Urinary leukotriene E4 as a biomarker in NSAID-exacerbated respiratory disease (N-ERD): a systematic review and meta-analysis

Malcolm Marquette[1,2*], Bhavesh V Tailor[2*], Philip C Calder[3,4], Peter J Curtis[5], Yoon Loke[2], Andrew M Wilson[1,2]

[1]Department of Respiratory Medicine, Norfolk & Norwich University Hospital, Norwich, UK
[2]Norwich Medical School, University of East Anglia, Norwich, UK
[3]School of Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, UK
[4]NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust and University of Southampton, Southampton, UK
[5]Department of Nutrition and Preventive Medicine, Norwich Medical School, University of East Anglia, Norwich, UK

*denotes equal contribution

Correspondence:
Malcolm Marquette, University of East Anglia Norwich Medical School Norwich NR4 7TJ, m.marquette@uea.ac.uk

Abstract:

Purpose of Review

Non-steroidal exacerbated respiratory disease (N-ERD) currently requires aspirin challenge testing for diagnosis. Urinary leukotriene E4 ($uLTE_4$) has been extensively investigated as potential biomarker in N-ERD. We aimed to assess the usefulness of $uLTE_4$ as a biomarker in the diagnosis of N-ERD.

Recent Findings

N-ERD, formerly known as aspirin-intolerant asthma (AIA), is characterised by increased leukotriene production. $uLTE_4$ indicates cysteinyl leukotriene production, and a potential biomarker in N-ERD. Although several studies and have examined the relationship between $uLTE_4$ and N-ERD, the usefulness of $uLTE_4$ as a biomarker in a clinical setting remains unclear.

Findings

Our literature search identified 38 unique eligible studies, 35 were included in the meta-analysis. Meta-analysis was performed (i.e., pooled standardised mean difference (SMD) with 95% confidence intervals (95% CI)) and risk of bias assessed (implementing Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy (Cochrane DTA)). Data from 3376 subjects was analysed (1354 N-ERD, 1420 ATA, and 602 HC). $uLTE_4$ was higher in N-ERD vs ATA (n=35, SMD: 0.80; 95% CI: 0.72-0.89). uLTE4 increased following aspirin challenge in N-ERD (n=12, SMD: 0.56; 95% CI: 0.26-0.85) but not ATA (n=8, SMD: 0.12; CI: -0.08-0.33). This systematic review and meta-analysis showed that $uLTE_4$ is higher in N-ERD than ATA or HC. Likewise, people with N-ERD have greater increases in $uLTE_4$

following aspirin challenge. However, due to the varied $uLTE_4$ measurement and result reporting practice, clinical utility of these findings is limited. Future studies should be standardised to increase clinical significance and interpretability of the results.

Keywords:

Asthma, N-ERD, non-steroidal anti-inflammatory respiratory disease, aspirin-intolerance, Samter's, Urinary leukotrienes E4.

Compliance with Ethical Standards:

Conflict of Interest:

The authors declare that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements) or non-financial interest (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.

Author Contribution:

All authors substantially contributed to conception and design of the study, acquisition of the data, or analysis and interpretation of the data; drafted the article or revised it for important intellectual content; gave final approval of the version to be submitted; and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Introduction:

NSAID-exacerbated respiratory disease (N-ERD) or aspirin exacerbated respiratory disease (AERD), formerly known as aspirin-intolerant asthma (AIA) and Samter's triad, is a phenotype of asthma characterised by increased leukotriene production and leukotriene driven inflammation[1]. N-ERD is the name used henceforth as it is the term accepted in current clinical practice[2]

N-ERD is clinically characterised by the presence of asthma, chronic rhinosinusitis with nasal polyposis, and exacerbation of respiratory symptoms on exposure to substances having cyclo-oxygenase 1 (COX-1) inhibiting activity[1,3]. The prevalence of N-ERD is reported to be 7% of asthmatics overall and approximately 15% in those who have severe asthma[4]. However, it occurs in 30-40% of those with asthma and nasal polyposis[5]. Accurate diagnosis of this asthma phenotype requires provocation testing, which involves nasal, oral or inhaled challenge with aspirin[6,7]. These procedures, whilst being clinically validated, do carry some inherent risks including significant bronchospasm and are thus not recommended for patients with severe airways disease. For these patients, diagnosis of N-ERD has typically relied on medical history alone, which increases the risk of misdiagnosing N-ERD, and the likelihood of providing inappropriate health management, by withholding the use of this class of medication in non-NERD individuals[2]. Consequently, it is considered highly desirable to identify a robust, accessible, and safe biomarker of N-ERD.

Given that leukotriene status is heightened in N-ERD, there is significant interest in establishing their utility as candidate biomarkers for the diagnosis and disease/treatment monitoring in N-ERD. More specifically, urinary leukotriene E4 ($uLTE_4$) excretion has been

identified as a surrogate marker of leukotriene production *in vivo* and is preferred to other leukotrienes (e.g. Leukotrienes B_4, C_4, and D_4), which have a short half-life and are difficult to measure[8,9]. To this extent, Hagan et al [10] reviewed the role of uLTE4 in the diagnosis of N-ERD in 2016. This is the only previous systematic review, of 10 studies, and showed $uLTE_4$ as a biomarker for N-ERD. However, the inclusion criteria for that review[10] required the availability of primary level data to carry out the necessary analysis, and a proportion of full text manuscripts were not available to the authors.

