# Supplementary Material

## Sputum Induction

Sputum was induced using hypertonic saline and collected in accordance with the European Respiratory Society guidelines. Sputum plugs were selected from expectorates, processed with dithioerythritol, to separate cells from the fluid phase, homogenised, filtered, using a 100 µm cell strainer (BD Falcon) to remove mucus, centrifuged at 10 G to remove supernatant and the cell pellet resuspended in phosphate buffered saline. A total cell count was made using a haemocytometer and cell viability determined using trypan blue exclusion.

# FIGURE E1 – Distribution of Blood Tests in Relation to Monoclonal Antibody Start Dates



# TABLE E1 – Distribution of included blood tests and the start dates of Monoclonal Antibody therapies in Biologic Naïve patients.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Year | Number of Blood Tests | Mepolizumab | Omalizumab | Benralizumab |
| 2006 | 489 | 0 | 0 | 0 |
| 2007 | 377 | 0 | 3 | 0 |
| 2008 | 568 | 0 | 4 | 0 |
| 2009 | 449 | 0 | 5 | 0 |
| 2010 | 506 | 0 | 6 | 0 |
| 2011 | 531 | 0 | 6 | 0 |
| 2012 | 632 | 0 | 3 | 0 |
| 2013 | 653 | 8 | 5 | 0 |
| 2014 | 767 | 0 | 13 | 0 |
| 2015 | 1037 | 0 | 19 | 0 |
| 2016 | 991 | 0 | 20 | 0 |
| 2017 | 1016 | 6 | 18 | 0 |
| 2018 | 982 | 49 | 8 | 0 |
| 2019\* | 606 | 18 | 8 | 5 |
| *sum* | *9604* | *81* | *118* | *5* |

\*last blood test from 31/07/2019 so figures represent only 8 months of 2019

# TABLE E2 – Comparison of Patients with at least one blood test and patients with no available blood tests

|  |  |  |  |
| --- | --- | --- | --- |
|  | At least one blood test N=471 | No blood tests available N=30 | P value |
|  | *Missing* |  | *Missing*  |
| Age (at enrolment)  | 52.0 [39.0,63.0] | *0* | 46.0 [32.0,58.0] | *0* | Ns |
| BMI  | 29.8 [25.7,35.3] | *4 (8.0%)* | 29.9 [25.2,39.8] | *2 (8.0%)* | Ns |
| Gender (female) | 306 (65.0%) | *0* | 19 (76.0%) | *0* | Ns |
| Smoker (ever) | 225 (47.9%) | *1 (0.2%)* | 9 (36.0%) | *0* | Ns |
| Age of Diagnosis | 19.0 [4.0,40.0] | *21 (4.5%)* | 17.5 [2.0,29.5] | *1 (4.0%)* | Ns |
| 4 or more OCS past year | 162 (38.4%) | *49 (10.4%)* | 11 (45.8%) | *1 (4.0%)* | Ns |
| Atopic (positive SPT) | 250 (67.8%) | *110 (23.3%)* | 14 (82.4%) | *8 (32.0%)* | Ns |
| FeNO | 18.1 [9.3,35.0] | *107 (22.7%)* | 24.0 [18.3,46.3] | *4 (16.0%)* | P<0.05 |
| Post BD FEV1 | 75.3 [59.4,92.7] | *155 (32.9%)* | 68.5 [54.3,83.2] | *5 (20.0%)* | Ns |
| Post BD FEV1/FVC ratio | 68.0 [58.0,78.0] | *155 (32.9%)* | 65.0 [54.2,74.2] | *5 (20.0%)* | Ns |
| Total IgE | 85.5 [20.1,269.1] | *105 (22.3%)* | 50.8 [24.0,198.2] | *11 (44.0%)* | Ns |
| ACQ6 | 2.7 [1.5,3.7] | *32 (6.8%)* | 2.5 [1.7,3.5] | *2 (8.0%)* | Ns |

Continuous variables expressed as median [Q1, Q3] with differences measured by Mann Whitney U test. Categorical variables expressed as n (%) with differences measured by Chi Square test. OCS = oral corticosteroids, FeNO = fraction of nitric oxide in exhaled breath, BD = bronchodilator, FEV1 = forced expiratory volume in 1 second, FVC = Forced vital capacity, IgE = immunoglobulin E, ACQ = asthma control test

