Journal of Breath Research

ACCEPTED MANUSCRIPT

The role of measuring exhaled breath biomarkers in sarcoidosis: A systematic review

To cite this article before publication: Dayle Terrington *et al* 2019 *J. Breath Res.* in press https://doi.org/10.1088/1752-7163/ab1284

The Role of Measuring Exhaled Breath Biomarkers in Sarcoidosis: A Systematic Review

Dayle L. Terrington[1], Conal Hayton[2,3], Adam Peel[1], Stephen J. Fowler[2,3], William Fraser[1], Andrew M. Wilson[1]

Affiliations:

[1] Norwich Medical School, University of East Anglia, Norwich, United Kingdom.

[2] Division of Infection, Immunity and Respiratory Medicine, School of Biological Sciences, University of Manchester, Manchester, United Kingdom.

[3] North West Lung Centre, Manchester University NHS Foundation Trust, Manchester, United Kingdom.

Contact for Correspondence:

Dr Dayle Terrington
Clinical Research Fellow in Respiratory Medicine
Bob Champion Research and Education Building
Norwich Medical School
University of East Anglia
Norwich
NR4 7UQ

Telephone: 01603 593294

Email: D.terrington@uea.ac.uk

Abstract

Introduction: Sarcoidosis is a chronic granulomatous disease of unknown aetiology with a variable clinical course and prognosis. There is a growing need to identify non-invasive biomarkers to differentiate between clinical phenotypes, identify those at risk of disease progression and monitor response to treatment.

Objectives: We undertook a systematic review and meta-analysis to evaluate the utility of breath-based biomarkers in discriminating sarcoidosis from healthy controls, alongside correlation with existing non breath-based biomarkers used in clinical practice, radiological stage, markers of disease activity and response to treatment.

Methods: Electronic searches were undertaken during November 2017 using PubMed, Ebsco, Embase and Web of Science to capture relevant studies evaluating breath-based biomarkers in adult patients with sarcoidosis.

Results: 353 papers were screened; 21 met the inclusion criteria and assessed 25 different biomarkers alongside VOCs in exhaled breath gas or condensate. Considerable heterogeneity existed amongst the studies in terms of participant characteristics, sampling and analytical methods. Elevated biomarkers in sarcoidosis included 8-isoprostane, carbon monoxide, neopterin, TGF-β1, TNFα, CysLT and several metallic elements including chromium, silicon and nickel. Three studies exploring VOCs were able to distinguish sarcoidosis from controls. Meta-analysis of four studies assessing alveolar nitric oxide showed no significant difference between sarcoidosis and healthy controls (2.22ppb; 95% CI -0.83, 5.27) however, a high degree of heterogeneity was observed with an I^2 of 93.4% ($p<0.001$). Inconsistent or statistically insignificant results were observed for correlations between several biomarkers and radiological stage, markers of disease activity or treatment.

Conclusions: The evidence for using breath biomarkers to diagnose and monitor sarcoidosis remains inconclusive with many studies limited by small sample sizes and lack of standardisation. VOCs have shown promising potential but further research is required to evaluate their prognostic role.

Background

Sarcoidosis is a chronic multi-system disease of unknown aetiology characterised by the presence of non-caseating epithelioid cell granulomas (1). Almost any organ can be affected with involvement of the lung parenchyma and intrathoracic lymph nodes occurring in 90% of patients (2). The exact aetiology remains inconclusive and is likely to involve both genetic and environmental factors resulting in an exaggerated immune response to an unidentified antigen (3). Infective agents such as mycobacterium and *Propionibacterium acnes* have been postulated as possible precipitating antigens (4).

The clinical course and prognosis of sarcoidosis is extremely variable with spontaneous remission occurring in up to 50% of patients within 2 years of diagnosis and in the vast majority of cases within 5 years (3). Up to a third may develop a chronic and progressive disease (1), resulting in increased morbidity or mortality due to pulmonary fibrosis, cardiac or neurological involvement. Current treatment strategies focus around immunosuppression with corticosteroids, however, the evidence for this remains weak (5). In addition, such treatment is often prolonged and may give rise to significant side effects.

Currently there is a distinct lack of clinically useful and robust biomarkers with sufficient sensitivity and specificity to help aid diagnosis, predict patterns of clinical behaviour, determine prognosis or monitor response to treatment. Decisions to commence treatment in those with pulmonary disease are often challenging and frequently based upon clinical symptoms or pulmonary function tests alone. Various biomarkers have been assessed in urine, serum and bronchoalveolar lavage fluid but lack adequate sensitivity and specificity for clinical use (6). Many are not routinely available in clinical practice or require invasive sampling methods and are therefore unsuitable for serial monitoring.

There is a growing need to develop non-invasive biomarkers which not only aid in diagnosis, but also measure disease activity and guide treatment decisions particularly as medicine evolves and becomes more personalised to the individual patient. Collecting and analysing components in exhaled breath is one such method (7) and may involve analysing either exhaled gas or breath condensate (8).

A wide range of potential biomarkers in exhaled breath have been assessed in sarcoidosis including nitric oxide, eicosanoids, inflammatory cytokines, markers of granulomatous inflammation and volatile organic compounds (VOCs). To date no previous systematic review has been performed to evaluate the current evidence of such markers and their use in sarcoidosis. The purpose of this systematic review was to examine the available evidence for the role of breath-based biomarkers and their utility in the diagnosis and management of sarcoidosis patients.

Methods

Study Design

This systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement (9) and was registered with the International Prospective Register of Systematic Reviews (PROSPERO) (Registration number: CRD42017076233). There were three main objectives for this systematic review which included 1) identification of a breath-based biomarker able to differentiate sarcoidosis from healthy controls, 2) differentiate between varying clinical phenotypes as well as 3) correlation with pre-existing markers of disease activity used in clinical practice or response to treatment.

Search Strategy

A literature search was performed using PubMed, Embase, Web of Science and Ebcso with the following search strategy: 'sarcoidosis' OR 'sarcoid' OR 'interstitial lung disease' OR sarcoidosis [MeSH Terms] AND 'breathomics' OR 'exhaled breath test' OR 'exhaled breath' OR 'breath biomarker' OR breath test [MeSH]. Bibliographies and reference lists were also manually screened to identify

additional papers. Initial electronic searches were undertaken during November 2017 and repeated in October 2018. No additional papers were identified during the repeated search.

Eligibility Criteria

The titles and abstracts of all papers identified through literature searches were reviewed and assessed for suitability and relevance by two independent reviewers (DT and CH). Discrepancies were resolved by discussion and involvement of a third reviewer (AMW). Papers considered eligible for inclusion were any studies that specifically evaluated any biomarker in exhaled breath in sarcoidosis and compared this to either healthy controls or differing clinical phenotypes (based upon radiological stage, pulmonary function test parameters, active vs. inactive or fibrotic vs. non-fibrotic disease). Other comparators of interest included treatment with immunosuppression and correlation with any current non breath-based biomarkers used in clinical practice and measured in either serum or bronchoalveolar lavage fluid. Additional inclusion criteria included 1) participants aged $\geq$18 years; 2) confirmation of the diagnosis of sarcoidosis as per internationally agreed standards or guidelines, multidisciplinary discussion or biopsy proven as well as 3) presented in full text and published in English. To ensure the broadest possible collection of evidence for evaluation, all study designs were included.

Data Extraction and Quality Assessment

Data extraction was performed independently by two reviewers (DT and CH) using a customised data collection form designed to capture the relevant information. The data collected included the main author's surname, country and year of publication, number of participants and healthy controls including baseline characteristics such as age, gender, ethnicity, smoking status, disease activity, radiological stage and treatment regime if available. Additional information gathered related to the biomarker of interest and included sampling and analytical methods and differences in the concentrations reported between the two groups or comparators of interest. The quality and overall risk of bias was assessed using the QUADAS-2 assessment tool (10). This consists of four key domains

covering patient selection, index and reference tests and flow of patients through the study. For the purposes of this review, the index test was the breath biomarker under investigation and the reference standard was a diagnosis of sarcoidosis either histologically or as per internationally agreed criteria. Where possible, meta-analysis was performed using Open Meta Analyst (11). Where a particular breath biomarker was assessed in two or more studies and meta-analysis was not possible, a narrative review has been provided.

Results

Electronic searches identified a total of 353 papers, 97 of which were duplicates. Two further studies were identified through bibliography and reference searches. Titles and abstracts were reviewed for relevance and full text was retrieved for 41 studies, 21 of which met the full inclusion criteria. The PRISMA screening flow diagram is shown in Figure 1.

Overall Characteristics of the Studies

Of the 21 studies identified, three were longitudinal in design (12-14), whereas the remaining 18 were cross-sectional case-controlled studies. Publication dates ranged from 1997 to 2017. The sample sizes were relatively small across all studies and ranged from 5 to 87 participants with sarcoidosis. Considerable heterogeneity existed between the studies in terms of participant characteristics, diagnostic methods, classification of disease activity and treatment status, particularly in studies which assessed the same biomarker. Study characteristics are shown in Tables 1, 2 and 3.

Alongside expired VOCs, 25 different individual breath-based biomarkers were assessed across the studies. Ten studies measured biomarkers in exhaled breath condensate (EBC), whereas the remaining eleven directly assessed biomarkers in expired breath gas. Four studies also examined the breath biomarker of interest in relation to the concentration in bronchoalveolar lavage fluid (BALF) and blood.

The sampling methods also differed considerably between studies, as did the methodology for biomarker analysis (Table 2). EBC was sampled using a variety of collection systems and whilst all

studies described methodology of collection, only four cited reference to standardised recommendations (12) (15) (16) (17). A variety of chemiluminescence analysers were used to measure nitric oxide, with three studies citing sampling as per published recommendations (14, 18, 19). VOCs were assessed using different methods such as an electric nose, mass spectrometry, ion mobility spectrometry and a colorimeter sensor array.

Utility of breath-based biomarkers in differentiating sarcoidosis from healthy controls

Twenty studies evaluated the biomarker of interest in differentiating sarcoidosis from healthy controls, however, many were not adequately matched for age and gender between the two cohorts.

Markers of oxidative stress

Biomarkers of interest related to oxidative stress were assessed across five studies (Table 4). Four studies assessed 8-isoprostane in EBC; three were cross-sectional case-controlled studies and one was longitudinal in design (12) (16) (17) (20). In all studies 8-isoprostane was significantly higher in participants with sarcoidosis. The sarcoid cohorts varied between the studies with regards to disease duration and treatment (Table 1). Due to the heterogeneous methods of reporting it was not possible to pool data for meta-analysis.

Another study assessed exhaled carbon monoxide and found that the mean concentration was significantly elevated in sarcoidosis compared to healthy controls (21).

Metallic elements

Two studies explored the concentration of metallic elements in EBC (7) (22) (Table 5). Corradi *et al* (22) evaluated various metallic elements across a variety of interstitial lung diseases, twenty-two of which had biopsy proven sarcoidosis. The median concentration of nickel, chromium and silicon was significantly higher amongst participants with sarcoidosis. Using multinomial logistic regression, metallic patterns in EBC could distinguish healthy controls from sarcoidosis, however overlap with other interstitial lung diseases occurred in 64% of cases. Mohan *et al* (7) reported the mean

concentration of calcium in EBC was significantly elevated in healthy controls compared to sarcoidosis patients diagnosed according to international guidelines. Similar findings were observed by Corradi *et al* with respect to calcium, however the increase failed to reach statistical significance.

Cytokines and markers of inflammation

Four studies assessed cytokines and markers of inflammation in EBC including cysteinyl leukotrienes (CysLT) (16), leukotriene B4 (LTB4) (16), tumour necrosis factor-α (TNFα) (7), neopterin (23), transforming growth factor-β_1 (TGF-β_1) (23) and interleukin-27 (IL-27) (24) (Table 5). CysLT was detectable in 75% (21/28) of participants with sarcoidosis and below the limit of detection in all controls. LTB4 was detectable in 79% (22/28) of sarcoidosis patients compared to 76% (13/17) of healthy controls, however, no statistically significant difference was observed between the two groups. TNFα was detectable in all participants, with the exception of one subject in each cohort and was significantly elevated in those with sarcoidosis. Neopterin was detectable in 44% (7/16) of participants with sarcoidosis and 10% (2/20) of healthy controls, whereas TGF-β_1 was detectable in all subjects. Mean concentration of both neopterin and TGF-β_1 was significantly higher in those with sarcoidosis. No statistically significant difference was observed in the mean concentration of IL-27 between sarcoidosis and controls.