Therefore, in this present study we sought to update the work carried out by Hagan et al[10], whilst reviewing and analysing the broader literature on this subject to compare the baseline $uLTE_4$ levels in patients with N-ERD, aspirin tolerant asthma (ATA), and healthy control (HC) subjects. In addition, we aimed to determine the impact of aspirin challenge testing on $uLTE_4$ concentration in N-ERD and ATA individuals and the diagnostic accuracy of baseline $uLTE_4$ measurements to predict aspirin intolerance in patients with asthma. In keeping with Hagan et al[10], we analysed the different assays separately, given the variations in these techniques.

Methods:

Literature search

The protocol for the review was published in the PROSPERO database (CRD42021228674) and developed with reference to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) 2020 guidelines[11]. A systematic search of MEDLINE, EMBASE, EMCARE, CINAHL and PsycINFO was undertaken by a medical librarian in conjunction with one reviewer (B.V.T.) from database inception to 31st December 2021. In contrast to the previous review, a comprehensive search strategy was implemented which captured all studies reporting baseline uLTE4 levels in N-ERD and ATA groups, irrespective of whether these studies reported primary level data to answer our primary research question. No filters were used. The strategies were peer reviewed by a second reviewer (M.M.) prior to final execution of the search. Reference lists from included studies and review articles that were identified through the database searches were hand searched to identify additional articles for possible inclusion. Both Healthcare Databases Advanced Search (HDAS) and Rayyan were used to identify duplicate records and additional duplicates were manually removed before screening for inclusion. Articles were screened by two independent reviewers (B.V.T., M.M.). Disagreements between the reviewers were resolved through discussion. The full search strategy can be found in Online Resource 1.

Study eligibility

The following medical diagnosis terminologies i.e., N-ERD/AERD, Samter's triad and AIA, have been interchangeably used in the literature to describe the population of interest and were included within the search criteria to ensure completeness of data capture and synthesis.

Original research studies recruiting human subjects with asthma utilising $uLTE_4$ as a biomarker (*index test*) to differentiate N-ERD from ATA were considered for inclusion. Diagnosis of N-ERD required at least one of the following two criteria to be met (*reference standard*): a) positive aspirin challenge, either historic (case-control study design) or performed prospectively (singe-gate design); b) unequivocal history of asthma exacerbation following ingestion of aspirin and/or other NSAIDs. There were no age restrictions.

The following exclusion criteria were applied: publication types other than primary studies (review articles, case reports, conference abstracts, book chapters and letters to the editor); papers published in languages other than English if a translation could not be found. Studies concerning aspirin challenge testing of asthmatic patients were excluded if baseline (pre-challenge) $uLTE_4$ data was not reported in the published article, in supplementary material, or on request from the corresponding author of the publication.

Study outcomes

The primary study outcome was to determine whether $uLTE_4$ concentration at baseline in N-ERD is different from ATA and (non-asthmatic) HC subjects, using a between-group comparison. Secondary outcomes were a) to determine the diagnostic accuracy of baseline uLTE4 measurements to predict aspirin intolerance in patients with asthma; and b) to determine the change in $uLTE_4$ concentration in N-ERD and ATA following aspirin challenge testing.

Data extraction

Two reviewers (B.V.T., M.M.) independently extracted the following data from included studies: author(s); year of publication; country of origin; source of funding; demographic

characteristics (*n*, sex, age); clinical characteristics (inclusion/exclusion criteria, co-morbidities, definition of asthma, baseline pulmonary function); index test (method of $uLTE_4$ analysis, original units, nature of urine collection); reference standard (clinical history/aspirin challenge/both, criteria for N-ERD); mean and standard deviation (SD) of $uLTE_4$ at baseline for N-ERD, ATA and HC; diagnostic test accuracy (if reported – area under curve, cut-off value, sensitivity, specificity, positive predictive value, negative predictive value); mean and SD of $uLTE_4$ following aspirin challenge testing for N-ERD and ATA (if performed). Two attempts at requesting missing data from the corresponding authors of included studies were made by contacting them via e-mail. Disagreements in data extraction were resolved through discussion.

If relevant data concerning baseline and/or post-challenge $uLTE_4$ were presented in published figures but not specified as summary data in the accompanying text or supplementary materials, the underlying numerical data was extracted from relevant figures using WebPlotDigitizer (v4.4, California, USA), a web-based semi-automated extraction tool[12].

Risk of bias assessment

A modified version of the QUADAS tool from the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy was used to assess the methodological quality of included studies[13]. This was performed independently by two reviewers (B.V.T., M.M.), with disagreements resolved through discussion.

Data synthesis and meta-analysis

A descriptive synthesis of included studies was performed and structured around the review objectives. Studies reporting the mean and SD of $uLTE_4$ at baseline (+/- post-challenge) for N-

ERD, ATA, and HC were included in our meta-analysis. If the extracted data were described as the median with range, or the median with interquartile range, then the data were converted to mean and SD using established approximation methods[14]. Data presented in separate subgroups were combined using established formulae from the Cochrane Handbook for Systematic Reviews of Interventions[15]. Pooled standardised mean difference (SMD) and 95% confidence intervals (CI) were calculated. We investigated the presence of statistical heterogeneity among included studies by using the I^2 test. The random-effects model was used if there was significant heterogeneity ($I^2 > 50\%$), otherwise the fixed-effects model was used to combine the results. To explore possible sources of heterogeneity, meta-regression analysis was performed, with variables including publication year, country of study origin, sample size, male percentage, and baseline lung function. Any p values of < 0.05 were considered statistically significant.