## TABLE E3 – Comparison of patients with less than 10 blood test results (excluded from analysis) and patients with 10 or more blood tests (included in analysis)

|  |  |  |  |
| --- | --- | --- | --- |
|  | Less than 10 (n=236) | 10 or more (n=235) | P value |
|  | *Missing* |  | *Missing*  |
| Age (at enrolment)  | 51.0 [38.0,62.0] | *0* | 56.0 [40.5,64.0] | *0* | P<0.05 |
| BMI  | 28.6 [25.1,34.1] | *4 (1.7%)* | 30.7 [26.7,36.5] | *0* | P<0.05 |
| Gender (female) | 146 (61.9%) | *0* | 160 (68.1%) | *0* | ns |
| Smoker (ever) | 108 (46.0%) | *1* | 117 (49.8%) | *0* | Ns |
| Age of Diagnosis | 15.0 [3.0,34.8] | *6 (2.5%)* | 22.0 [6.0,43.2] | *15 (16.4%)* | P<0.05 |
| 4 or more OCS past year | 84 (39.8%) | *25* | 78 (37.0%) | *24* | Ns |
| Atopic (positive SPT) | 140 (71.1%) | *39* | 110 (64.0%) | *63* | Ns |
| FeNO | 19.0 [9.7,38.7] | *47 (19.9%)* | 17.3 [9.0,29.6] | *60 (25.5%)* | Ns |
| Post BD FEV1 | 75.0 [58.5,92.1] | *75 (31.8%)* | 76.2 [60.7,92.7] | *80 (34.0%)* | Ns |
| Post BD FEV1/FVC ratio | 69.0 [58.0,77.8] | *75 (31.8%)* | 67.0 [58.0,78.0] | *80 (34.0%)* | Ns |
| Total IgE | 99.7 [33.2,276.4] | *46 (19.5%)* | 56.6 [13.5,249.9] | *59 (25.1%)* | P<0.05 |
| Latest Blood Eosinophil within last 12 months | 0.2 [0.1,0.3] | *28 (11.9%)* | 0.2 [0.1,0.3] | *8 (3.4%)* | Ns |
| ACQ6 | 2.5 [1.5,3.7] | *11 (4.7%)* | 2.7 [1.7,3.8] | *21 (8.9%)* | Ns |

Continuous variables expressed as median [Q1, Q3] with differences measured by Mann Whitney U test. Categorical variables expressed as n (%) with differences measured by Chi Square test. OCS = oral corticosteroids, FeNO = fraction of nitric oxide in exhaled breath, BD = bronchodilator, FEV1 = forced expiratory volume in 1 second, FVC = Forced vital capacity, IgE = immunoglobulin E, ACQ = asthma control test

## **TABLE E4** – Comparison of clinical features between groups defined by frequency of blood eosinophilia including missing values