Nitric oxide

Exhaled nitric oxide was evaluated across six studies, four cross-sectional studies (18) (19) (25) (26) and two longitudinal observational studies (13) (14) involving between 10 and 52 participants (Table 6). Studies were heterogeneous across most participant characteristics, in particular ethnicity, smoking status and treatment exposure which limits the comparability and generalisability. The analytical platform and sampling methods also differed considerably across all six studies and only three (14) (18) (19) carried out sampling in accordance with current available recommendations (27) (28).

Two studies measured nitric oxide at various flow rates and calculated alveolar concentration of nitric oxide ($C_{Alv}NO$) (14) (19), whereas one study exclusively reported mean alveolar nitric oxide (13). Of the remaining studies, one reported mean peak, alveolar and end-tidal nitric oxide (26), one reported the mean end expiratory nitric oxide (18) and the final study reported exhaled nitric oxide during the plateau phase of expiration when carbon dioxide was 70-80% of the maximum (25). Only two studies reported significantly elevated nitric oxide in participants with sarcoidosis compared to healthy controls which ranged from (Mean ± SEM) 6.7 ± 0.5 *versus* 5.2 ± 0.73ppb p=0.05 and 9.8 ± 0.4 *versus* 4.1 ± 0.2ppb p<0.001 (13) (18).

Meta-analysis was performed on the four studies which reported alveolar nitric oxide to determine if alveolar nitric oxide differed between patients with sarcoidosis and healthy controls using a random effects model (Figure 2). When pooling data (n=95), the mean difference was 2.22ppb (CI -0.83, 5.27) which was statistically insignificant. The I^2 test was 93.4% (p<0.001) indicating a high degree of heterogeneity, which most likely reflects the fact that pooled data included a mixed population incorporating those with active and inactive disease, alongside those with newly diagnosed sarcoidosis and pre-existing disease of variable durations.

Volatile organic compounds

The role of VOCs in discriminating participants with sarcoidosis from healthy controls was evaluated across four studies, (29) (30) (31) (32) (Table 7). One study (29) employed ion mobility spectrometry to identify characteristic peak VOC profiles in patients with confirmed sarcoidosis (n=5) compared to those with suspected sarcoidosis (n=4) who received an alternative diagnosis. Using principle component analysis, those with confirmed sarcoidosis had a highly congruent distribution of metabolites compared to those without sarcoidosis.

Fijten and colleagues (31) performed gas chromatography and time-of-flight mass spectrometry in a two-part discovery and validation study. Nine discriminatory VOCs were found to predict sarcoidosis with 79.4% accuracy with a sensitivity and specificity of 75.4% and 92.5% respectively with sampling

undertaken during 2010-2012. The receiver operating characteristic (ROC) curve had an area under the curve (AUC) of 91.3%. During the validation phase, sampling took place at a different centre in the Netherlands during 2015 and the overall prediction rate was found to be 74.1% accurate with a sensitivity of 68.0% and specificity of 79.3%. The ROC curve had an AUC of 76.4%.

A further study (32) evaluated a colorimeter array capable of identifying 36 VOCs in the detection of non-small cell lung cancer. In the clinical control group which included 20 subjects with sarcoidosis, a random forest model was generated from 70% of subjects which was validated with the remaining 30%. When comparing sarcoid patients against all other study subjects, the model error rate was 10% with a validation sensitivity and specificity of 16.7% and 81.1% respectively ($p=0.69$). Using electric nose technology, Dragonieri *et al* (30) were able to differentiate untreated pulmonary sarcoidosis from healthy controls with a cross-validated accuracy of 83.3% ($p<0.001$).

Considerable variation existed amongst the studies with respect to disease duration and smoking status which may affect the VOC profiles obtained; two recruited exclusively non-smokers or ex-smokers in subjects with sarcoidosis (30) (32), however one study also included three smokers in the control group (32). Fijten and colleagues (31) recruited current smokers in the discovery cohort, whereas the validation cohort consisted entirely of non-smokers, however subgroup analysis was performed to ensure this had no confounding effect. Furthermore, this was the only study which attempted to control for the confounding effect of exposure to exogenous VOCs in the environment by performing sampling of controls in the same location as patients and recruiting spouses as healthy controls where possible. In addition, recruitment of spouses and partners as healthy controls also helped to minimise the influence of dietary exposure on the breath profiles.

Miscellaneous breath-based biomarkers

Total protein was explored in two studies (7) (23) both of which identified elevated concentrations in EBC, however only one demonstrated statistical significance when comparing to healthy controls (Table 5). Angiotensin converting enzyme (ACE) was detectable in 2/16 participants with sarcoidosis

and 3/20 healthy controls, and whilst mean values were not presented, the authors concluded no statistically significant difference between the two groups (23). Hepatocyte growth factor (HGF) was evaluated by Piotrowski *et al* (2010c) (33) and was detectable in 56% (36/64) of participants with sarcoidosis and 87% (13/15) of healthy controls, however no significant difference was observed between the two groups.

Utility of breath-based biomarkers in differentiating between clinical phenotypes, disease activity, radiological stage or in correlation with traditional serological biomarkers used in clinical practice

Ten studies classified patients into those with and without active disease, however, the criteria used for defining disease activity varied considerably amongst the studies (Table 3). Whilst most studies reported radiological stage, several did not assess or correlate the breath biomarker of interest in relation to the radiological stage. Correlation of the breath biomarker with pulmonary function tests or current serological biomarkers such as calcium, peripheral lymphocytes and ACE was also variable across the studies.

Markers of oxidative stress

No significant difference was reported in the mean concentration of carbon monoxide between those with active and inactive sarcoidosis. Furthermore, no correlation was observed with pulmonary function tests, serum ACE, radiological stage or clinical activity.

Two studies evaluated the utility of 8-isoprostane in assessing disease activity according to the percentage of lymphocytes in BALF with conflicting results. Psathakis *et al* (20) reported significantly elevated 8-isoprostane levels in those with active disease on the basis of clinical symptoms and markers of disease activity including lymphocytic alveolitis (>18% lymphocytes in BALF) compared to healthy controls and those with inactive disease. No significant difference was observed between those with inactive disease and healthy controls and there was no correlation with radiological stage.

In contrast, Piotrowski *et al* (2007) (16) observed no such findings with respect to the percentage of lymphocytes in BALF, however, there was a trend of increasing 8-isoprostane with increasing radiological stage although this did not reach statistical significance. Elevated 8-isoprostane correlated with the percentage and number of eosinophils in BALF. In two further studies, 8-isoprostane was higher in those with stage III disease compared to stage I disease (12) and when comparing stage III disease against healthy controls (17). No other significant difference was observed between radiological stages. Levels of 8-isoprostane in Piotrowski *et al* (2010b) (12) were detectable in 26/40 (65%) of participants with sarcoidosis and 8/34 (24%) of healthy controls and subjects with undetectable 8-isoprostane were assigned a value half of the detection limit. Undetectable 8-isoprostane occurred more frequently in controls and those with stage I disease compared to stages II and III disease and the authors concluded that during follow-up at 6-12 months, the chance of remission (relative risk) was 3.33 (95% confidence interval; 1.20-5.78) when 8-isoprostane was undetectable at baseline which was statistically significant.

When evaluating somatostatin-receptor scintigraphy in assessing disease activity compared to traditional markers of disease activity, 8-isoprostane was significantly higher in those with a positive scan, compared to a negative scan indicative of inactive disease (15).

Correlations between 8-isoprostane and various pre-existing biomarkers was not observed amongst the studies, with the exception of Psathakis *et al* (20) where 8-isoprostane correlated positively with serum ACE and Piotrowski *et al* (2010a) (17) where 8-isoprostane correlated negatively with the percentage of lymphocytes in BALF (See Table 4).

Metallic elements

In the two studies which explored metallic elements, Corradi *et al* (22) reported a positive correlation between iron in EBC and diffusion capacity of the lung for carbon monoxide (DLCO). The authors also reported no correlation with respect to disease severity, however the criteria used to classify disease severity was not specified. Mohan *et al* (7) reported no significant correlations between calcium and

radiological stage. Negative correlations were reported between EBC calcium and forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) (See Table 5).

Cytokines and markers of inflammation

All studies with the exception of Loke *et al* (24) discussed the breath biomarker of interest in relation to radiological stage and reported no significant correlations were observed. In Piotrowski *et al* (2007) (16) there was a general trend of increasing LTB4 with increasing radiological stage, however this failed to reach statistical significance. Both neopterin and TGF-β_1 demonstrated a general trend of increasing biomarker concentration with declining FEV_1 (23). Alongside radiological stage, Piotrowski and colleagues (16) also classified participants with sarcoidosis according to percentage of lymphocytes in BALF, however no significant difference was observed in the mean concentration of CysLT or LTB4 in those with and without lymphocytic alveolitis (>18% lymphocytes) (See Table 5).

Nitric oxide

Across the six studies, four (13) (14) (18) (26) classified participants into those with and without active disease. Criteria and methods used to define and classify disease activity differed amongst the studies. In two studies, all participants had newly diagnosed sarcoidosis and were presumed to have active disease on the basis of clinical symptoms, extra pulmonary manifestations and elevated serum ACE in one study (13) and based upon clinical symptoms and elevated lymphocytes in BALF in the other (26). Two studies (14) (18) reported no significant difference between individuals with and without active disease. Five studies reported no significant differences in exhaled nitric oxide in relation to radiological stage (14) (18) (19) (25) (26). With the exception of a weak correlation between exhaled nitric oxide and DLCO (r=0.52, p=0.029) in Ziora *et al,* (18) and a negative correlation between $C_{AlV}NO$, DLCO (r=-0.40, p=0.012) and FVC (r=-0.52, p=0.001) in Choi *et al,* (14) there were no other associations observed in any study between nitric oxide and any marker of disease activity or pre-existing biomarker (See Table 6).

Volatile organic compounds

No studies assessed or reported differences in VOC profiles in relation to radiological stage, disease activity or correlation with pre-existing biomarkers (See Table 7).

Miscellaneous breath-based biomarkers

Of the two studies that evaluated concentration of total protein in EBC, one (7) reported no significant difference between participants of differing radiological stage, whereas in the remaining study (23) the relationship between total protein and radiological stage was not assessed. In Piotrowski *et al* (2010c) (33) all participants were considered to have active disease however there was no correlation observed with HGF or any parameter measured (See Table 5).

The effect of treatment on the breath-based biomarker

Six studies recruited participants that were treatment naïve (16) (17) (18) (25) (26) (33). In one study treatment status was not specified (32). Fourteen studies recruited participants receiving a range of treatments including inhaled and oral corticosteroids through to immunosuppressive agents. Only nine studies reported outcomes or differences in the breath biomarker with respect to the effect of treatment (7) (12) (13) (14) (19) (21) (22) (23) (30).

Markers of oxidative stress

No significant correlation was found between exhaled carbon monoxide and subjects receiving steroid therapy (21). Only one of the studies reporting 8-isoprostane assessed treatment effect; two recruited treatment naïve participants (16) (17) and the remaining two studies did not report the effect of treatment (20) (15). In the longitudinal study by Piotrowski *et al* (2010b) (12) all participants were treatment naïve at baseline and during follow-up over 6-12 months eight participants received treatment. Those treated with corticosteroids had significantly lower concentrations of 8-isoprostane ($p=0.02$), however the authors reported that a similar effect was not observed in those who underwent spontaneous remission (Table 4).

Metallic elements

Corradi *et al* (22) reported no significant difference in the concentration of metallic elements in EBC was observed between treated and untreated participants (See Table 5).

Cytokines and markers of inflammation

Of the studies which assessed inflammatory cytokines, three recruited participants receiving treatment (7) (23) (24). In the remaining study (16) all participants were treatment naïve at enrolment. No significant difference was reported in neopterin or TGF-β_1 between subjects receiving treatment compared to those not receiving treatment (23). Mohan and colleagues (7) reported that a patient with severe disease receiving treatment with intravenous, oral and inhaled corticosteroids had TNFα which was undetectable. The effect of treatment on IL-27 was not assessed or reported (24) (See Table 5).

