01011970 (DDMMYYYY)	
	DOV	0	-	Date of visit	01062012 (DDMMYYYY)	01072014 (DDMMYYYY)	Visit record
Grip tests	HGR	2	N/s	Hand Grip Rate	1	1000	According to SOP 161 "Measuring Strength and Endurance Using the MIE Pinch/Grip Digital Analyser and Using the Clinical Analysis System (CAS) Software", version 1, 8th August 2011 and recorded on the grip test printouts.
	HFR	2	N/s	Hand Fatigue Rate	1	100	
	HF	2	%	Hand Fatigue	1	100	
	HRR	2	N/s	Hand Release Rate	1	2000	
	HGmE	2	N	Hang Grip max in Endurance	1	500	
	Targ	2	N	Target value	50	250	
	Elap	2	s	Elapsed time	5	200	
	TOT	2	%	Time on target	0	100	
BIA row data	IMP5	0	kHz	Impedance at 5 kHz	200	950	According to SOP 117 "Measuring Bioelectrical Impedance using the Bodystat QuadScan 4000" version 2, 19th March 2012 and recorded on the Bodystat printout. If not available, the machine should be interrogated for the data. In the rare event that data is still not available, results have been copied from the anthropometrics work sheet (some values missing then).
	IMP100	0	kHz	Impedance at 100 kHz	200	950	
	IMP200	0	kHz	Impedance at 200 kHz	200	950	
	Resist	0	ohm	Resistance	200	900	
	React	1	ohm	Reactance	20	100	
	PhA	1	degrees	Phase angle	1	15	
BIA body composition	BFM	1	kg	Body fat mass	1	60	
	DLW	1	kg	Dry lean weight	5	30	
	TBW	2	L	Total body water	15	80	
	ECW	1	L	Extracellular water	5	30	
	ICW	1	L	Intracellular water	5	50	
	BCM	1	Kg	Body cell mass	5	60	
	3SW	1	L	Third space water	-5	5	
	MFA	1	cm	Mid forearm circumference	10	30	Anthropometric worksheet

Database quality assurance plan

Strategy to provide high quality of nutrition data gathered in Epi-Hip 001 collected in the Nutrition Database

Name of project	**Epi-Hip 001**
Document characteristic	**Database quality assurance plan for nutrition data collected within the Epi-Hip 001 in the Nutrition Database.**
Date of creation	**13/06/2013**
Date of modification	**18/12/2013**
Version	**2.0**
Authors	**Malwina Wojtas**

To ensure the EPI HIP Nutrition Database as secure and robust, as required in the Data Management Plan, a selection of procedures have been put in place. The first action was the identification of risk sources. The second action was defining an appropriate way to prevent and interact with risk sources. The last action was defining the method of data check and data correction.

Identifying risk of error

Within the process of creating the Nutritional Database, there are a number of ways in which error may be introduced at each stage of data handling process.

	Risk source	Prevention	
1	Incorrect value entered due to **typing error**	Performing data check.	Study staff that are working on the Nutritional Database have to be introduced to all documentation of the database, especially Database Specification where all variables are precisely defined and characterised to avoid discrepancies in the data entry and data handling.
2	Incorrect value entered due to **incorrect source** chosen	A source of each value is specified in the Database specification file.	
3	Incorrect value entered due to **incorrect coding** model	Coding models are specified in the Database specification file.	
4	**Incorrect database** chosen to work on	All current database versions are kept separately from the previous version.	

Data check

A data check is a process of comparing values in the database with original source information (CRF). The process of the data check has been organised in two steps: 1) Logical data check; and 2) 5% data check.

LOGICAL DATA CHECK

The first step of the data check is to confirm that each entered value is logical in terms of what result would be expected for that particular variable. This activity is performed in 2 stages: Firstly, analysis of unit and expected values (i.e. same value for each subject should be within specific range, which is specified in the data dictionary – upper and lower limit), for example, height is expected to be within 145-199cm. If one of the values represents 1.75 it suggests incorrect unit, but when the value would be 78 it suggest typing error.