In a change to the planned data synthesis as registered in Prospero, summary receiver-operating characteristic (SROC) modelling was not performed since individual data points were largely missing from included studies. Hence, evaluation of test diagnostic accuracy was not possible.

All data were extracted and stored in an Excel data file (Microsoft Excel for Mac; Microsoft Corporation, USA). Review Manager version 5.4 (The Cochrane Collaboration, Copenhagen, Denmark) and R software version 4.0.1 (R Foundation for Statistical Computing, Vienna, Austria) were used for conducting the meta-analysis.

Results:

Study selection

A total of 660 articles were identified [December 2021], with 547 article titles and abstracts reviewed following de-duplication. Of these, 491 articles were ineligible for full-text review. A total of 38 eligible full-text articles were reviewed (Fig. 1). Each article described a unique study. We performed qualitative synthesis of all included studies (n=38) and meta-analysis of 35 studies. Three of the studies which did not meet the criteria for inclusion in the meta-analysis did not have the required effect size data to allow for such an analysis.

Study characteristics

Included studies (n=38) were published between 1991 and 2021, across 8 countries [study numbers as follows: Japan (n=13), Poland (n=11), United States (n=5), South Korea (n=3), Sweden (n=2), United Kingdom (n=2), Italy (n=1), Switzerland (n=1)]. A total of n=1354 N-ERD, n=1420 ATA, and n=602 HC subjects were represented across the included studies, with n= 1010 (36.5%) males. In 19 studies, patients with N-ERD were study-defined N-ERD and/or there was clear documentation concerning co-morbid chronic rhinosinusitis and/or nasal polyposis status. In the remaining studies (n=19), the terminology AIA was used without reference to presence of nasal polyposis. The main characteristics of included studies are summarised in Table 1.

Across all the studies included in this review, $uLTE_4$ concentration was measured using one of 4 different techniques: (i) Amersham-enzyme immunoassay (A-EIA) (n=8), (ii) Cayman-enzyme immunoassay (C-EIA) (n=18), (iii) mass spectrometry (MS) (n=7), and (iv)

radioimmunoassay (RIA) (n=6), with *Sanak et al*., reporting results with both C-EIA and MS (thus represented twice in these overview data)[16].

Twenty-seven studies used positive aspirin challenge alone (inhaled, intravenous, nasal, or oral) as the reference standard to diagnose N-ERD, two studies used convincing clinical history of asthma exacerbation secondary to ingestion of aspirin alone, and the remaining nine studies used either positive challenge or convincing clinical history. Further details on the aspirin challenge criteria and methodology for $uLTE_4$ measurement are found in Table 2.

Key findings

Studies with different $uLTE_4$ measurement methodologies were combined. Thirty-five studies including 1127 N-ERD and 1191 ATA reported that the baseline concentration of $uLTE_4$ was significantly higher in N-ERD (SMD: 0.80, 95% CI = 0.72 to 0.89; I^2 = 42%, Fig. 2)[16–50]. Fifteen studies including 780 ATA and 452 HC reported that the baseline concentration of $uLTE_4$ was significantly higher in ATA (SMD: 0.45, 95% CI = 0.17 to 0.74; I^2 = 78%, Fig. 3)[16,19,21–26,30,32,35,36,38,43,49]. The concentration of $uLTE_4$ increased following aspirin challenge in N-ERD (12 studies, n = 314 SMD: 0.56; 95% CI = 0.26 to 0.85, Fig. 4) [25,33–35,37–41,44,46,47] but not ATA (8 studies, n = 187, SMD: 0.12; 95% CI = -0.08 to 0.33, Fig. 5) [16,19,21–26,30,32,35,36,38,43].

Meta-regression and Risk of Bias

Heterogeneity observed between studies in this meta-analysis was low. Despite this, we performed meta-regression analysis to assess the contribution of several covariates on effect size across studies included in pooling of effect size for baseline $uLTE_4$ in N-ERD vs ATA comparison. I^2 for this analysis was low (42%). Meta-regression revealed that country of study

had an impact on effect size (I^2 = 13.05%). Furthermore, by identifying different study sites and including this in the multiple regression analysis, we found that this would account for an I^2 of 100%, suggesting that heterogeneity across studies in this meta-analysis is related to site. There was no significant impact on the effect size when other covariates (publication year, percentage male participants, baseline lung function, and methodology for $uLTE_4$ measurement) were analysed by means of meta-regression, and hence no significant impact on heterogeneity between studies was noted.

Risk of bias assessed by means of the QUADAS tool from the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy[13], was acceptable across all studies; however 37.8% of quality assessment items were unfulfilled (Fig. 6 & 7). The following risk of bias items were poorly reported across all studies (reported in <30% overall): spectrum of representative patients (10.5%) and independent interpretation of index and reference standard tests (0%).

Discussion:

Our meta-analysis of 35 studies demonstrated a statistically significant higher baseline concentration of $uLTE_4$ in patients with N-ERD compared to those with ATA and HC, adding an addition 25 studies to the previous review. These findings corroborate current knowledge regarding the importance of leukotriene status in patients with N-ERD, and again identify $uLTE_4$ as a potential biomarker in N-ERD diagnosis and disease monitoring. For the subset of studies reporting $uLTE_4$ measurements before and after aspirin challenge testing, a significant rise in $uLTE_4$ was seen in patients with N-ERD, but not those with ATA. This is the first meta-analysis which evaluates the change in $uLTE_4$ concentrations following aspirin challenge in N-ERD compared to ATA, and the results are consistent with previous literature demonstrating that the magnitude of nasal and/or respiratory reactions to provocative aspirin challenges in asthmatics is associated with both the degree of baseline $uLTE_4$ elevation and the rise in $uLTE_4$ during a challenge[51,52].