Nitric oxide

Three studies (13) (14) (19) included participants treated with inhaled or oral corticosteroids or a combination of steroids and immunosuppression. Moodley *et al* (13) reported that nitric oxide decreased from 9.8 ± 0.4 to 5.9 ± 1.4ppb, p=<0.01 at six weeks follow-up in eight patients treated with 40mg prednisolone once daily. In contrast, Choi *et al* (14) observed no consistent pattern of change in nitric oxide at any flow rate, $C_{Alv}NO$ or flux of nitric oxide from the airway wall in six patients who received treatment and completed follow-up over 3-23 weeks duration. Cameli *et al* (19) reported no significant differences between treated and untreated participants and furthermore, Ziora *et al* (18) reported no significant differences between those with and without an indication for commencing treatment (See Table 6).

Volatile organic compounds

Three studies (29) (30) (31) included participants treated with steroids or immunosuppression, however only one study reported the effect of treatment on the VOC profile. Dragonieri and

colleagues (30) reported that participants with untreated sarcoidosis could be discriminated from healthy controls with a cross-validated accuracy of 83.3%, however breath-prints from patients with treated and untreated sarcoidosis could be discriminated less easily (See Table 7).

Quality Assessment and Risk of Bias

Included studies were all of relatively small sample size and of variable quality. Outcomes of the QUADAS-2 quality assessment are shown in Figures 3 and 4. Unclear risk of bias existed for the vast majority of studies with respect to participant selection due to the lack of clarity surrounding recruitment methods. It is unclear as to whether consecutive or random sampling occurred in order to enrol participants, or if highly selected patients with sarcoidosis were recruited. The vast majority of studies were cross-sectional and case-controlled in design and frequently there was inadequate matching for age and gender amongst control groups. There was considerable variation in sampling and analytical techniques all of which influence the results obtained in breath analysis making comparability difficult (28) (34) (35). Sample sizes were small and ranged from 5-87 participants with no power calculation reported in any study. With the exception of one study (31), all were single-centre which ultimately limits the generalisability. Finally, in all studies it was unclear whether breath sampling and analysis was conducted by an investigator blinded to the participants' disease status.

Discussion

This systematic review demonstrates that the evidence for the role of breath-based biomarkers in sarcoidosis remains inconclusive. Twenty-one studies were identified through electronic searches which assessed 25 individual breath-based biomarkers and expired VOCs. Many studies were of small sample size and varying quality with considerable heterogeneity in patient characteristics and populations recruited. In addition, the lack of gold standard for defining disease activity also contributed to the variability of the findings reported, particularly in studies which assessed the same biomarker such as exhaled nitric oxide and 8-isoprostane. With the exception of three studies, all were

cross-sectional in design and therefore the lack of longitudinal data makes it difficult to draw firm conclusions with regards to the clinical relevance and utility of potential biomarkers.

Another concern was the variation in length of disease duration which ranged from those newly diagnosed to 11 years, particularly in studies which did not attempt to assess for active disease. Only seven studies (13) (14) (16) (17) (20) (25) (26) recruited participants with newly diagnosed disease which was likely to be active. Whilst sarcoidosis is often a self-limiting disease, resolving spontaneously within two years (3) the prolonged time lag between diagnosis and breath-sampling in several studies may have confounded results leading to false negatives.

Amongst the studies which evaluated EBC and exhaled nitric oxide there was lack of standardisation in breath sampling and analytical techniques. Alongside many patient related factors, dilution of EBC and biomarkers at the lower limit of detection have been postulated as a cause of variability amongst studies (36). These disparities have been recently addressed by the European Research Society task force with recommendations put forward to standardise sampling and analytical techniques (34) (35). Of the ten EBC studies included in this review, six assigned an arbitrary value of half the lower limit of detection for biomarkers that were undetectable and specified the number of participants in which this applied to. In the remaining studies, Psathakis *et al* (20) did not specify a detection threshold, nor the proportion of subjects in which 8-isoprostane was undetectable or how this was handled during analysis, whereas, in Mohan *et al,* (7) TNFα was detectable in all but one subject from each cohort (sarcoid and controls). In contrast, Piotrowski *et al* (2012) (15) and Piotrowski *et al* (2010a) (17) specified a detection threshold of 5pg/ml and assigned an arbitrary value of half this lower limit in those with undetectable 8-isoprostane, however it is unclear as to the number of participants to which this applied. If these samples were comprised of mainly healthy controls or subjects with less severe clinical phenotypes of sarcoidosis, then assigning them a higher value could potentially nullify any possible differences observed between the groups of interest, in particular when comparing disease

severity and radiological stages. Whilst the concentration may be below the limit of detection it is also important to consider that lack of detection may reflect the true absence of the biomarker.

Breath biomarkers investigated in sarcoidosis included markers of oxidative and nitrosative stress, cytokines, macrophage activation, metallic elements and VOCs. Oxidative stress has been implicated in a variety of interstitial lung diseases including sarcoidosis (37). Markers of oxidative stress elevated in sarcoidosis included carbon monoxide and 8-isoprostane. Other markers such as hydrogen peroxide and ethane have also been reported to be higher in sarcoidosis and other forms of interstitial lung disease (38) (39). Given its low specificity alongside the influence of passive and active smoking, exhaled carbon monoxide is unlikely to be of clinical utility (21). 8-isoprostane is a prostaglandin-like compound produced by free-radical oxidation of arachidonic acid (40). Elevated 8-isoprostane has been identified in both serum and BAL in sarcoid patients, however was not found to correlate with markers of disease activity including FVC, DLCO or oxygen saturation (41). Evidence from EBC supports this (12) (16) (17) (20), reflecting a role for oxidative stress in the pathophysiology of sarcoidosis. However, its use as a discriminative diagnostic biomarker is uncertain as 8-isoprostane has been shown to be elevated in other forms of interstitial lung disease including idiopathic pulmonary fibrosis (42), systemic sclerosis (43) and non-fibrotic lung diseases such as asthma (44). Conflicting results were observed with respect to disease activity, and whilst there was a trend for increasing 8-isoprostane with advancing radiological stage, this was not statistically significant in one study. Piotrowski *et al* (2010b) (12) reported that undetectable 8-isoprostane at baseline may predispose to increased chances of early remission, however, it was also observed that such patients could present during follow-up with elevated 8-isoprostane in the absence of clinical signs and symptoms of disease. The authors concluded that given the short period of follow-up it was unclear if such patients were at risk of future relapse or flare. Further research with larger powered studies and longer follow-up periods are therefore required to assess its potential role as a prognostic marker.

Several cytokines including TNFα and TGF-β_1 have been shown to be implicated in the pathogenesis of sarcoidosis and augmentation of inflammation (6). Additionally, TNFα is elevated in those with active disease (45). Whilst TNFα and TGF-β_1 in EBC were significantly elevated in sarcoidosis compared to controls in keeping with previous research, neither correlated with clinical activity or radiological stage, however, there was a trend for increasing TGF-β_1 and declining FEV_1 which was not statistically significant and again most likely reflected insufficient power to detect significant differences. Neopterin is a metabolite of guanosine triphosphate secreted from activated macrophages (6). Serum and urinary levels appear to correlate with disease activity, radiological stage and likelihood of spontaneous remission (6) (46) (47). Although neopterin was significantly higher amongst those with sarcoid, it was only detected in 7 of 16 patients and again failed to correlate with disease activity, radiological stage or response to treatment. This indicates the need for not only a larger cohort study, but also a more sensitive detection method for biomarkers which may only be present in extremely dilute concentrations.

Nitric oxide is a marker of nitrosative stress which has largely been evaluated as a biomarker in asthma (28). It has also been shown to be elevated in a variety of interstitial lung diseases (48). Multiple factors have been shown to influence the fraction of exhaled nitric oxide including spirometric manoeuvres, exercise, concurrent steroid medication , inhaled corticosteroids and atopy (28). In addition, smoking lowers exhaled nitric oxide (49). Given the heterogeneity across the six studies assessing nitric oxide coupled with inadequate control for many of these confounding factors it is likely this gave rise to the conflicting results observed. Whilst four studies recruited non-atopic healthy controls, Choi *et al* (14) also recruited participants with concurrent asthma. The effect of atopy was only evaluated in one study, Wilsher *et al* (25), which demonstrated significantly elevated levels of nitric oxide in atopic sarcoid patients compared to non-atopic sarcoid patients. It is unclear as to whether or not atopy confounded the positive findings observed in both Ziora *et al* and Moodley *et al* (13) (18). Both Wilsher *et al* and Choi *et al* also included current smokers in comparison to control groups consisting of non-smokers which may have confounded results. Differences in baseline participant characteristics and

treatment exposures may have also accounted for the differing results obtained. Moodley and colleagues recruited only African and Asian participants, nearly all of whom had extra-pulmonary manifestations with raised serum ACE. This cohort are more likely to have represented those with more severe disease possibly accounting for higher mean baseline concentrations of exhaled nitric oxide in comparison to those studies which exclusively recruited Caucasians. Cameli *et al* reported no differences between untreated and those treated with oral steroids, however, in Choi *et al*, 69% of participants with sarcoidosis were prescribed inhaled corticosteroids and it is unclear if this supressed the level of exhaled nitric oxide as no subgroup analysis took place with the 31% not receiving inhaled corticosteroid. Similarly to 8-isoprostane, the lack of specificity of exhaled nitric oxide coupled with multiple factors influencing levels of nitric oxide limit its use as a diagnostic tool. Furthermore, there is no robust evidence to support its use for prognostication or monitoring in sarcoidosis.

There has been a growing interest into VOCs as a potential source of biomarkers in respiratory disease (50) (51). Highly sensitive analytical techniques such as gas chromatography-mass spectrometry have facilitated discovery and identification of novel VOCs in a range of respiratory diseases including pneumonia, asthma, chronic obstructive pulmonary disease, lung cancer and idiopathic pulmonary fibrosis (52) (53) (54). Of all the breath-based biomarkers evaluated in this review, VOCs have shown promising potential, particularly as a diagnostic tool to distinguish sarcoidosis from health.

Fijten *et al* (31) identified a set of nine compounds which discriminated between sarcoidosis and healthy controls. These included isoprene, 2-methylpentane, benzene, 3-methylhexane, p-Benzoquinone, phenol, D-Limonene and dibenzofuran all of which were present in lower concentrations in sarcoidosis. In contrast, the concentration of iodomethylcyclopentane was elevated in exhaled breath from sarcoidosis patients compared to controls.

Isoprene is a by-product of normal cholesterol synthesis and prominent in exhaled breath (55). Reduced exhaled isoprene has been observed in lung cancer and may therefore be of limited use as a biomarker in sarcoidosis (56) (57). The branched alkanes 3-methylhexane and 2-methylpentane are

thought to represent pollutants and their biosynthesis is thought to relate to changes in oxidative stress (31). Interestingly, despite representing the greatest importance in the classification model with the highest statistical significance, their concentration was found to be lower in sarcoidosis compared to controls. This is conflicting in relation to other markers of oxidative stress such as 8-isoprostane and hydrogen peroxide which are elevated in sarcoidosis and interstitial lung diseases. Phenol is metabolised endogenously from benzene and previous research has demonstrated background emission from Tedlar bags (58). Fijten and colleagues therefore remarked that the role of phenol as a discriminatory VOC in sarcoidosis requires interpretation with caution. Dibenzofuran and iodomethylcyclopentane have not previously been identified in exhaled breath and therefore the presence in patients with sarcoidosis warrants further exploration (31). Dragonieri *et al* (30) reported the breath prints between healthy controls and treated sarcoidosis patients were indistinguishable suggesting normalisation of the breath profile following treatment. VOCs could therefore be used to monitor treatment response. Further longitudinal data is required to explore how the VOC profile changes over time and correlates with disease activity, pulmonary function tests and serological markers. In addition, rigorous validation should be undertaken as only one study evaluating VOCs, Fijten *et al,* sought to externally validate their dataset by sampling at both a different time and location.