Secondly, a logical data check should be performed. That is a process of data check based on data relationships between selected data. Discrepancies in the values suggest typing error. Relations between data which are used to perform second step logical data check are presented in Table 6.

Table 6 Relationships between data and acceptable differences

Data compared	Against	Acceptable difference	Explanation of acceptable differences
BMI	Sum of BFMI + FFMI	0	All three values derived from Bodystat machine and should be identical. Any difference should suggest typing error
FFMI	BLM / Ht.m^2	±0.3	Height typed into Bodystat machine can have just one decimal place, which may cause insignificant difference in calculated FFMI
BFMI	BFM / Ht.m^2	±0.3	Height typed into Bodystat machine can have just one decimal place, which may cause insignificant difference in calculated BFMI
TBW	Sum of ICW+ECW+ThdSpaceWater	0	All values derived from Bodystat machine and should be identical. Any difference should suggest typing error
Wt.kg	Sum of BFM + BLM	0	All values derived from Bodystat machine and should be identical. Any difference should suggest typing error

5% DATA CHECK

The second level of data clearing is the 5% data check against CRF values. For that purpose, a random patient's data are compared to the corresponding CRFs. The data check is organised sheet-wise as follows:

- Based on enrolment visit the number of subjects should be assessed (including only subjects who had typed results, excluding withdrawn subjects)
- Based on the total group size 5% of that value should be calculated
- When the number of cases that should be checked is calculated, a random selection of subjects should be selected (online randomisation website should be used)
- Based on the numbers chosen by an online randomisation machine subjects should be selected to 5% data check.
- Each subject that has been included in the 5% data check should be compared with the CRF for each three monthly visits for each value.

After performing the data check a report should be prepared to analyse what type of error is most common and to detail the appropriate steps needed to prevent this error in the future.

Post-entry analysis

When the data have been entered and cleaned, the post-entry analysis must be performed. The aim of the analysis is to produce a report summarising the number of participants, the number of missing values by variable and list all of the values by variable outside of the pre-defined range (listed in the data dictionary). The report should be produced for each datasheet (month 0, 3, 6, etc.) and for each dataset (Nutrition, Vitamins).

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Appendix PData analysis plan

AERIS ANALYSIS PLAN v3.0

21st March 2016

Nutritionally driven phenotyping in COPD

Malwina Maria Wojtas

CONTENTS

1. BACKGROUND, OBJECTIVES & HYPOTHESES

BACKGROUND

The traditional view of COPD pathophysiology is mainly concerned with consistent airflow limitations, but in recent years studies have shown systemic effects of the disease. Recently changes in body composition, especially loss of muscle mass and abnormal muscle structure and/or function were often reported in COPD cohorts.

It has been demonstrated that as health deteriorates and body composition worsens, the ability to recover also deteriorates and risk of mortality rises. In COPD this process is accelerated by exacerbations. A history of multiple hospital admissions is related to poorer muscle function and increased risk of hospital readmissions. Moreover exacerbations further deteriorate muscle dysfunction and weight loss during the follow-up period and are related to a higher risk of having new exacerbation and higher risk of mortality. This creates a vicious loop where exacerbations cause wasting and wasting causes exacerbations however there is lack of understanding of mechanisms leading to this state.

AIM

To explore relationships between nutritional markers and COPD progression and identify distinct nutritional phenotypes of COPD

Main objectives A to E are listed below, with secondary objectives in the analysis plan table on pages 3 to 6.

MAIN OBJECTIVES

A To describe the distribution of and associations between nutritional status, appetite, and physical capacity markers

B To examine change in nutritional status, appetite, and physical capacity markers over time

C To examine the ability of nutritional status, appetite and physical capacity markers to predict future exacerbation risk

D To evaluate the suitability of current lean-marker and nutrient cut-points

E To identify nutritional COPD phenotypes and assess their usefulness

HYPOTHESIS

COPD patients with poor nutritional status are more predisposed to worse clinical outcomes than COPD patients with good nutritional status.