This study has a number of limitations. Because individual data points were largely missing from most studies, sensitivity and specificity testing was not possible. Four studies did provide some data of interest[8,9,16,38], but this was insufficient to carry out this analysis. The corresponding authors of the rest of the included studies were contacted via e-mail asking for this data, but there was no response from any of them. Studies included were published between 1991 to 2021, a total span of 30 years, and this will invariably carry with it a variation in practice of $uLTE_4$ measurement. Although, our meta-regression analysis did not identify year of publication as contributing to heterogeneity across studies, four different methodologies were used to measure $uLTE_4$ across the studies included. However, to account for this, a separate comparison analysis for studies using each of the methods was performed and then

the studies were combined. This analysis has revealed that despite the different methodologies, there was no significant heterogeneity across studies (Fig. 2), meaning that different methodologies were not shown to have a significant impact on effect size. Although the different methodologies did not appear to result in heterogeneity, there was a large number of methodologies used and methods of reporting the data. The country of publication had an effect on heterogeneity but not when site was included in the multiple regression. This suggests that site was responsible for the heterogeneity, presumably due to a composite of methodology, definition of N-ERD and population sampled. Greater standardisation of the procedure and reporting is required in clinical research and clinical practice.

There was also variation in the way asthma was defined across studies, with American Thoracic Society (ATS) criteria, Global Initiative for Asthma (GINA) guidelines, National Heart, Lung and Blood Institute criteria, and physician diagnosis all used. In 17 studies, definition of asthma was not specified. This is important given that it will dictate the characteristics of the population being studied. Similarly, the definition of aspirin intolerance varied across studies. Although most studies performed aspirin challenge testing (either retrospectively or prospectively), there was considerable variation in the challenge agent employed and the diagnostic cut-off for a positive test (i.e., fall in FEV_1 relative to baseline). Approximately half of studies included in the meta-analysis (18/35) provided clear documentation of co-morbid chronic rhinosinusitis and/or nasal polyposis status, or the aspirin-intolerant cohort was defined as N-ERD. The remaining studies did not provide such population characteristics. In several studies, summary data concerning $uLTE_4$ levels were not stated in the published text or supplementary materials and had to be derived from figures using a web-based extraction tool. This invariably is an estimation of the data. Similarly, for studies where the reported data was described as median with range or interquartile range, this required conversion to mean and

SD using published approximation methods. This is important because of the potential impact this has on the accuracy of the results and the impact this could have on the weight of the individual studies, and therefore the overall study results. We therefore feel that standardisation of result reporting should also be implemented.

One of the most important features of this meta-analysis is the enforced use of the standardised mean difference. This summary statistic is used when the measurement scales of the various papers are too diverse to be pooled in a meta-analysis, and thus they have to be converted to a common statistical denominator, or statistical units. The use of the standardised difference means that we cannot know the absolute difference between groups, nor can we define a diagnostic cut off. This is important especially when considering developing study protocols going forward with the aim of establishing sensitivity and specificity. This work has identified the need for standardisation of such protocols to move closer towards achieving clinical significance. Our results show that all the methodologies employed to measure $uLTE_4$ yielded comparable results across studies. Mass spectrometry has been described in a number of publications as the gold standard for the measurement of leukotrienes in biological fluids [53,54], however, access to MS and cost might impact its availability in the clinical setting, whereas, enzyme immunoassays might be more readily available. We feel that these are important considerations to make going forward in the protocol development for research of this subject area. This would allow calculation of the absolute mean difference in clinically useful terms rather than the slightly abstract concept of a standardised mean difference. The current heterogeneity in methods and measurement makes it impossible to come up with clinically relevant recommendations on the use of such diagnostic technology.

It should also be noted that most studies have been conducted in specialist centres and excluded participants with uncontrolled asthma or participants reporting a respiratory tract infection or asthma exacerbation in the preceding 6 weeks. While this provides a well-defined cohort for research purposes, our findings may not be generalisable to patients undergoing testing in routine clinical practice, especially since N-ERD is most prevalent among patients with severe asthma.

Overall, the risk of bias was acceptable across all studies. However, in all included studies, it was not reported whether study authors were blinded to baseline $uLTE_4$ data (*index test*) when performing aspirin challenge testing or obtaining clinical history of aspirin intolerance (*reference standard*). The primary aim of many included studies was not to determine test diagnostic accuracy, which may account for this. It is also unclear how much a lack of blinding could affect interpretation of aspirin challenge testing since challenges are normally undertaken following a set protocol with a pre-determined diagnostic cut-off.

The finding of a significant rise in uLTE4 following aspirin challenge testing is in keeping with the central role leukotriene release as a cause of upper and lower airway symptoms[55]. Daffern et al. showed that rise in uLTE4 following challenge was related to severity of airflow obstruction post challenge. However, interestingly, the rise doesn't seem to be attenuated by inhibition of 5-lipoxygenase which should reduce leukotriene production[51,56].