Limitations

There are several limitations to this systematic review. Firstly, only studies published in English language were included. The search strategy identified three further studies, two of which were published in Polish (39) (59) and one in German (60). Secondly, a rigorous inclusion criteria was applied with respect to the methods surrounding diagnosis of sarcoidosis. As such, this resulted in the exclusion of two papers due to insufficient details surrounding the diagnostic criteria for sarcoidosis (61, 62). We attempted to contact the authors for further clarification, however did not receive any correspondence. One of these papers by Antczak *et al* (62) aimed to correlate eicosanoids in BALF and

EBC in a variety of respiratory diseases including sarcoidosis. Furthermore, and similar to Psathakis *et al,* those with active disease and a lymphocytic alveolitis >18% had significantly higher concentrations of 8-isoprostane in EBC and BAL fluid compared to those with non-active disease. This strict inclusion criteria was justified as with all reviews surrounding a diagnostic test of accuracy in order to improve validity. Finally, due to variation in reporting methods and a lack of homogeneity, it was not possible to combine, for meta-analysis, all studies which evaluated the same biomarker.

Conclusions and directions for future research

Whilst various breath-based biomarkers have been assessed in sarcoidosis, the strength of the evidence remains weak and inconclusive. This reflects the poor quality and variability in terms of design, methodology and small sample sizes across the spectrum of studies. Under-powering alongside difficulties in identifying biomarkers likely to be highly diluted in breath samples may have both contributed in failures to detect statistically significant differences. It seems unlikely that a single component in exhaled breath would be of diagnostic value due to lack of sensitivity and specificity. Further research is required to assess the utility of these biomarkers in the prognostic setting. Early research involving VOCs has shown promising potential and may provide a unique breath-print which could not only aid diagnosis, but also aid clinical phenotyping to identify those at risk of chronic disease or relapse, and also monitor response to treatment. Ideally, studies should involve much larger sample sizes across multiple centres and be longitudinal in design thus allowing sequential monitoring of breath-biomarkers over time. Standardised and validated methods should also be established in both the sampling and analysis of breath components.

Funding & Acknowledgements

No funding was received for this systematic review and this research is being undertaken as part of a PhD programme (DT). The authors would like to thank Dr Jocelyn Keshet-Price for her review of the final manuscript.

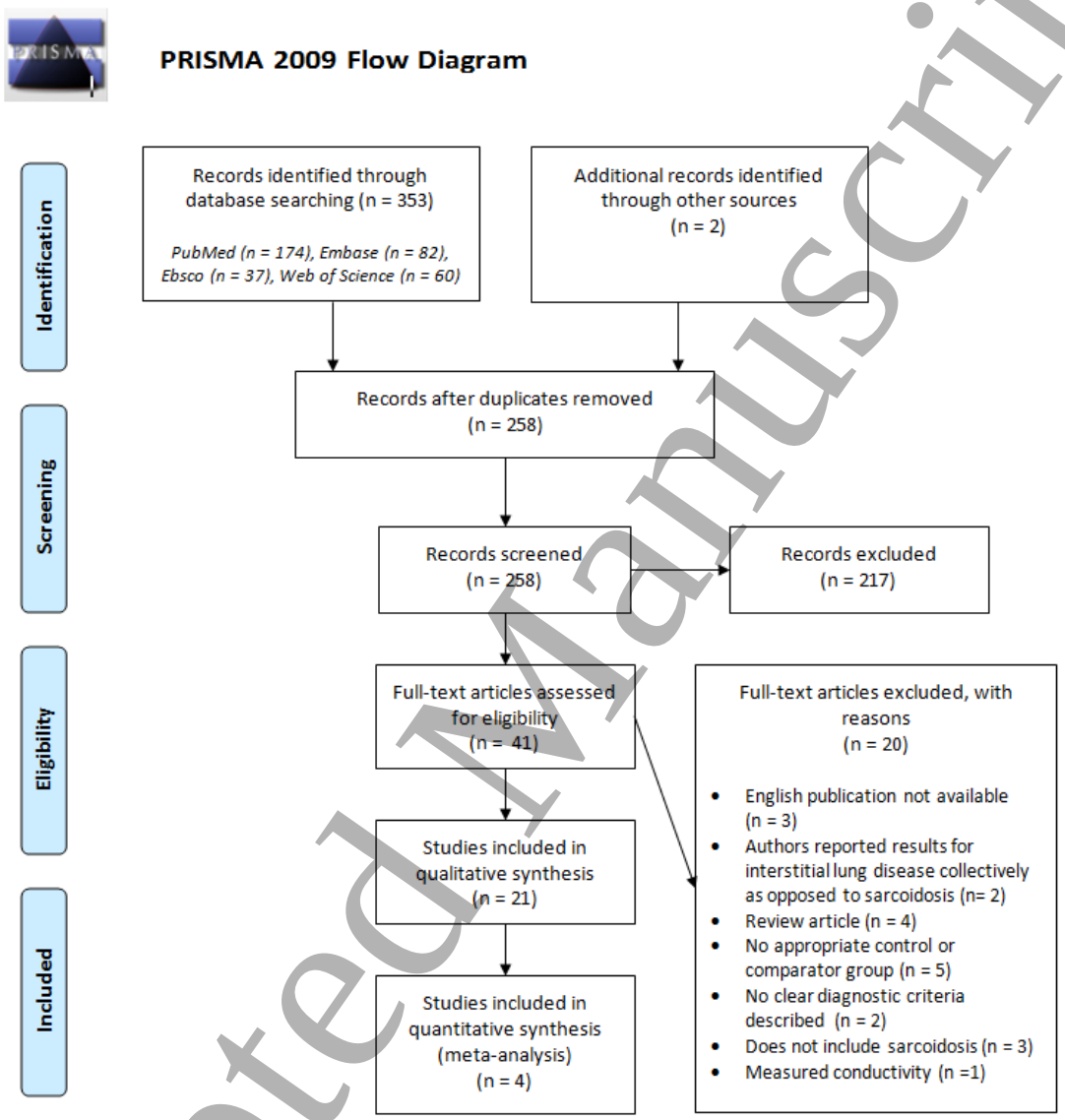

Figure 1: PRISMA 2009 Flow diagram.

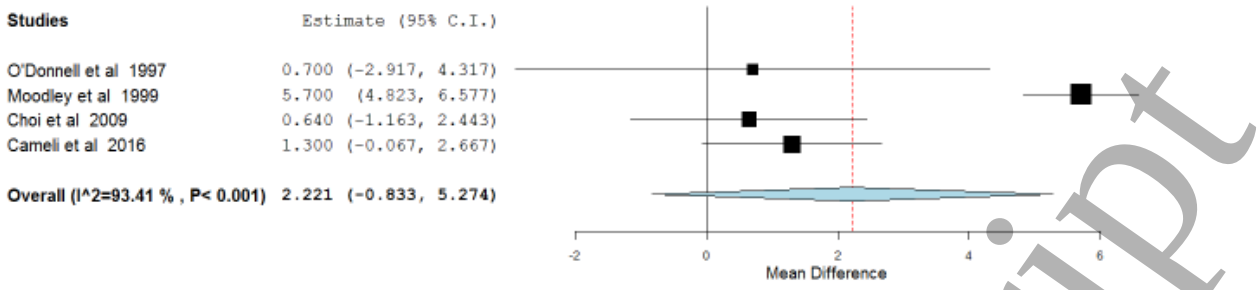

Figure 2: Continuous random-effects meta-analysis of the mean difference of alveolar nitric oxide in sarcoidosis compared to healthy controls. Study weights: O'Donnell *et al* 20.18%, Moodley *et al* 27.52%, Choi *et al* 25.64% and Cameli *et al* 26.66%.

Author / Year	Country	Study Design	Biomarker	Sarcoidosis (n), age (years), % male	Healthy Controls (n), age (years), % male	Ethnicity	Smoking Status (current/ex/never) (Sarcoidosis) *(Healthy Controls)*	Diagnostic Methods	Disease Duration	Radiological Stage (0/I/II/III/IV)	Treatment Exposure
O'Donnell *et al* (1997) (26)	Ireland	Cross-sectional case-control	Nitric oxide (Peak, end-tidal and $C_{ALV}NO$)	n=10 35.5 (22-63)* 80%	n=12 28.2 (22-44)* 66%	Irish Caucasian (n=10)	Not specified *All non-smokers*	Biopsy (n=10)	Newly diagnosed (n=10)	0/4/3/2/1	All treatment naïve
Moodley *et al* (1999) (13)	South Africa	Longitudinal	Nitric oxide (Alveolar)	n=12 41 ± 2.1† 50%	n=21 42 ± 3.8† 48%	African (n=10) Asian (n=2)	Not specified *All non-smokers*	Biopsy (n=12)	Newly diagnosed (n=12)	0/2/9/1/0	All treatment naïve at baseline. At six weeks follow-up n=8 received steroid treatment
Ziora *et al* (2004) (18)	Poland	Cross-sectional case-control	Nitric oxide (Mean end expiratory)	n=27 26-67* 67%	N=11 31-52* 73%	Caucasian (n=27)	All non-smokers *All non-smokers*	Biopsy (n=27)	Disease duration <2 years	0/6/10/11/0	All treatment naïve. Treatment indicated in n=12
Psathakis *et al* (2004) (20)	Greece	Cross-sectional case-control	8-isoprostane	n=30 48 ± 14 33%	n=12 39 ± 9.0 42%	Not specified	All non-smokers *All non-smokers*	Biopsy (n=30)	Newly diagnosed (n=7), Established (n=23) with a duration of 3±2 years	4/6/11/8/1	Oral steroids (n=9)
Wilsher *et al* (2005) (25)	New Zealand	Cross-sectional case-control	Nitric oxide (Plateau of end expiration when carbon dioxide 70-80% of maximum)	n=52 42 (23-66)* 56%	n=44 Age & gender not specified	Caucasian (n=41), Indian (n=5), Polynesian (n=4), African (n=1), Asian (n=1)	7/5/40 *All non-smokers*	Biopsy or clinico-radiologically	Newly diagnosed (n=15), Established disease (n=37). Duration not specified	3/13/21/14/1	All treatment free at enrolment and during preceding 3 months

Piotrowski *et al* (2007) (16)	Poland	Cross-sectional case-control	8-isoprostane CysLT LTB4	n=28 39.2 ± 2.0† 64%	n=17 39.8 ± 2.6† 53%	Not specified	All non-smokers *All non-smokers*	Biopsy (n=23), clinic-radiologically as per guidelines (n=5) (1)	Newly diagnosed (n=28), disease duration <3 months	0/8/10/10/0	All treatment naïve
Mazzone *et al* (2007) (32)	USA	Cross-sectional case-control	VOCs	n=20 53 (41-75)* 25%	n=21 55 (36-70)* 52%	Not specified	0/4/16 *3/9/9*	Biopsy and clinic-radiologically	Not specified	Not specified	Not specified, however treatment status was not criteria for exclusion
Westhoff *et al* (2007) (29)	Germany	Cross-sectional	VOCs	n=5 49 (30-67)* Incomplete data for gender	Alternative diagnoses n=4 63.5 (60-65)* Incomplete data for gender	Not specified	Not specified *Not specified*	Biopsy (n=4) Clinico-radiologically (n=1)	Not specified	Incomplete data	Oral steroids (n=1), otherwise not stated
Ciarleglio *et al* (2008) (21)	Italy	Cross-sectional case-control	Carbon monoxide	n=78 52 ± 13 32%	n=25 39 ± 20 32%	Not specified	Non-smokers for ≥12 months *Non-smokers for ≥12 months*	As per international guidelines (63)	Not specified	14/19/20/25/0	Oral steroids (n=33)
Choi *et al* (2009) (14)	USA	Longitudinal	Nitric oxide (Various flow rates, $C_{ALV}NO$, $J_{AW}NO$)	n=42 46.0 ± 12.2 (active) 51.7 ± 10.7 (inactive) 64%	n=20 45.4 ± 10.3 50%	Caucasian (n=25), African American (n=17)	1/1/6 (active) 3/11/20 (inactive) *All never smokers*	Biopsy (n=42)	New and established. Duration 48.6 ± 73.7 months (active), 92.2 ± 107.3 months (inactive).	6/11/19/3/3	All treatment naïve at baseline to oral steroids, n=29 treated with ICS. At 3-23 week-follow-up n=6 received treatment with steroids ± methotrexate