2. ANALYSIS PLAN TABLE

Main Objectives	GSK Datasets	Time points	Secondary objectives	research questions	Methods
A. Distribution and associations between markers		Enrolment	A1. Explore distribution of nutritional status markers, appetite, and physical capacity variables (univariate)	A1.1 Are markers of nutritional status normally distributed?	**Descriptive statistics** including histograms, means, medians, SD and IQR for continuous variables, and bar charts and percentages for categorical variables.
				A1.2 What proportion of patients have lean mass below healthy ranges?	**Descriptive statistics** including frequency and percentage
				A1.3 What proportion of patients have low appetite score?	**Descriptive statistics** including frequency and percentage
			A2. Describe associations between nutritional status, appetite, physical capacity, and other variables	A2.1 Do current smokers have lower average fat free mass index than ex-smokers?	**Independent T test** / **Mann Whitney test** with smoking status as the grouping variable and fat free mas index as the continuous variable
				A2.2 Is being overweight associated with poorer grip strength?	**Independent T test** / **Mann Whitney test** with overweight as the grouping variable and grip strength as the continuous variable. **Chi-square test** for categorical grip strength
				A2.3 Is being lean depleted, associated with poorer grip endurance (high fatigability)?	**Independent T test** / **Mann Whitney test** with lean depletion as the grouping variable and grip endurance as the continuous variable. **Chi-square test** for categorical grip endurance.
				A2.4 Do patients with worse 6 minute walk score have lower lean mass?	**Scatterplot** and **Spearman's/Pearson's correlation coefficient**
				A2.5 Do patients with lower appetite have higher exacerbation history?	**Independent T test** / **Mann Whitney test** with high/low appetite score as the grouping variable and exacerbations in previous year as the continuous variable. **Scatterplot** and **Spearman's/Pearson's correlation coefficient** for continuous appetite score
				A2.6 Are patients with poor appetite score (CNAQ<28) more likely to be smokers?	Crosstabulation and Chi-Square test between poor appetite score and smoking status
				A2.7 Is body composition different in GOLD groups when adjusted for history of exacerbations?	**ANCOVA / linear regression** with GOLD group as a categorical variable and body composition as a continuous variable, adjusting for number of exacerbations in the previous year.
				A2.8 Is appetite associated with nutritional status after controlling for smoking status?	**ANCOVA / linear regression** with poor appetite as a categorical variable and nutritional status as a continuous variable, asjusting for smoking status.