Conclusion:

The true prevalence of N-ERD is unclear and it is likely to be significantly underdiagnosed especially in those individuals with mild respiratory symptoms, and because of difficulty accessing specialist centres for diagnostic confirmation[2,4]. An accurate diagnosis of N-ERD

is important, as this can have an impact on both treatment modalities and management of co-morbid chronic diseases such as ischaemic heart disease and chronic pain. Including $uLTE_4$ in the diagnostic algorithm for patients suspected to suffer from N-ERD, would be especially useful in individuals who may be at higher risk of adverse reactions from aspirin challenge testing because of increased risk such as FEV_1 <70%, or nasal pathology (precluding nasal aspirin challenge test)[2]. This safe, non-invasive biomarker for N-ERD may reduce clinician time needed for aspirin challenge testing and would be cost-effective. Future research should be directed at evaluating diagnostic specificity and sensitivity to establish biomarker diagnostic accuracy and employing standardised methods of $uLTE_4$ measurements to ensure any results yielded are more readily translatable to impact clinical practice.

Figures:

Fig. 1 Flowchart showing process of article selection for inclusion

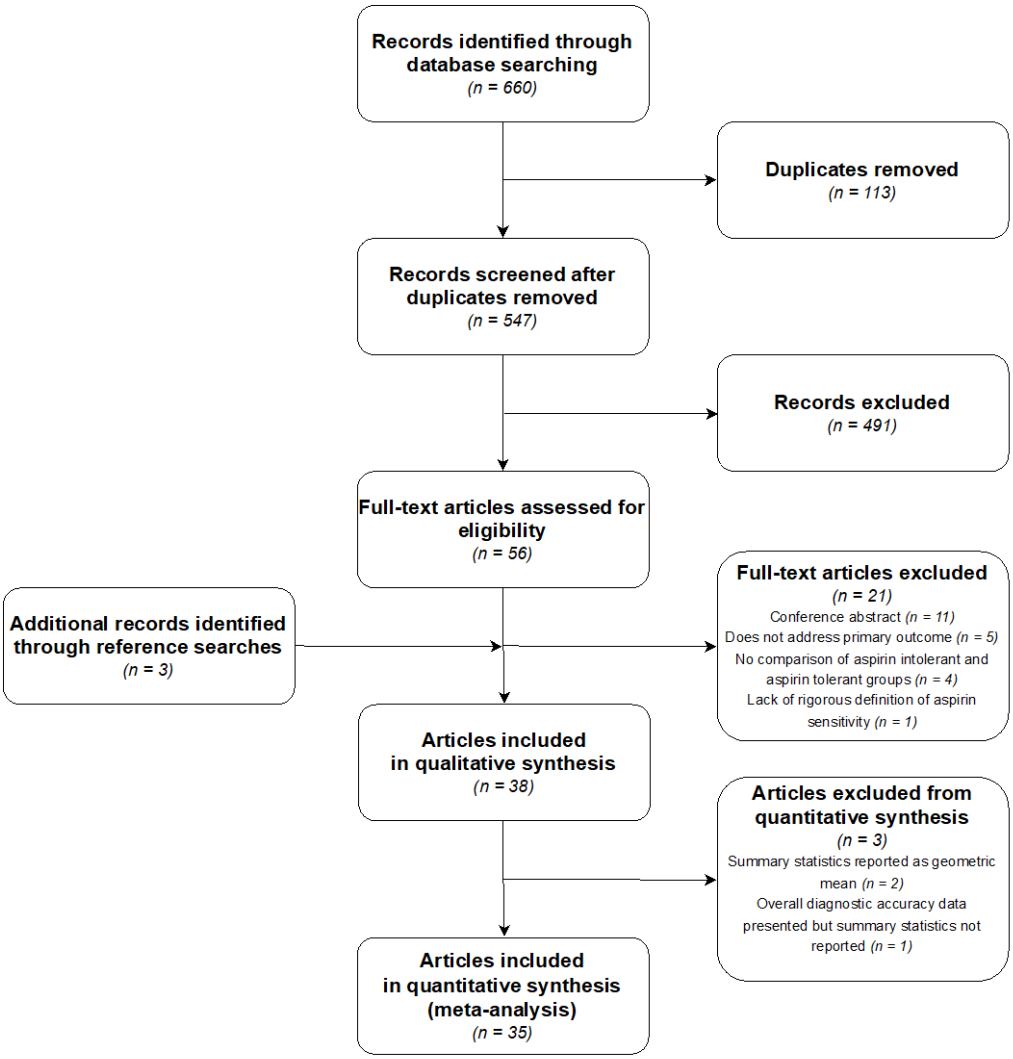


Fig. 2 Forest Plot of baseline uLTE$_4$ for N-ERD vs ATA [35 studies]