Corradi *et al* (2009) (22)	Italy	Cross-sectional case-control	Metallic elements: Aluminium Lead Nickel Chromium Silicon Cobalt Calcium Zinc Iron Copper Selenium Manganese Molybdenum	n=22 53.5 ± 13.9 64%	n=33 55 ± 10.8 76%	Not specified	0/16/6 - 16.5 (5.3-20.0) pack years *All non-smokers*	Biopsy (n=22) as per guidelines (1)	Not specified	Not specified	Oral steroids (n=6), Combined steroids & immunosuppression (n=4)
Piotrowski *et al* (2010a) (17)	Poland	Cross-sectional case-control	8-isoprostane	n=29 40.2 ± 2.0 55%	n=34 39.8 ± 2.6 41%	Not specified	All non-smokers *All never smokers*	Biopsy (n=29)	Newly diagnosed (n=29)	0/9/14/6/0	All treatment naïve
Piotrowski *et al* (2010b) (12)	Poland	Longitudinal	8-isoprostane	n=40 39 ± 11 43%	n=34 45 ± 10 44%	Caucasian (n=40)	All non-smokers *All never smokers*	Biopsy or clinico-radiologically as per guidelines (1)	Median of 10 (4-60) weeks duration from symptom onset to baseline visit	0/23/9/8/0	All treatment naïve for ≥12 months at baseline. At follow-up (n=8) received treatment with steroids.
Piotrowski *et al* (2010c) (33)	Poland	Cross-sectional case-control	HGF	n=64 39±2.0 (stage I)† 42±2.0 (stage II)† 40±3.0 (stage III)†	n=15 Age & gender not specified	Not specified	0/14/50 - <10 pack years *All never smokers*	Biopsy or clinico-radiologically	Established disease duration ranging from 48 ± 25 – 120 ± 41 weeks	Stages I – III (n for each stage not specified)	All treatment naïve at time of sampling

				Gender not specified							
Piotrowski *et al* (2012) (15)	Poland	Cross-sectional	8-isoprostane	n=32 43 ± 12 53%	(Sarcoidosis patients with negative somatostatin scintigraphy considered negative controls) n=12 41.0 ± 10.0 50%	Not specified	All non-smokers	Biopsy (n=32) as per guidelines (1)	Not specified	4/15/8/3/2	Oral steroids (n=9)
Ahmadzai *et al* (2013) (23)	Australia	Cross-sectional case-control	Neopterin TGF-β_1 ACE Total protein	n=16 48.5≠ 69%	n=22 37≠ 59%	Not specified	0/9/7 *1/5/16*	As per international guidelines (1)	Established disease (n=16), disease duration 7.5 ± 6.7 years	1/6/6/1/2	Oral steroids (n=3), steroids and methotrexate (n=1)
Dragonieri *et al* (2013) (30)	Netherlands	Cross-sectional case-control	VOCs	n=31 48.4 ± 9.0 (untreated) 49.7 ± 7.9 (treated) 55%	n=25 39.6 ± 14.1 44%	Not specified	0/0/31 *All never smokers*	Biopsy or clinico-radiologically	Established disease, duration 6.8 ± 5.5 years (untreated) and 11.1 ± 6.5 years (treated)	1/2/5/2/1 (untreated) 2/2/2/1/13 (treated)	Oral steroids (n=12), steroids & azathioprine (n=4), methotrexate (n=1) or hydroxychloroquine (n=3)
Loke *et al* (2015) (24)	Australia	Cross-sectional case-control	IL-27	n=18 50.3 ± 10.5 61%	n=21 39.7 ± 19.7 33%	Not specified	1/7/10 *0/6/15*	As per international guidelines (1)	Established disease, duration 6.72 ± 6.71 years	2/2/8/0/6	Combinations of steroids, azathioprine or mycophenolate (n=4)

Cameli *et al* (2016) (19)	Italy	Cross-sectional case-control	Nitric oxide (Various flow rates, $C_{ALV}NO$)	n=31 55.7 ± 12.6 Gender not specified	n=30 62.0 ± 4.7 Gender not specified	Not specified	Ex-smokers (n=5) - 1.8 ± 5.7 pack years *7 ex-smokers*	As per international guidelines (1)	Not specified	0/0/17/14/0	Oral steroids (n=11)
Mohan *et al* (2016) (7)	Australia	Cross-sectional case-control	TNFα Calcium Total protein	n=17 50.06 ± 2.62[†] 65%	n=23 43.48 ± 2.93[†] 61%	Not specified	0/8/9 *0/8/15*	Physician led and radiological staging as per international guidelines (1)	Not specified	7/0/6 (Stages II-III)/4	Combinations of oral/ inhaled steroids or immunosuppression (n=12)
Fijten *et al* (2017) (31)	Netherlands	Cross-sectional case-control discovery/ validation study	VOCs	n=87 (discovery) 50.6 ± 10.6 57% n=25 (validation) 53.4 ± 11.5 64%	n=26 50.5 ± 10.9 38% n=29 51.2 ± 9.8 41%	Not specified	7/12/68 (discovery) 0/0/25 (validation) *3/10/13 (discovery)* *Incomplete data (validation cohort)*	Biopsy or clinico-radiologically as per guidelines (64)	Established disease, with sampling during 2010-2012 for discovery cohort and 2015 for validation cohort.	18/12/24/10/23 (discovery) 5/3/7/3/7 (validation)	n=40 (discovery) n=19 (validation)

Table 1: Characteristics of included studies. Data expressed as mean and standard deviation unless otherwise stated. *Mean and Range; [†]Mean and SEM; [≠]Median; [††]Median (25-75[th] percentile); [¥]Mean and 95% confidence intervals. $C_{ALV}NO$, Calculated alveolar concentration of exhaled nitric oxide; CysLT, Cysteinyl leukotrienes; LTB4, Leukotriene B4; VOCs, Volatile organic compounds; $J_{AW}NO$, Flux of nitric oxide from the airway wall; HGF, Hepatocyte growth factor; TGF-β_1, Transforming growth factor beta; ACE, Angiotensin converting enzyme; IL-27, Interleukin-27; TNFα, Tumour necrosis factor; ICS, Inhaled corticosteroids; vs, versus; USA, United States of America.

Author (Year)	Biomarkers	Biological Sample	Collection Device	Method of Analysis	Detection Limit
O'Donnell *et al* (1997)(26)	Nitric oxide (Peak, end-tidal & $C_{ALV}NO$)	Exhaled Breath	Logan Research LR2000 Chemiluminescence Analyser	Chemiluminescence	1ppb
Moodley *et al* (1999) (13)	Nitric oxide (Alveolar)	Exhaled Breath	Logan Research LR2000 Chemiluminescence Analyser	Chemiluminescence	1-5000ppb
Ziora *et al* (2004) (18)	Nitric oxide (Mean end expiratory)	Exhaled Breath	Seivers '280' Chemiluminescence Analyser	Chemiluminescence	<1ppb
Psathakis *et al* (2004) (20)	8-isoprostane	EBC	Customised Device	Specific enzyme based immunoassay (Cayman Chemicals; Ann Arbor, Michigan)	Not specified
Wilsher *et al* (2005) (25)	Nitric oxide (Plateau of end expiration when carbon dioxide 70-80% of maximum)	Exhaled Breath	Logan Research LR2000 Chemiluminescence Analyser	Chemiluminescence	1-5000ppb
Piotrowski *et al* (2007) (16)	8-isoprostane CysLT LTB4	EBC	EcoScreen Device	Specific enzyme based immunoassay (Cayman Chemicals; Ann Arbor, Michigan)	8-isoprostane 5pg/mL CysLT 13pg/mL LTB4 4.43pg/mL
Mazzone *et al* (2007) (32)	VOCs	Exhaled Breath	Colorimetric Sensor Device	Colorimetric assay sensitive for 36 VOCs (colour changes converted into numerical vectors)	Lower ppm to upper ppb
Westhoff *et al* (2007) (29)	VOCs	Exhaled Breath	Direct port connected to a 6-way valve and multi-capillary column on Ion mobility spectrometer	Ion mobility spectrometer	Not specified
Ciarleglio *et al* (2008) (21)	Carbon monoxide	Exhaled Breath	Smokerlyzer Analyser	Electrochemical sensor	1ppm
Choi *et al* (2009) (14)	Nitric oxide (Various flow rates, $C_{ALV}NO$, $J_{AW}NO$)	Exhaled Breath	Logan Research LR1800 Chemiluminescence Analyser	Chemiluminescence	1-5000ppb
Corradi *et al* (2009) (22)	Metallic elements	EBC	TURBO-DECCS Portable Condenser	Inductively coupled plasma mass spectrometry	0.005µg/L
Piotrowski *et al* (2010a) (17)	8-isoprostane	EBC	EcoScreen Device	Specific enzyme based immunoassay Cayman Chemicals; Ann Arbor, Michigan)	5pg/mL
Piotrowski *et al* (2010b) (12)	8-isoprostane	EBC	EcoScreen Device	Specific enzyme based immunoassay Cayman Chemicals; Ann Arbor, Michigan)	5pg/mL

Piotrowski *et al* (2010c) (33)	Hepatocyte growth factor	EBC	EcoScreen Device	Specific Immunoassay Kit (BioSource Europe S.A., Belgium)	20pg/mL
Piotrowski *et al* (2012) (15)	8-isoprostane	EBC	EcoScreen Device	Specific enzyme based immunoassay Cayman Chemicals; Ann Arbor, Michigan)	5pg/mL
Ahmadzai *et al* (2013) (23)	Neopterin TGF-β_1 ACE Total protein	EBC	Customised Device	Neopterin: ELISA (GenWay Biotech. San Diego, USA), TGF-β_1: ELISA (GE Healthcare, Buckinghamshire, UK), ACE: Colorimetric assay (Buhlmann Laboratories, Switzerland), Total protein: QuantiPro bicinchoninic assay (Sigma-Aldrich, Sydney, Australia)	Neopterin 0.7nmol/L TGF-β_1 15pg/mL ACE 2.6 U/L Total protein 4mg/mL
Dragonieri *et al* (2013) (30)	VOCs	Exhaled Breath	Tedlar Bag	Electric Nose sensitive to 32 organic polymers (Cyranose 320, Smith Detectors, USA)	Not specified
Loke *et al* (2015) (24)	IL-27	EBC and blood	EcoScreen Device	ELISA (R&D Systems, USA)	156.3pg/mL
Cameli *et al* (2016) (19)	Nitric oxide (Various flow rates, $C_{ALV}NO$)	Exhaled Breath	Modified Hypair FeNO Device	Chemiluminescence	Not specified
Mohan *et al* (2016) (7)	TNFα Calcium Total protein	EBC	EcoScreen Device	TNFα: ELISA (Invitrogen, Camarillo, USA), Calcium: Inductively coupled plasma mass spectroscopy (PerkinElmer, Melbourne, Australia), Total Protein: QuantiPro bicinchoninic assay (Sigma-Aldrich, Sydney, Australia)	TNFα 0-32pg/mL Calcium 5.0µg/L Protein 1-20µg/mL
Fijten *et al* (2017) (31)	VOCs	Exhaled Breath	Tedlar Bag (5L for discovery study and 3L for validation study)	Thermal Desorption followed by Gas Chromatography and Time-of-Flight mass spectrometry (Differing instruments were utilised between the discovery and validation studies)	Not specified

Table 2: Biomarker sampling and analytical methods. Same key as per table 1; EBC, Exhaled breath condensate; ELISA, Enzyme-linked immunosorbent assay; ppb, parts-per-billion; ppm, parts-per-million; pg, picograms; µg, micrograms.