Appendix

Main Objectives	GSK Datasets	Time points	Secondary objectives		research questions		Methods
A. (contd.)		Exacerbation	A3.	Describe appetite score in the cohort at exacerbation	A3.1	What proportion of patients have low appetite score at first exacerbation?	**Descriptive statistics** including histogram, count, percentage
					A3.2	Are severe first exacerbations associated with worse appetite than less severe first exacerbations?	**Independent t test** / **Mann Whitney test** with exacerbation severity as the grouping variable, and appetite score as the continuous variable.
B. Change in markers over time		Enrolment & quarterly visits	B1.	Description of changes in nutritional status and appetite over time	B1.1	What proportion of patients maintain lean mass and what proportion lose lean mass between enrolment and months 3, 6, 9, and 12?	**Line graph** for each continuous nutritional/appetite marker, and **Paired T tests / Wilcoxon signed rank test / McNemar's test** between enrolment and each subsequent quarterly visit. **Descriptive statistics** including the mean difference between enrolment and each quarterly visit, and the proportion of individuals whose nutritional/appetite markers deteriorate
					B1.2	Do patients who lose lean mass also lose grip strength and grip endurance?	**Scatterplot** and **Spearman's/Pearson's correlation coefficient** of the difference between enrolment and month 12 in lean mass and grip strength.
					B1.3	Do patients with greatest loss of appetite score have also greatest loss of lean mass and lung function?	**Scatterplot** and **Spearman's/Pearson's correlation coefficient** of the difference between enrolment and month 12 in appetite score and lung function.
					B1.4	Do patients with low grip strength at enrolment have lower lung function at month 12?	**Scatterplot** and **Spearman's/Pearson's correlation coefficient** of grip strength at enrolment and lung function in month 12.
		Quarterly visit vs quarterly visit	B2.	Examine whether exacerbations are associated with a worsening of nutritional markers	B2.1	Does grip strength deteriorate between the quarterly visit prior to an exacerbation, and the quarterly visit following an exacerbation?	**Paired T test/Wilcoxon signed rank test** for continuous markers, **McNemar's** test for categorical markers.
		Enrolment vs month 12	B3.	Explore if severity of structural and lean marker depletion at enrolment is associated with change in these markers over 12 months	B3.1	Is deterioration in grip strength more rapid for individuals who already have poor grip strength?	**Multivariate linear regression** with change in marker as the outcome, and enrolment level of the marker as main exposure.
			B4	Identify to what extent change in appetite is associated with change in nutritional status	B4.1	As appetite declines, does nutritional status also decline?	**Scatterplot** and **Multivariate linear regression** with change in nutritional status between enrolment and month 12 as the outcome, and change in appetite as the main exposure, controlling for enrolment levels.

Main Objectives	GSK Datasets	Time points	Secondary objectives		research questions		Methods
C. Prediction of exacerbation risk		Enrolment & exacerbation frequency/rate	C1.	To determine if structural and functional lean markers are useful predictors of future exacerbations	C1.1	Which structural and functional lean markers are useful predictors of future exacerbations?	**Negative binomial regression** with exacerbation frequency/rate as the outcome, or **CPH regression** with TTFE as the outcome, and structural or functional lean marker as the main exposure. Analysis conducted separately for each marker.
			C2.	Determine if appetite is a useful predictor of future exacerbations	C2.1	How useful is appetite at predicting future exacerbations?	**Negative binomial regression** with exacerbation frequency/rate as the outcome, or **CPH regression** with TTFE as the outcome, and appetite score as the main exposure.
					C2.2	Can selected questions from the CNAQ questionnaire predict future exacerbation risk to the same extent as the full questionnaire?	**Negative binomial regression,** with exacerbation frequency/rate as the outcome, or **CPH regression** with TTFE as the outcome, individual CNAQ questions as the main exposure(s).
			C3.	Determine if nutritional biochemistry are useful predictors of future exacerbations	C.3.1	Which nutrients are useful predictors of future exacerbations?	**Negative binomial regression** with exacerbation frequency/rate as the outcome, or **CPH regression** with TTFE as the outcome, and nutrients level as the main exposure.
D. Evaluation of current lean-marker cut-points		Enrolment	D1.	Evaluate the suitability of existing lean-marker cut-points in reflecting clinical outcome	D1.1	How does the proportion of individuals defined as lean-depleted vary according to which cut-point is used? What is the accuracy of current cut-offs?	**Bland and Altman comparison** to assess limits of agreement between different measures of lean depletion. **Descriptive statistics** including frequency and proportion of lean-depleted for different cut-points.
		Enrolment & exacerbation frequency/rate			D1.2	Are different cut-points useful in predicting future exacerbations? What is the clinical value of current cut-offs?	**Negative binomial regression** with regression with exacerbation rate as the outcome, or **CPH regression** with TTFE as the outcome, and lean-depleted as a binary main exposure. Analysis repeated for different cut-offs.
					D1.3	What is the sensitivity and specificity of existing cut-offs in predicting poor clinical outcome? Can a better cut-off be identified?	**ROC curve analysis.** Monthly exacerbation rate of >=0.17 denotes poor clinical outcome.