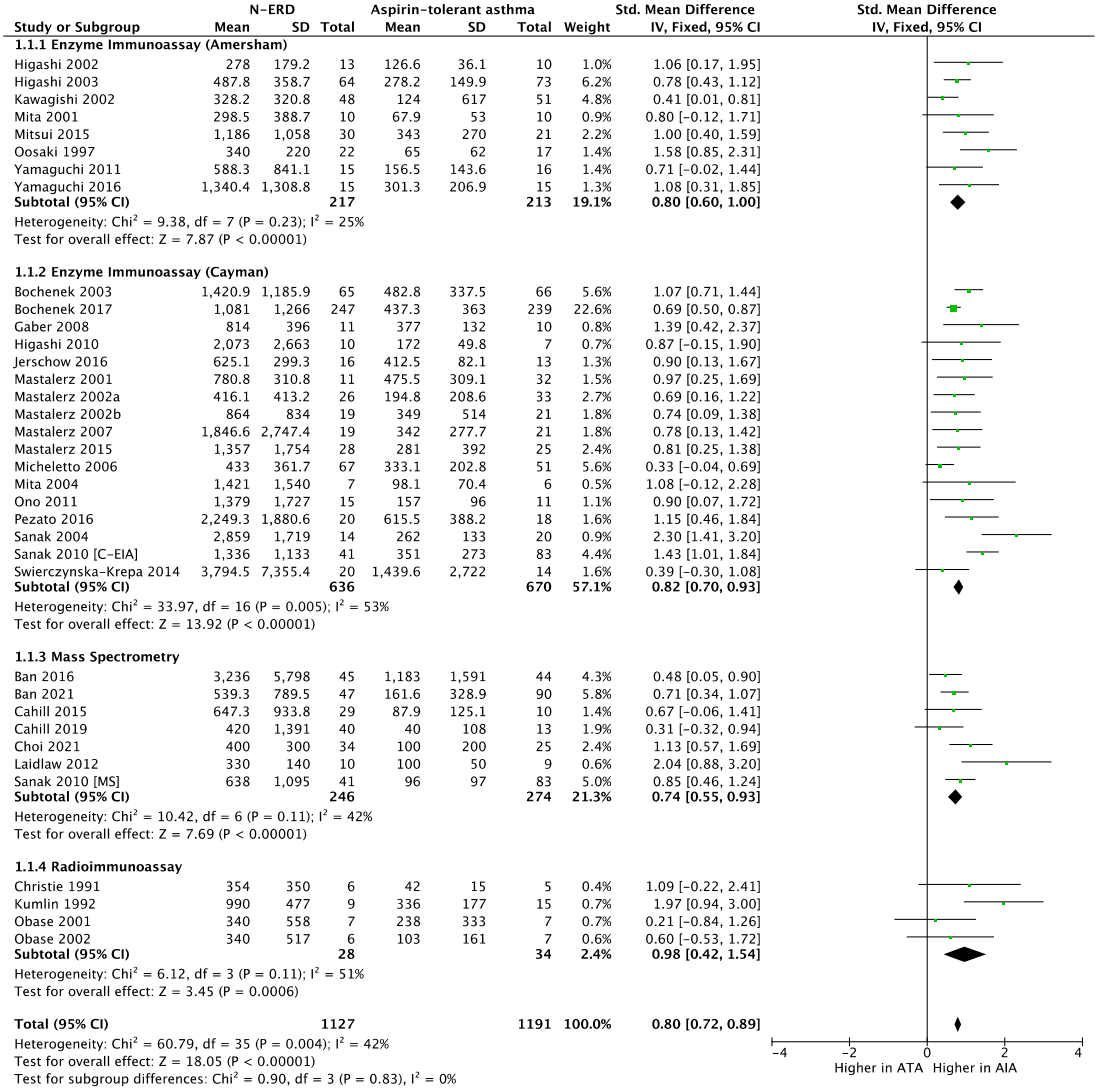


Fig. 3 Forest Plot of baseline uLTE$_4$ for ATA vs HC [15 studies]

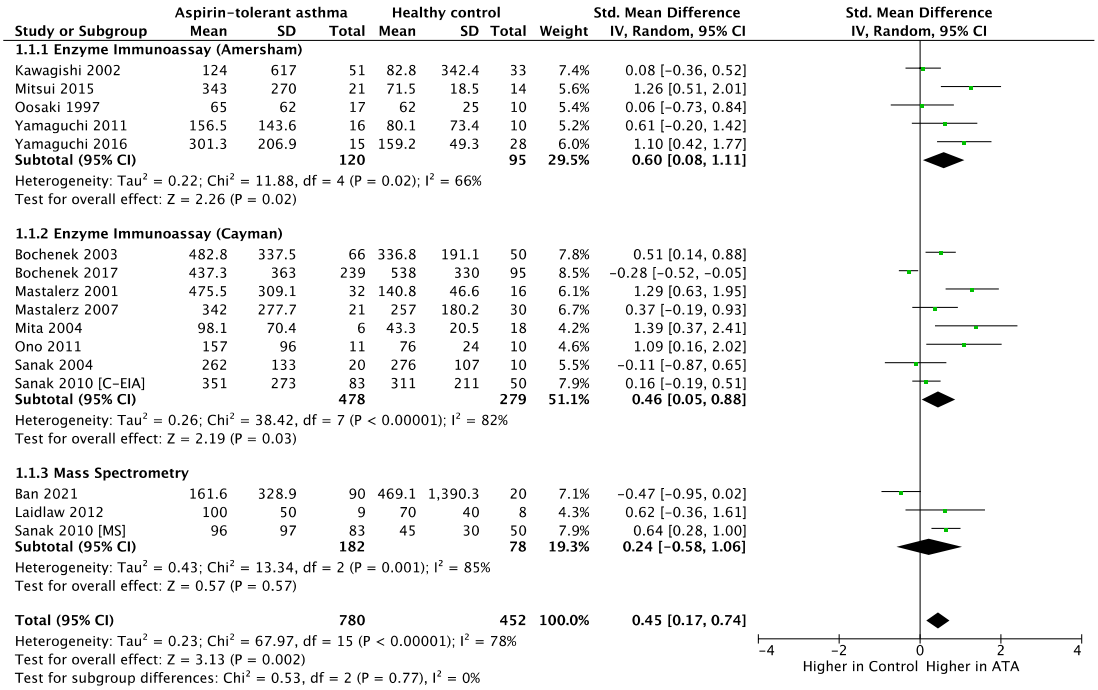


Fig. 4 Forest Plot of uLTE$_4$ Pre- and Post-aspirin challenge in N-ERD [12 studies]