Author (Year)	Biomarker	(n) Total	(n) Active Disease	Specific Criteria used to Define Participants with Active Disease
O'Donnell *et al* (1997)(26)	Nitric oxide (Peak, end-tidal and $C_{ALV}NO$)	10	10	Clinical symptoms, abnormal radiology, serological markers (ACE), BAL (elevated lymphocytic count, CD4:CD8 ratio).
Moodley *et al* (1999) (13)	Nitric oxide (Alveolar)	12	12	Clinical symptoms, extra-pulmonary manifestations, abnormal radiology, serological markers (ACE), BAL (CD4:CD8 ratio) and FEV_1.
Ziora *et al* (2004) (18)	Nitric oxide (Mean end expiratory)	27	21	Clinical symptoms, radiological features, serological markers and BAL based upon the American Thoracic Society, European Respiratory Society and World Association of Sarcoidosis and other Granulomatous Disorders criteria (1).
Psathakis *et al* (2004) (20)	8-isoprostane	30	14	Clinical symptoms alongside any one of the following: radiological progression over preceding three months on chest radiograph or presence of ground glass opacification on high resolution computerised tomography, serological markers (ACE, calcium, liver enzymes), BAL (lymphocytic alveolitis >18%, CD4:CD8 ratio >3.5) or decline in pulmonary function tests over preceding three months ($\geq$10% or $\geq$200ml decline in lung volumes or $\geq$15% or $\geq$3ml/min/mmHg for DLCO).
Piotrowski *et al* (2007) (16)	8-isoprostane CysLT LTB4	28		BALF lymphocytic counts (lymphocytic alveolitis >18% n=19, lymphocytic counts <17% n=9).
Ciarleglio *et al* (2008) (21)	Carbon monoxide	78	47	Clinical symptoms alongside any of the following: extra-pulmonary manifestations, evidence of radiological progression, serological markers (ACE, calcium), BAL (CD4:CD8 ratio >2.5) or decline in pulmonary function tests.
Choi *et al* (2009) (14)	Nitric oxide (Various flow rates, $C_{ALV}NO$, $J_{AW}NO$)	42	8	Active disease was evident when $\geq$3 of the following criteria were met over a 6-12 week period: progression of respiratory symptoms, exercise desaturation $\geq$10% using pulse oximetry, decline in FVC or DLCO $\geq$10% or radiological progression.
Piotrowski *et al* (2010b) (12)	8-isoprostane	40		Disease activity assessed during follow-up using a scoring system according to the following: clinical symptoms, presence of Lofgren syndrome, extra-pulmonary manifestations, radiological progression on chest radiograph, serological markers (ACE, calcium, CRP), urinary calcium and decline in pulmonary function tests.
Piotrowski *et al* (2010c) (33)	Hepatocyte growth factor	64	64	Clinical symptoms, evidence of radiological progression, serological markers (ACE, calcium), BAL (elevated lymphocytic counts).
Piotrowski *et al* (2012) (15)	8-isoprostane	32	20	Somatostatin receptor scintigraphy scan. A negative scan (n=12) was consistent with inactive disease.

Table 3: Criteria used to define and assess disease activity in studies which classified participants into those with and without active disease. Same key as per table 1; BAL, Bronchoalveolar lavage; FEV1, Forced expiratory volume in one second; FVC, Forced vital capacity; DLCO, Diffusion capacity for carbon monoxide; CRP; C-reactive protein.

Author (year)	Biomarker	Differentiating Sarcoidosis from Healthy Controls (Sarcoidosis *versus* healthy controls)	Differentiating Between Radiological Stage or Disease Activity	Correlation with Pre-existing Clinical Biomarkers	Effects with Respect to Treatment
Psathakis *et al* (2004) (20)	8-isoprostane	All sarcoidosis patients vs healthy controls: 64.23 (37.00-91.46) vs 20.75 (16.06-25.44) pg/mL; p = 0.04.[¥] Inactive sarcoidosis vs healthy controls 22.94 (15.89-29.99) vs 20.75 (16.06-25.44) pg/mL; p = >0.05[¥]	Active vs inactive sarcoidosis: 111.40 (62.56-160.30) vs 22.94 (15.89-29.99) pg/mL; p = <0.001[¥] No significant differences reported according to radiological stage (p = 0.2)	8-isoprostane correlated positively with serum ACE (r =0.69, p = <0.0001) No correlations reported in relation to pulmonary function tests and serum calcium (p = >0.05)	Not assessed or reported
Piotrowski *et al* (2007) (16)	8-isoprostane CysLT LTB4	8-isoprostane: 13.95 ± 2.59 vs 2.67 ± 0.16pg/mL; p = 0.0003[†]	No difference reported according to clinical activity or radiological stage *(p = >0.05)* 8-isoprostane increased with radiological stage, however did not reach statistical significance *(p = >0.05)*	No correlations reported in relation to DLCO *(p = >0.05)*	Treatment naïve
Ciarleglio *et al* (2008) (21)	Carbon monoxide	3.3 (2.9-3.8) vs 1.4 (1.2-1.7)ppm; p<0.001[¥]	Active sarcoidosis vs healthy controls: 3.3 (2.9-3.8) vs 1.4; (1.3-1.7)ppm; p = <0.001[¥] Inactive sarcoidosis vs healthy controls: 2.7 (2.1-3.3) vs 1.4 (1.3-1.7)ppm; p = <0.001[¥] No significant differences reported according to clinical activity or radiological stage *(p-values not reported)*	No significant correlations reported in relation to pulmonary function tests or ACE *(p-values not reported).*	No significant differences reported in relation to steroid treatment *(p-values not reported)*
Piotrowski *et al* (2010a) (17)	8-isoprostane	6.20; 2.50-16.95 vs 2.50; 2.50-3.90pg/mL; p = <0.05[††]	No significant differences reported according to radiological stage, however significance was highest when comparing stage III disease with healthy controls: 26.35; 3.90-33.85 vs 2.50; 2.50-3.90pg/ml; p = <0.01 [††]	8-isoprostane correlated negatively with percentage of lymphocytes in BAL fluid (r = -0.40, p = 0.03)	Treatment naïve
Piotrowski *et al*	8-isoprostane	8.50; 2.50-17.40 vs 2.50; 2.50-3.90pg/mL, p = 0.001[††]	8-isoprostane concentration was significantly higher in stage III compared to stage I disease (p = 0.03)	No significant correlations reported in relation to pulmonary function tests or	At follow-up 8-isoprostane significantly decreased in those treated with steroids (p = 0.02).

(2010b) (12)				laboratory parameters (ACE, BAL cell counts, CRP, Ca^{2+}) *(p = >0.05)*	No differences were observed in those who underwent spontaneous remission *(p = >0.05)*
Piotrowski *et al* (2012) (15)	8-isoprostane	No healthy controls included; those with negative somatostatin scintigraphy scans were considered to have inactive sarcoidosis.	8-isoprostane in those with a positive vs negative scintigraphy scans: 19.1 ± 19.8 vs 5.4 ± 3.5pg/mL (p = 0.02)	Not assessed or reported	Not assessed or reported

Table 4: Results for studies evaluating markers of oxidative stress. Results presented as mean and standard deviation unless otherwise stated. Same key as per tables 1-3.

Author (year)	Biomarker	Differentiating Sarcoidosis from Healthy Controls (Sarcoidosis *versus* healthy controls)	Differentiating Between Radiological Stage or Disease Activity	Correlation with Pre-existing Clinical Biomarkers	Effects with Respect to Treatment
Piotrowski *et al* (2007) (16)	8-isoprostane CysLT LTB4	CysLT : 27.82 ± 6.65 vs 6.5 ± 0.0pg/mL; p = <0.0001[†] LTB4: 23.14 ± 3.35 vs 20.09 ± 4.85pg/mL; p = 0.49[†]	No significant difference reported according to clinical activity or radiological stage *(p = >0.05).* LTB4 increased with radiological stage, however did not reach statistical significance *(p = >0.05)*	No significant correlations reported in relation to DLCO *(p = >0.05)*	Treatment naïve
Corradi *et al* (2009) (22)	Metallic elements: Aluminium Lead Nickel Chromium Silicon Cobalt Calcium Zinc Iron Copper Selenium Manganese Molybdenum	Ni, Cr and Si were significantly higher in sarcoidosis compared to controls whereas Co, Zn, Se and Cu were all significantly lower in those with sarcoidosis (p<0.05). No significant difference was observed in Al, Pb, Ca, Fe, Mn and Mo between sarcoidosis and controls. Using multinomial logistic regression metallic patterns in EBC, including Cobalt (p=0.015), Nickel (p=0.002) and Selenium (p=0.043) could distinguish healthy controls from sarcoidosis, however overlap occurred in up to 63.6% of cases between sarcoidosis and other forms of ILD.	No significant differences reported in relation to disease severity *(p = >0.05)*	Iron concentration correlated positively with DLCO (r=0.46,p = <0.05)	No significant differences reported in relation to treatment
Piotrowski *et al* (2010c) (33)	Hepatocyte growth factor	40.9 ± 4.8 vs 52.1 ± 8.1pg/mL, p = >0.05[†]	No significant differences reported according to clinical activity or radiological stage *(p = >0.05)*	No significant correlations reported in relation to pulmonary function tests, BAL lymphocyte counts, ACE, CRP, calcium and 24 hour urinary calcium *(p = >0.05)*	Treatment naïve
Ahmadzai *et al* (2013) (23)	Neopterin TGF-β_1 ACE Total protein	Neopterin: 0.57 ± 0.45 vs 0.41 ± 0.22nmol/L, p = 0.04 TGF- β_1: 115.5 ± 79.6 vs 82.3 ± 16.26pg mol^{-1}, p = 0.048 ACE detected in 2/16 sarcoidosis patients vs 3/20 controls, p = 0.91	No significant difference reported according to radiological stage with neopterin (p = 0.76) or TGF- β_1 (p = 0.79)	No significant correlations reported in relation to pulmonary function tests, however a general trend existed for both increasing neopterin (r^2 = 0.36) and TGF- β_1 (r^2 = 0.297) with decreasing FEV_1	No significant differences reported in relation to treatment

		Total protein: 17.8 ± 38.5 vs 8.1 ± 2.3µg/mL, p = 0.27			
Loke *et al* (2015) (24)	IL-27	1.8 ± 3.0pg/mL vs 3.1 ± 4.2pg/mL, p = 0.57	Not assessed or reported	Not assessed or reported	Not assessed or reported
Mohan *et al* (2016) (7)	TNFα Calcium Total protein	TNFα: 3.37 ± 0.38 vs 2.59 ± 0.40pg/mL, p = 0.037† Calcium: 73.88 ± 13.35 vs 116.50 ± 12.19µmol/L, p = 0.018† Total protein: 19.51 ± 4.52 vs 10.60 ± 1.31µg/mL, p = 0.020†	No significant difference reported according to radiological stage with either TNFα (p = 0.47), protein (p = 0.36) or calcium (p = 0.17)	No significant correlation reported in relation to serum calcium and EBC calcium (r=0.070, p = 0.81) Negative correlation was observed with EBC calcium concentration and FEV_1 and FVC (r^2= 0.32, p = 0.023 and r^2=0.39, p = 0.009 respectively)	Not assessed or reported, however authors stated that a patient with the most severe disease treated with inhaled, oral and intravenous steroids had undetectable TNFα

Table 5: Results for studies evaluating cytokines, metallic elements and miscellaneous biomarkers. Results presented as mean and standard deviation unless otherwise stated.

Same key as per tables 1-3; Ni, Nickel; Cr, Chromium; Si, Silicon; Co, Cobalt; Zn, Zinc; Cu, Copper; Se, Selenium; Al, Aluminium; Pb, Lead; Ca, Calcium; Fe, Iron; Mn, Manganese; Mo, Molybdenum.

Author (year)	Nitric Oxide Flow Rate	Differentiating Sarcoidosis from Healthy Controls (Sarcoidosis *versus* healthy controls)	Differentiating Between Disease Activity or Radiological Stage	Correlation with Pre-existing Clinical Biomarkers	Effects with Respect to Treatment
O'Donnell *et al* (1997) (26)	Peak, end-tidal and $C_{ALV}NO$	7.8 ± 4.4 vs 7.1 ± 4.2ppb; p = 0.70	No difference observed in peak, alveolar or end tidal nitric oxide when comparing hilar lymphadenopathy alone to interstitial infiltration on chest radiographs (p = 0.80, 0.44 and 0.46 respectively)	No significant correlation reported with serum ACE, BAL leucocyte counts or DLCO	Treatment naïve
Moodley *et al* (1999) (13)	Alveolar	9.8 ± 0.4 vs 4.1 ± 0.2ppb; p = <0.001[†]	Not reported	No significant correlation reported with serum ACE (r=0.13), FEV_1(r=-0.06), BAL CD4:CD8 ratio (r=0.07); (p = >0.05)	Following 6 weeks treatment, nitric oxide fell from 9.8 ± 0.4 to 5.9 ± 0.7ppb; p = 0.01
Ziora *et al* (2004) (18)	Mean end expiratory	6.7 ± 0.50 vs 5.17 ± 0.73ppb, p = 0.05[†]	No difference reported according to disease activity (p = 0.43) or radiological stage when comparing stages I &II combined against stage III (p = 0.99)	Nitric oxide correlated positively with DLCO (r=0.515,p = 0.029). No significant correlations reported between nitric oxide and FVC (r=0.163, p = 0.417), BAL lymphocyte counts (r=0.180, p = 0.449)	No significant differences identified between those with and without indications for commencing treatment (p = 0.124)
Wilsher *et al* (2005) (25)	Plateau of end expiration when carbon dioxide 70-80% of maximum	Median 6.8ppb (range 2.4-21.8) vs 6.3ppb (1.6-28.0); *(p-value not reported)*	No significant differences reported according to disease severity as assessed upon HRCT CT scoring; *(p-value not reported)*	No significant correlation reported in relation to pulmonary function tests; *(p-value not reported)*	Treatment free at enrolment
Choi *et al* (2009) (14)	Various flow rates, $C_{ALV}NO$, $J_{AW}NO$	Active sarcoidosis: 3.8 ± 3.3 vs 4.7 ± 3.0ppb; *p = >0.05* Inactive sarcoidosis: 5.7 ± 4.2 vs 4.7 ± 3.0ppb; *p = >0.05*	No significant differences reported with any flow rate of exhaled nitric oxide, $C_{ALV}NO$ or $J_{AW}NO$ in relation to clinical activity or radiological stage.	$C_{ALV}NO$ correlated negatively with FVC (r=-0.52,p = 0.001) and DLCO (r=-0.40, p = 0.012). No significant correlations reported in relation to ACE.	No consistent differences observed in nitric oxide in relation to treatment at follow-up.