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Never (39) | Rare (65) | Intermittent (65) | Persistent (66) | p value |
|  | *Missing* |  | *Missing* |  | *missing* |  | *missing* |
| Age (at enrolment; years)  | 56.0 [37.0,63.0] | *0* | 52.0 [41.0,61.0] | *0* | 54.0 [36.0,64.0] | *0* | 60.0 [48.2,65.0] | *0* | Ns |
| BMI  | 31.5 [28.8,36.0] | *0* | 32.9 [27.8,40.5] | *0* | 28.7 [25.2,34.7] | *0* | 30.2 [27.5,35.4] | *0* | Ns |
| Sex (female) | 31 (79.5%) | *0* | 45 (68.2%) | *0* | 43 (65.2%) | *0* | 41 (62.1%) | *0* | Ns |
| Smoker (ever) | 16 (41.0%) | *0* | 31 (47.0%) | *0* | 39 (59.1%) | *0* | 33 (50.0%) | *0* | Ns |
| Age of Asthma Diagnosis (years) | 20.0 [7.8,44.5] | *3* | 21.0 [8.0,35.2] | *7* | 21.0 [3.0,43.5] | *2* | 27.0 [10.5,51.5] | *3* | Ns |
| Current Inhaled Corticosteroid (BDP equivalent) | 2050.0 [1625.0,3000.0] | *9* | 3000.0 [2000.0,3820.0] | *10* | 3000.0 [1840.0,3840.0] | *12* | 3000.0 [2000.0,3000.0] | *9* | Ns |
| Asthma Biologics (ever) | 9 (23.1%) | *0* | 21 (32.3%) | *1* | 33 (50.8%) | *1* | 31 (47.0%) | *0* | Ns |
| 4 or more OCS courses in past year | 11 (30.6%) | *3* | 24 (39.3%) | *5* | 23 (39.7%) | *8* | 21 (36.8%) | *9* | Ns |
| Hospitalised for asthma in past year | 14 (35.9%) | *0* | 28 (43.1%) | *1* | 20 (30.8%) | *1* | 19 (28.8%) | *0* | Ns |
| Atopic (positive SPT to any aeroallergen) | 19 (59.4%) | *7* | 26 (55.3%) | *19*  | 31 (72.1%) | *23*  | 35 (67.3%) | *14* | Ns |
| FeNO | 13.0 [5.7,19.0] | *14* | 12.8 [7.5,24.4] | *15* | 20.0 [9.0,54.0] | *12* | 23.0 [13.8,51.1] | *19* | P<0.001 |
| Total IgE | 11.6 [0.0,64.3] | *7* | 23.3 [13.4,68.7] | *23* | 153.5 [39.2,474.7] | *14* | 102.5 [21.5,561.6] | *16* | P<0.001 |
| ACQ6 | 2.7 [1.7,3.7] | *6* | 2.5 [1.5,3.5] | *2* | 2.5 [1.7,3.5] | *5* | 2.4 [1.2,3.7] | *8* | Ns |
| Rhinitis | 24 (70.6%) | *5* | 43 (70.5%) | *5* | 34 (63.0%) | *12* | 38 (64.4%) | *7* | Ns |
| Nasal Polyposis (on CT) | 0 | *35* | 1 (14.3%) | *59* | 2 (16.7%) | *54* | 5 (50.0%) | *56* | Ns |
| Eczema | 9 (23.1%) | *0* | 15 (24.2%) | *4* | 18 (27.3%) | *0* | 15 (22.7%) | *0* | Ns |
| ABPA or SAFS | 0 | *0* | 2 (3.1%) | *2*  | 9 (13.8%) | *1* | 9 (13.6%) | *0* | P<0.05 |
| Bronchiectasis | 3 (7.7%) | *0* | 8 (12.5%) | *2*  | 11 (16.7%) | *0* | 15 (24.2%) | *0* | Ns |
| GORD | 29 (76.3%) | *1* | 47 (73.4%) | *2* | 40 (62.5%) | *2* | 46 (73.0% | *3* | Ns |
| Depression | 13 (37.1%) | *4* | 25 (39.7%) | *3* | 25 (43.9%) | *9* | 21 (33.3%) | *3* | Ns |
| Anxiety | 9 (26.5%) | *5* | 23 (37.1%) | *4* | 21 (38.2%) | *11* | 22 (33.8%) | *1* | Ns |
| Dysfunctional Breathing | 21 (55.3%) | *1* | 35 (55.6%) | *3* | 33 (51.6%) | *2* | 28 (43.8%) | *2* | Ns |
| Vocal Cord Dysfunction | 4 (11.4%) | *4* | 17 (27.4%) | *4* | 5 (8.8%) | *9* | 9 (15.3%) | *7*  | P<0.05 |
| Clinical Sulphite Sensitivity | 2 (5.1%) | *0* | 5 (7.7%) | *1* | 3 (4.6%) | *1* | 4 (6.1%) | *0* | Ns |
| Clinical Salicylate Sensitivity | 8 (20.5%) | *0* | 19 (29.7%) | *2* | 14 (21.5%) | *1* | 11 (16.7%) | *0* | Ns |
| Sleep Apnoea | 1 (2.6%) | *0* | 6 (9.5%) | *3* | 5 (7.6%) | *0* | 4 (6.2%) | *1* | Ns |