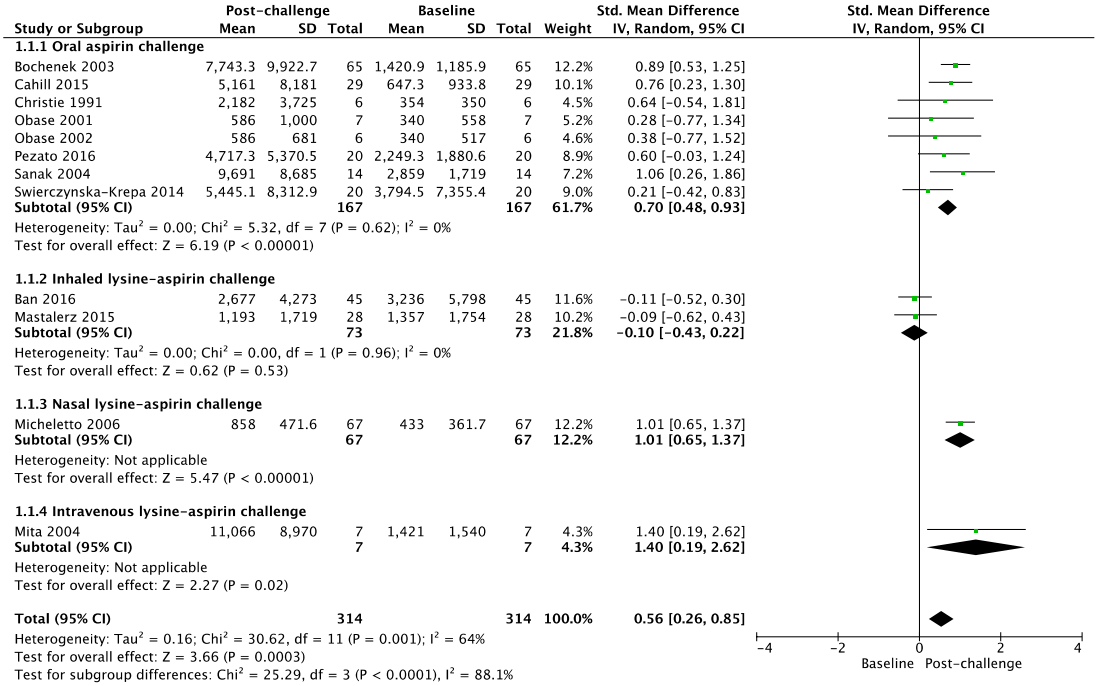


Fig. 5 Forest Plot of uLTE$_4$ Pre- and Post-aspirin challenge in ATA [8 studies]