Cameli *et al* (2016) (19)	Various flow rates, $C_{ALV}NO$	6.0 ± 3.1 vs 4.7 ± 2.3ppb; p = >0.05.	No significant differences reported according to radiological stage; *(p-value not reported)*	Not assessed or reported	No significant differences reported in relation to steroid treatment

Table 6: Results for studies evaluating exhaled nitric oxide. Where exhaled nitric oxide was measured and reported at various flow rates including calculation of $C_{ALV}NO$ and $J_{AW}NO$, only alveolar nitric oxide has been tabulated in the above result table when comparing participants with sarcoidosis to healthy controls. No significant differences were observed for nitric oxide at any other flow rates in the studies. Results presented as mean and standard deviation unless otherwise stated. Same key as per tables 1-3.

Author (year)	Differentiating Sarcoidosis from Healthy Controls (Sarcoidosis *versus* healthy controls)	Differentiating Between Radiological Stage or Disease Activity	Correlation with Pre-existing Clinical Biomarkers	Effects with Respect to Treatment
Mazzone *et al* (2007) (32)	Sarcoidosis vs all other groups: model error rate 10%, validation sensitivity 16.7% and specificity 81.1%, p = 0.69	Not assessed or reported	Not assessed or reported	Not assessed or reported
Westhoff *et al* (2007) (29)	Across all chromatograms, thirteen peaks were identified for discrimination, six of which appeared to be specific for a participant with sarcoidosis. Principle component analysis allowed separation of those with confirmed sarcoidosis compared to those who received a negative diagnosis.	Not assessed or reported	Not assessed or reported	Not assessed or reported
Dragonieri *et al* (2013) (30)	VOC profiles discriminated untreated pulmonary sarcoidosis from healthy controls with a cross validated accuracy (CVA) of 83.3% (p = <0.001).	Not assessed or reported	Not assessed or reported	Untreated and treated sarcoidosis could be less well discriminated against (CVA 74.2%). Breath prints were undistinguishable between treated sarcoidosis and controls (CVA 66.7%)
Fijten *et al* (2017) (31)	430 different VOCs identified across the samples; 9 VOCs in the discovery study were discriminatory (Isoprene, 2-methylpentane, benzene, 3-methylhexane, p-Benzoquinone, phenol, D-limonene, iodomethylcyclopentane and dibenzofuran) which could predict sarcoidosis with 79.4% accuracy (sensitivity and specificity 75.4% and 92.5% respectively). During the validation study overall prediction rate was 74.1% accurate (sensitivity and specificity 68% and 79.3% respectively).	Not assessed or reported	Not assessed or reported	Not assessed or reported

Table 7: Results for studies evaluating volatile organic compounds.

Same key as per tables 1-3.

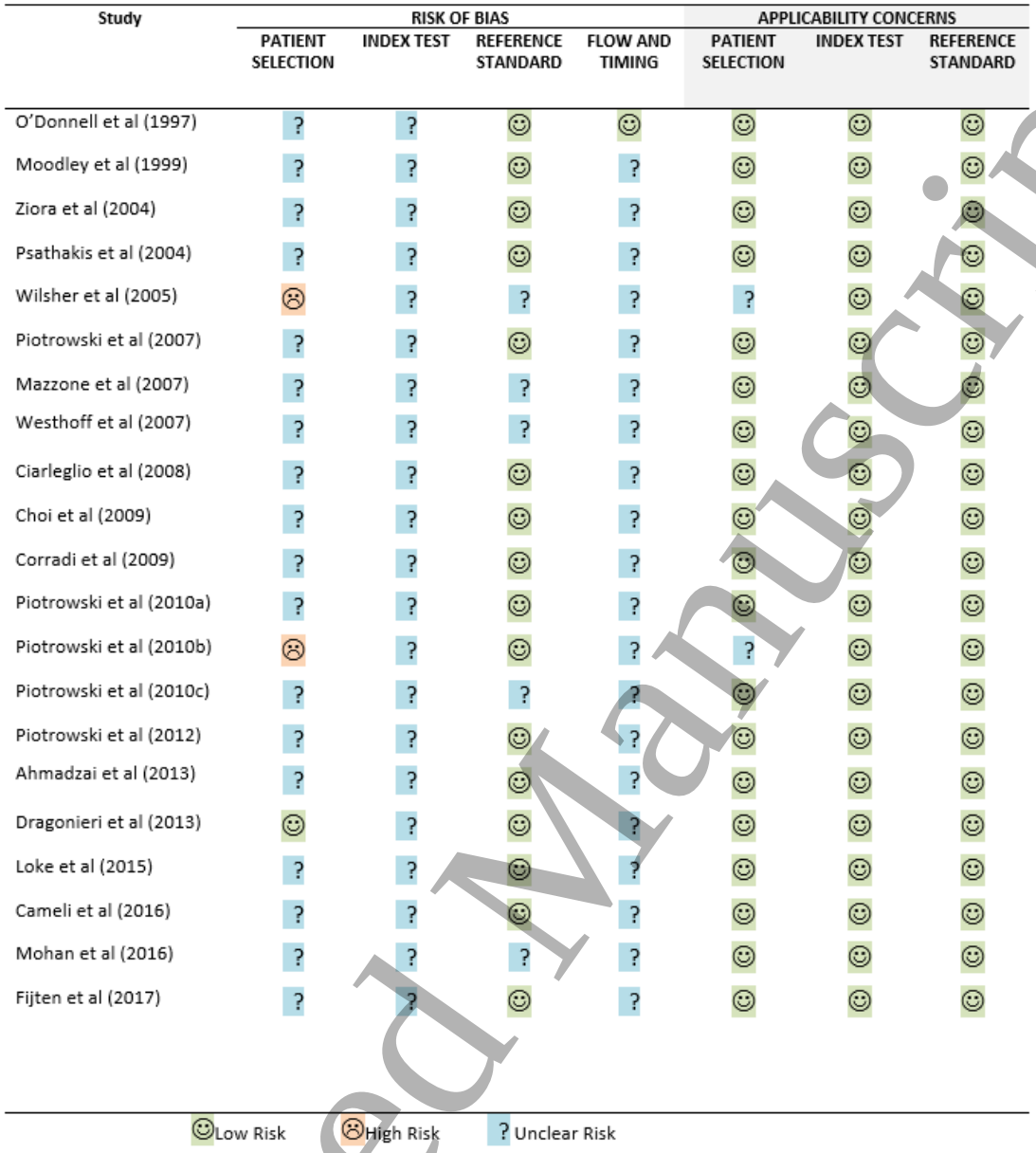

Figure 3: QUADAS-2 assessment

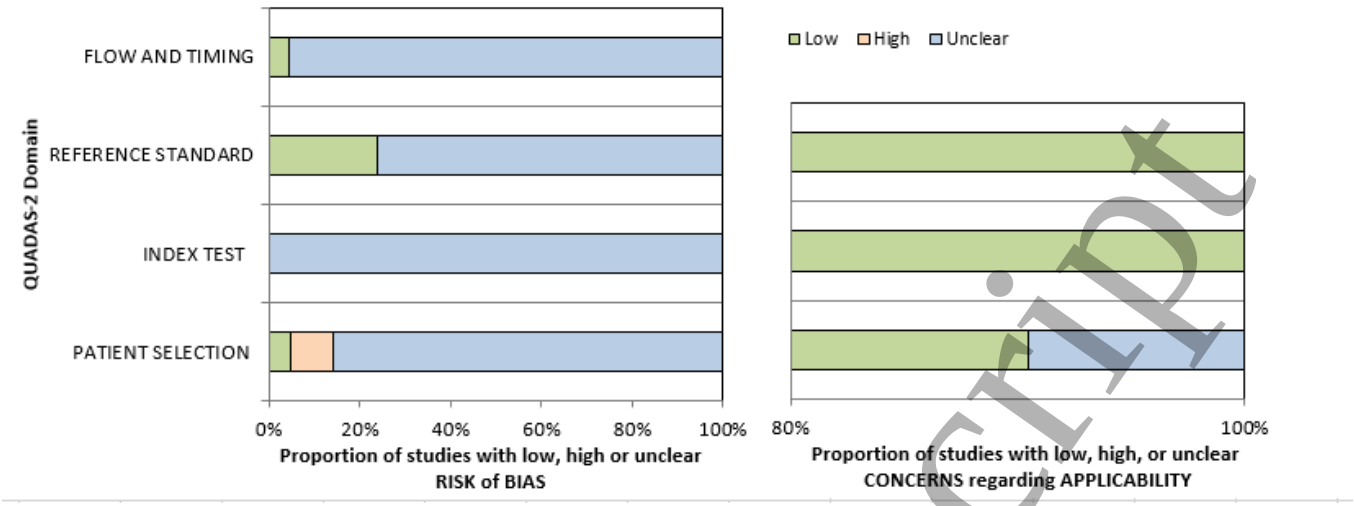

Figure 4: QUADAS-2 assessment

References

1. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. American journal of respiratory and critical care medicine. 1999;160(2):736-55.
2. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. The New England journal of medicine. 2007;357(21):2153-65.
3. Valeyre D, Prasse A, Nunes H, Uzunhan Y, Brillet PY, Muller-Quernheim J. Sarcoidosis. Lancet (London, England). 2014;383(9923):1155-67.
4. Esteves T, Aparicio G, Garcia-Patos V. Is there any association between Sarcoidosis and infectious agents?: a systematic review and meta-analysis. BMC pulmonary medicine. 2016;16(1):165.
5. Paramothayan S, Lasserson T. Treatments for pulmonary sarcoidosis. Respiratory medicine. 2008;102(1):1-9.
6. Ahmadzai H, Sheng Joshua Loke W, Huang S, Herbert C, Wakefield D, S. Thomas P. Biomarkers in sarcoidosis: a review2014. 93-106 p.
7. Mohan N, Akter R, Bryant K, Herbert C, Chow S, Thomas PS. Exhaled breath markers of alveolar macrophage activity in sarcoidosis. Inflammation research : official journal of the European Histamine Research Society [et al]. 2016;65(6):471-8.
8. Rattray NJ, Hamrang Z, Trivedi DK, Goodacre R, Fowler SJ. Taking your breath away: metabolomics breathes life in to personalized medicine. Trends in biotechnology. 2014;32(10):538-48.
9. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS medicine. 2009;6(7):e1000100.
10. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Annals of internal medicine. 2011;155(8):529-36.
11. Wallace BC, Dahabreh IJ, Trikalinos TA, Lau J, Trow P, Schmid CH. Closing the Gap between Methodologists and End-Users: R as a Computational Back-End. 2012. 2012;49(5):15.
12. Piotrowski WJ, Kurmanowska Z, Antczak A, Marczak J, Gorski P. Exhaled 8-isoprostane as a prognostic marker in sarcoidosis. A short term follow-up. BMC pulmonary medicine. 2010;10:23.
13. Moodley YP, Chetty R, Lalloo UG. Nitric oxide levels in exhaled air and inducible nitric oxide synthase immunolocalization in pulmonary sarcoidosis. The European respiratory journal. 1999;14(4):822-7.
14. Choi J, Hoffman LA, Sethi JM, Zullo TG, Gibson KF. Multiple flow rates measurement of exhaled nitric oxide in patients with sarcoidosis: a pilot feasibility study. Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG. 2009;26(2):98-109.
15. Piotrowski WJ, Bienkiewicz M, Frieske I, Marczak J, Antczak A, Gorski P, et al. Somatostatin receptor scintigraphy in sarcoidosis: relation to selected clinical and laboratory markers. Polskie Archiwum Medycyny Wewnetrznej. 2012;122(3):98-106.
16. Piotrowski WJ, Antczak A, Marczak J, Nawrocka A, Kurmanowska Z, Gorski P. Eicosanoids in exhaled breath condensate and BAL fluid of patients with sarcoidosis. Chest. 2007;132(2):589-96.
17. Piotrowski WJ, Kurmanowska Z, Antczak A, Marczak J, Ciebiada M, Gorski P. Exhaled 8-isoprostane in sarcoidosis: relation to superoxide anion production by bronchoalveolar lavage cells. Inflammation research : official journal of the European Histamine Research Society [et al]. 2010;59(12):1027-32.