Continuous variables expressed as median [Q1, Q3] with between group differences assessed by Kruskal Wallis testing. Categorical variables expressed as n (%) with between group differences assessed by Chi Square testing. BMI = Body mass index, OCS = oral corticosteroids, SPT = skin prick test, FeNO = fraction of nitric oxide in exhaled breath, BD = bronchodilator, FEV1 = forced expiratory volume in 1 second, pred = predicted, FVC = Forced vital capacity, FEF25-75 = Forced expiratory flow at 25% to 75% of FVC, IgE = immunoglobulin E, ACQ = asthma control test. Distribution of missing data available in Supplementary material; Table E5. Biologics include all NICE licensed anti-IgE, anti IL5 and anti-IL5r monoclonal antibodies for severe asthma. ABPA = Allergic Bronchopulmonary Aspergillosis, SAFS = Severe Asthma with Fungal Sensitisation, GORD = Gastrooesophageal Reflux Disease

## **TABLE E5** – Comparison of Post Bronchodilator Spirometry between groups defined by frequency of blood eosinophilia

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Never (39) | Rare(65) | Intermittent(65) | Persistent(66) | p value |
| *Missing cases* | *13* | *26* | *19* | *23* |  |
| Post BD FEV1%pred | 83.8 [67.9,92.3] | 83.5 [65.6,94.6] | 73.3 [58.0,92.8] | 72.3 [57.7,84.6] | Ns |
| Post BD FEV1/FVC ratio | 76.5 [64.5,82.0] | 75.0 [62.0,82.0] | 66.0 [54.0,75.5] | 64.0 [53.5,70.5] | P=0.001 |
| Post BD FEF25-75% pred | 69.2 [34.5,96.7] | 70.2 [32.3,93.7] | 39.6 [27.8,67.0] | 37.3 [24.1,58.1] | P=0.001 |

Continuous variables expressed as median [Q1, Q3] with between group differences assessed by Kruksal Wallis testing. BD = bronchodilator, FEV1 = forced expiratory volume in 1 second, pred = predicted, FVC = Forced vital capacity, FEF25-75 = Forced expiratory flow at 25% to 75% of FVC.

## **TABLE E6** – Comparison of Sputum Differential Cell Counts between groups defined by frequency of blood eosinophilia

Sputum differential cell counts were only captured when clinically indicated and thus only available in a small subset of patients. These tests were only performed as part of the Regional Severe Asthma Service, when patients had been optimised on therapy.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Never (11) | Rare (22) | Intermittent(25) | Persistent(24) | p value |
| Sputum Eosinophil % | 0.4 [0.2,1.2] | 0.1 [0.0,0.7] | 2.0 [0.5,6.5] | 4.4 [1.1,7.9] | P<0.001 |
| Sputum Neutrophil % | 39.6 [11.6,54.4] | 23.4 [6.2,46.3] | 44.0 [19.0,64.8] | 55.7 [32.2,68.7] | P<0.05 |

Continuous variables expressed as median [Q1, Q3] with differences measured by Kruskall Wallis test.

## **TABLE E7** –Inflammatory Phenotypes defined by Sputum Differential Cell Counts between groups defined by frequency of blood eosinophilia

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Never(11) | Rare(22) | Intermittent(25) | Persistent(24) |
| Paucigranular | 8 (72.7%) | 20 (90.9%) | 6 (24.0%) | 39 (47.6%) |
| Eosinophilic | 1 (9.1%) | 0  | 11 (44.0%) | 8 (33.3%) |
| Neutrophilic | 2 (18.2%) | 1 (4.5%) | 5 (20.0%) | 4 (16.7%) |
| Mixed | 0  | 1 (4.5%) | 3 (12.0%) | 7 (29.2%) |

Categorical variables expressed as n (%). Groups defined using 2% eosinophil and 61% neutrophil cutoffs

## **TABLE E8** – Contingency Tables for Proportions of Patients with Sputum Eosinophilia

|  |  |  |
| --- | --- | --- |
| Cross sectional (p<0.01) | Sputum Eosinophils <2% (n=43) | Sputum Eosinophils >2% (n=30) |
| Blood Eosinophils <300 cells/µL | 32 | 13 |
| Blood Eosinophils >300 cells/µL | 11 | 17 |
| Retrospective (p<0.05) | **(n=51)** | **(n=31)** |
| Never Blood Eosinophils >300 cells/µL | 10 | 1 |
| Historical Blood Eosinophils >300 cells/µL | 41 | 30 |

Categorical variables expressed as n (%)