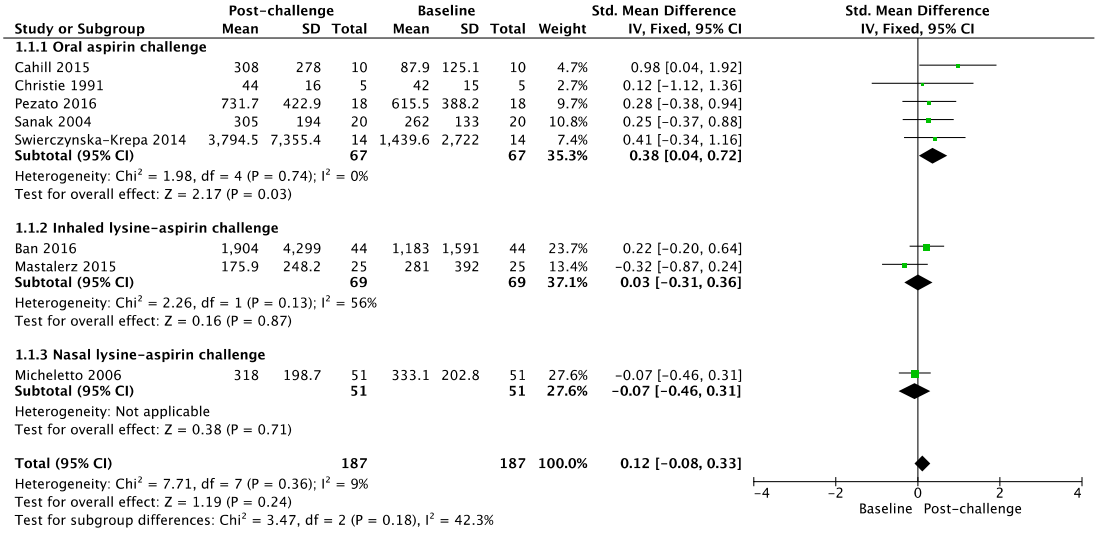


Fig 6. Risk of Bias Summary

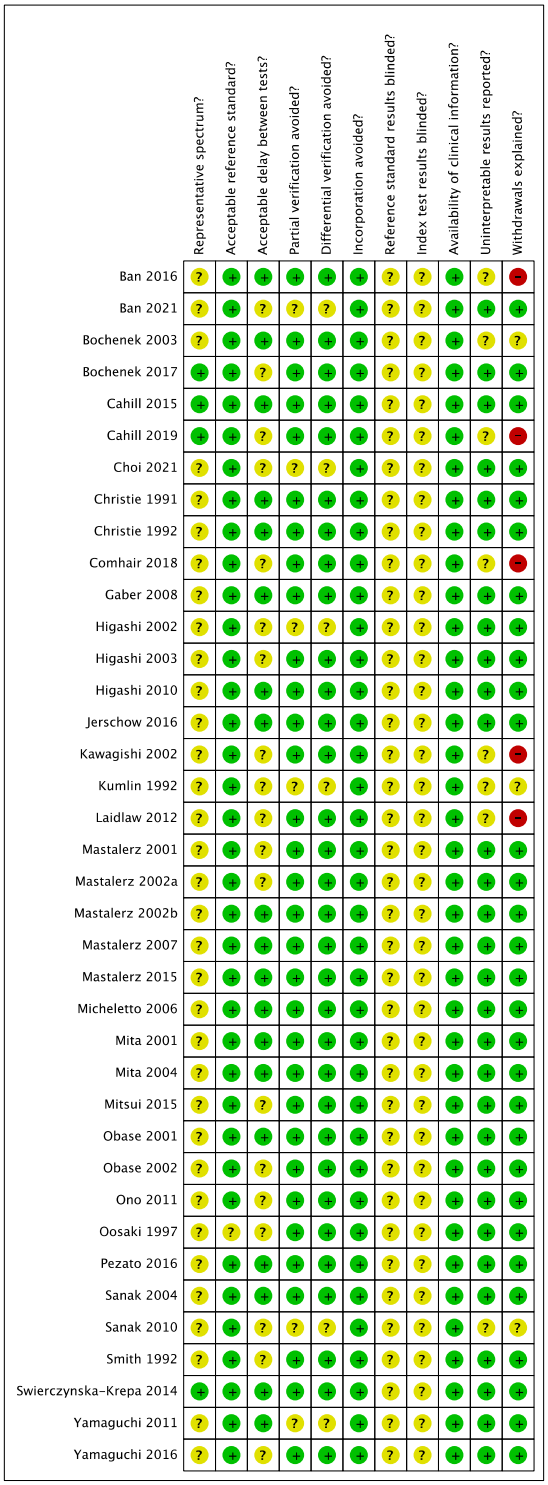
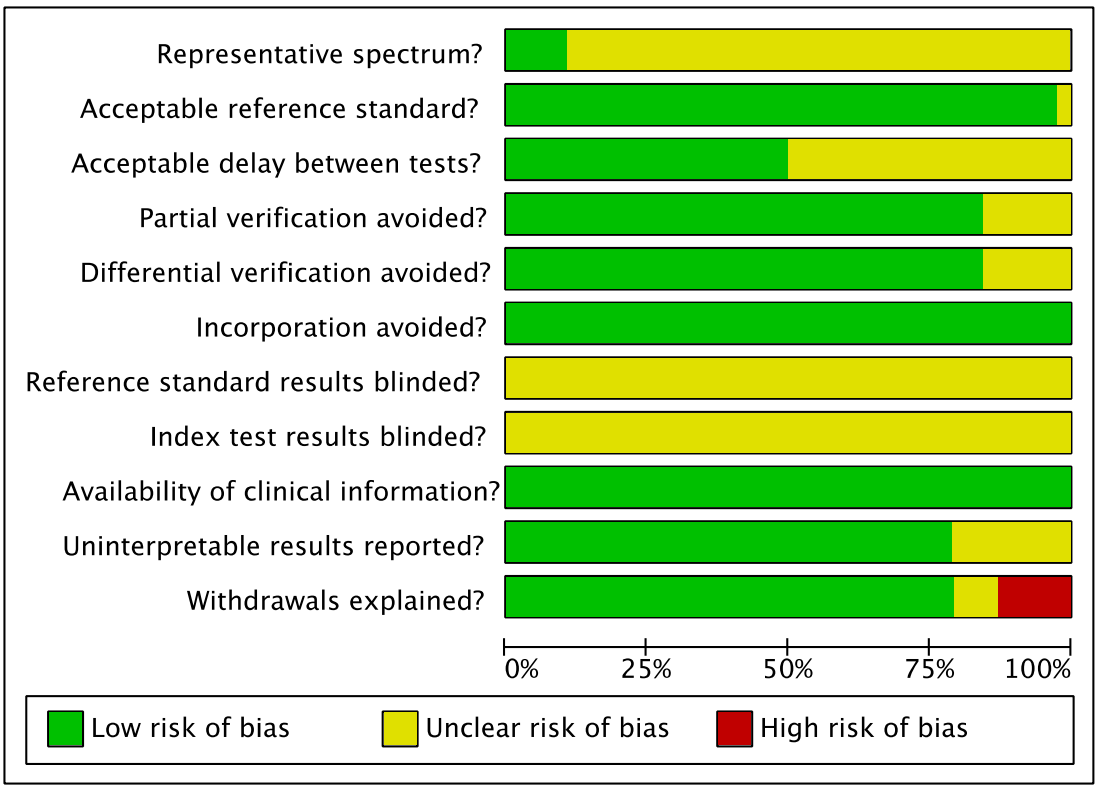


Fig 7. Risk of Bias Graph



Supplementary Information (SI):

Online Resource 1 Detailing the search strategy

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