18. Ziora D, Kaluska K, Kozielski J. An increase in exhaled nitric oxide is not associated with activity in pulmonary sarcoidosis. The European respiratory journal. 2004;24(4):609-14.
19. Cameli P, Barbagli E, Rottoli P. Exhaled nitric oxide is not increased in pulmonary sarcoidosis. Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG. 2016;33(1):39-40.
20. Psathakis K, Papatheodorou G, Plataki M, Panagou P, Loukides S, Siafakas NM, et al. 8-Isoprostane, a marker of oxidative stress, is increased in the expired breath condensate of patients with pulmonary sarcoidosis. Chest. 2004;125(3):1005-11.
21. Ciarleglio G, Refini RM, Pieroni MG, Martino VA, Bargagli E, Rottoli P, et al. Exhaled carbon monoxide in sarcoidosis. Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG. 2008;25(1):46-50.
22. Corradi M, Acampa O, Goldoni M, Adami E, Apostoli P, De Palma G, et al. Metallic elements in exhaled breath condensate of patients with interstitial lung diseases. Journal of breath research. 2009;3 (4) (no pagination)(046003).
23. Ahmadzai H, Cameron B, Chui J, Lloyd A, Wakefield D, Thomas PS. Measurement of neopterin, TGF-beta1 and ACE in the exhaled breath condensate of patients with sarcoidosis. Journal of breath research. 2013;7(4):046003.
24. Loke WS, Freeman A, Garthwaite L, Prazakova S, Park M, Hsu K, et al. T-bet and interleukin-27: possible TH1 immunomodulators of sarcoidosis. Inflammopharmacology. 2015;23(5):283-90.
25. Wilsher ML, Fergusson W, Milne D, Wells AU. Exhaled nitric oxide in sarcoidosis. Thorax. 2005;60(11):967-70.
26. O'Donnell DM, Moynihan J, Finlay GA, Keatings VM, O'Connor CM, McLoughlin P, et al. Exhaled nitric oxide and bronchoalveolar lavage nitrite/nitrate in active pulmonary sarcoidosis. American journal of respiratory and critical care medicine. 1997;156(6):1892-6.
27. Kharitonov S, Alving K, Barnes PJ. Exhaled and nasal nitric oxide measurements: recommendations. The European Respiratory Society Task Force. The European respiratory journal. 1997;10(7):1683-93.
28. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med. 2005;171(8):912-30.
29. Westhoff M, Litterst P, Freitag L, Baumbach JI. Ion mobility spectrometry in the diagnosis of sarcoidosis: results of a feasibility study. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society. 2007;58 Suppl 5(Pt 2):739-51.
30. Dragonieri S, Brinkman P, Mouw E, Zwinderman AH, Carratu P, Resta O, et al. An electronic nose discriminates exhaled breath of patients with untreated pulmonary sarcoidosis from controls. Respiratory medicine. 2013;107(7):1073-8.
31. Fijten R, Smolinska A, Drent M, Dallinga J, Mostard R, Pachen D, et al. The necessity of external validation in exhaled breath research: a case study of sarcoidosis. J Breath Res. 2017.
32. Mazzone PJ, Hammel J, Dweik R, Na J, Czich C, Laskowski D, et al. Diagnosis of lung cancer by the analysis of exhaled breath with a colorimetric sensor array. Thorax. 2007;62(7):565-8.
33. Piotrowski WJ, Kurmanowska Z, Antczak A, Marczak J, Gorski P. Hepatocyte growth factor in exhaled breath and BAL fluid in sarcoidosis. Pneumonologia i alergologia polska : organ Polskiego Towarzystwa Ftyzjopneumonologicznego, Polskiego Towarzystwa Alergologicznego, i Instytutu Gruzlicy i Chorob Pluc. 2010;78(3):187-91.
34. Horvath I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, et al. Exhaled breath condensate: methodological recommendations and unresolved questions. The European respiratory journal. 2005;26(3):523-48.
35. Horvath I, Barnes PJ, Loukides S, Sterk PJ, Hogman M, Olin AC, et al. A European Respiratory Society technical standard: exhaled biomarkers in lung disease. The European respiratory journal. 2017;49(4).
36. Konstantinidi EM, Lappas AS, Tzortzi AS, Behrakis PK. Exhaled Breath Condensate: Technical and Diagnostic Aspects. TheScientificWorldJournal. 2015;2015:435160.

37. Bargagli E, Olivieri C, Bennett D, Prasse A, Muller-Quernheim J, Rottoli P. Oxidative stress in the pathogenesis of diffuse lung diseases: a review. Respiratory medicine. 2009;103(9):1245-56.
38. Kanoh S, Kobayashi H, Motoyoshi K. Exhaled ethane: an in vivo biomarker of lipid peroxidation in interstitial lung diseases. Chest. 2005;128(4):2387-92.
39. Kwiatkowska S, Luczynska M, Grzelewska-Rzymowska I, Nowak D, Zieba M. [Comparison of oxidative stress markers in exhaled breath condensate and in serum of patients with tuberculosis and sarcoidosis]. Polski merkuriusz lekarski : organ Polskiego Towarzystwa Lekarskiego. 2005;19(109):37-40.
40. Liu T, Stern A, Roberts LJ, Morrow JD. The isoprostanes: novel prostaglandin-like products of the free radical-catalyzed peroxidation of arachidonic acid. Journal of biomedical science. 1999;6(4):226-35.
41. Malli F, Bardaka F, Tsilioni I, Karetsi E, Gourgoulianis KI, Daniil Z. 8-Isoprostane levels in serum and bronchoalveolar lavage in idiopathic pulmonary fibrosis and sarcoidosis. Food and Chemical Toxicology. 2013;61(Supplement C):160-3.
42. Psathakis K, Mermigkis D, Papatheodorou G, Loukides S, Panagou P, Polychronopoulos V, et al. Exhaled markers of oxidative stress in idiopathic pulmonary fibrosis. European journal of clinical investigation. 2006;36(5):362-7.
43. Tufvesson E, Bozovic G, Hesselstrand R, Bjermer L, Scheja A, Wuttge DM. Increased cysteinyl-leukotrienes and 8-isoprostane in exhaled breath condensate from systemic sclerosis patients. Rheumatology (Oxford, England). 2010;49(12):2322-6.
44. Peel AM, Crossman-Barnes CJ, Tang J, Fowler SJ, Davies GA, Wilson AM, et al. Biomarkers in adult asthma: a systematic review of 8-isoprostane in exhaled breath condensate. Journal of breath research. 2017;11(1):016011.
45. Ziegenhagen MW, Benner UK, Zissel G, Zabel P, Schlaak M, Muller-Quernheim J. Sarcoidosis: TNF-alpha release from alveolar macrophages and serum level of sIL-2R are prognostic markers. Am J Respir Crit Care Med. 1997;156(5):1586-92.
46. Ziegenhagen MW, Rothe ME, Schlaak M, Muller-Quernheim J. Bronchoalveolar and serological parameters reflecting the severity of sarcoidosis. The European respiratory journal. 2003;21(3):407-13.
47. Prior C, Frank A, Fuchs D, Hausen A, Judmaier G, Reibnegger G, et al. Urinary neopterin excretion in pulmonary sarcoidosis: correlation to clinical course of the disease. Clinica chimica acta; international journal of clinical chemistry. 1988;177(3):211-20.
48. Cameli P, Bargagli E, Refini RM, Pieroni MG, Bennett D, Rottoli P. Exhaled nitric oxide in interstitial lung diseases. Respiratory physiology & neurobiology. 2014;197:46-52.
49. Torén K, Murgia N, Schiöler L, Bake B, Olin A-C. Reference values of fractional excretion of exhaled nitric oxide among non-smokers and current smokers. BMC pulmonary medicine. 2017;17:118.
50. Boots AW, van Berkel JJ, Dallinga JW, Smolinska A, Wouters EF, van Schooten FJ. The versatile use of exhaled volatile organic compounds in human health and disease. Journal of breath research. 2012;6(2):027108.
51. van der Schee MP, Paff T, Brinkman P, van Aalderen WMC, Haarman EG, Sterk PJ. Breathomics in lung disease. Chest. 2015;147(1):224-31.
52. van de Kant KD, van der Sande LJ, Jobsis Q, van Schayck OC, Dompeling E. Clinical use of exhaled volatile organic compounds in pulmonary diseases: a systematic review. Respiratory research. 2012;13:117.
53. van Oort PM, Povoa P, Schnabel R, Dark P, Artigas A, Bergmans D, et al. The potential role of exhaled breath analysis in the diagnostic process of pneumonia-a systematic review. Journal of breath research. 2018;12(2):024001.
54. Yamada YI, Yamada G, Otsuka M, Nishikiori H, Ikeda K, Umeda Y, et al. Volatile Organic Compounds in Exhaled Breath of Idiopathic Pulmonary Fibrosis for Discrimination from Healthy Subjects. Lung. 2017;195(2):247-54.

55. de Lacy Costello B, Amann A, Al-Kateb H, Flynn C, Filipiak W, Khalid T, et al. A review of the volatiles from the healthy human body. Journal of breath research. 2014;8(1):014001.
56. Fuchs D, Jamnig H, Heininger P, Klieber M, Schroecksnadel S, Fiegl M, et al. Decline of exhaled isoprene in lung cancer patients correlates with immune activation. Journal of breath research. 2012;6(2):027101.
57. Bajtarevic A, Ager C, Pienz M, Klieber M, Schwarz K, Ligor M, et al. Noninvasive detection of lung cancer by analysis of exhaled breath. BMC cancer. 2009;9:348.
58. Beauchamp J, Herbig J, Gutmann R, Hansel A. On the use of Tedlar(R) bags for breath-gas sampling and analysis. Journal of breath research. 2008;2(4):046001.
59. Ziora D, Polonska A, Kaluska K, Rozentryt P, Trzeciak P. [Concentration of nitric oxide in exhaled air in patients with sarcoidosis--pilot study]. Pneumonologia i alergologia polska. 2002;70(5-6):290-5.
60. Schildge J. [Nitric oxide in exhaled breath of patients with interstitial lung diseases]. Pneumologie (Stuttgart, Germany). 2011;65(3):143-8.
61. Gregus M, Foret F, Kindlova D, Pokojova E, Plutinsky M, Doubkova M, et al. Monitoring the ionic content of exhaled breath condensate in various respiratory diseases by capillary electrophoresis with contactless conductivity detection. Journal of breath research. 2015;9(2):027107.
62. Antczak A, Piotrowski W, Marczak J, Ciebiada M, Gorski P, Barnes PJ. Correlation between eicosanoids in bronchoalveolar lavage fluid and in exhaled breath condensate. Disease markers.30(5):213-20.
63. Hunninghake GW, Costabel U, Ando M, Baughman R, Cordier JF, du Bois R, et al. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG. 1999;16(2):149-73.
64. Costabel U, Hunninghake GW. ATS/ERS/WASOG statement on sarcoidosis. Sarcoidosis Statement Committee. American Thoracic Society. European Respiratory Society. World Association for Sarcoidosis and Other Granulomatous Disorders. The European respiratory journal. 1999;14(4):735-7.