Main Objectives	GSK Datasets	Time points	Secondary objectives	research questions	Methods
E. Nutritional phenotypes		Enrolment & exacerbation frequency/rate	E1. Identify nutritional COPD phenotypes	E1.2 Identify one lean-marker, one appetite score, one functional marker, and one nutritional biochemistry marker, which are best at predicting future risk of exacerbation	**Negative binomial regression** with exacerbation frequency/rate as the outcome, or **CPH regression** with TTFE as the outcome, controlling for selected covariates identified in Section A.
				E1.1 Define optimal cut-points for the four markers identified in E1.2	**ROC curve analysis.** Monthly exacerbation rate of >=0.17 denotes poor clinical outcome.
			E2. Test usefulness of the phenotypes in characterising COPD outcomes	E2.1 Which of the 17 combinations of markers (4 binary markers) are best at predicting future risk of exacerbation?	**Negative binomial regression**, with exacerbation frequency/rate as the outcome, or **CPH regression** with TTFE as the outcome, nutritional phenotype (consistently poor, consistently good, mixed) as the main exposure. Possible additional analysis using SEM.
				E2.2 Do nutritional phenotypes have distinct nutritional biochemistry characteristics?	**ANOVA / Kruskal-Wallis** test. **Descriptive statistics** including histograms for nutritional markers stratified by phenotype.

3. TECHNICAL APPENDIX

Abbreviations: Analysis of Covariance (ANCOVA); Analysis of Variance (ANOVA); Council on Nutrition Appetite Questionnaire (CNAQ); Cox Proportional Hazards (CPH); Interquartile Range (IQR); Receiver Operating Characteristic (ROC); Standard Deviation (SD); Structural Equation Modelling (SEM); Time to First Exacerbation (TTFE);

Inclusion criteria: Only data from the first 12 months of the AERIS study will be considered in these analyses. The analyses will include data collected from individuals who later withdrew from the study, however certain analyses (involving change in markers and exacerbation frequency over 12 months) will be limited to those who remained in the study for at least 12 months.

Missing data: Missing data will not be imputed, and will be excluded on a casewise basis. Variables with a large proportion of missing values may be excluded. Checks will be made to ensure data is missing at random.

Multiple exacerbations per individual: One individual can have more than one exacerbation over the study period. Where this is an issue (objectives A3 and B2) analyses are limited to the first exacerbation per individual.

Cut-points: Markers will be considered in the analyses as both continuous and categorical variables. Where appropriate, gender-specific cut-points will be used to define categories. Previously published criteria will be used for variables like: 6 minute walk test (<0.8m/s), mid upper arm circumference (< 235mm (men); < 220mm (women)), or CNAQ score (≤28).

When no cut-off variables are available to stratify the results (e.g. endurance of grip test) tertiles or centiles approach will be used. For some of the variables (e.g. fat mass index) tertiles approach will be used additionally to published cut-offs, because of credibility of available criteria (cut-offs based on poor quality studies or non-COPD specific cohorts).

Multiplicity: A large number of nutritional markers will be considered in these analyses (see variable table). The number of markers used in the analyses will be reduced by the removal of markers which are not consistently associated with other markers (assessed in Objective A) and with clinical outcomes (Objective C).

B. Longitudinal change

- Objective B2
  - The nearest stable quarterly visit at least 14 days prior to/following exacerbation onset with valid data will be considered in these analyses
  - Only exacerbations which occur in isolation with a preceding stable quarterly visit and a following stable quarterly visit will be included. Multiple exacerbations with no stable quarterly visits separating them will be excluded.
  - The 'first' exacerbation per individual will be the first eligible exacerbation with a stable quarterly visit at least 14 days either side, and valid data on the required variables.

E. Nutritional phenotypes

- Only one key marker for each of the four categories (lean, appetite, functional, nutritional biochemistry) will be considered.
- A binary cut-point for each of these markers will be identified, resulting in 17 distinct phenotypes. Phenotypes with small numbers of individuals may be collapsed together.

The document was assisted by an attachment with a full list of variables included in each analysis, attachment not included in this appendix.

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