Literature Review:
Immunopathogenesis and the use of exhaled breath condensate analysis in sarcoidosis

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1. Abstract

Sarcoidosis is a multisystemic, inflammatory disorder with a histological hallmark of non-caseating epitheloid granulomas that can manifest in lungs, heart, skin, eyes and the nervous system. Although there has been extensive research regarding sarcoidosis, it currently remains a diagnosis of exclusion due to its non-specific signs and symptoms, undetermined aetiology and immunopathogenesis, as well as a lack of diagnostic gold standard. This leads to the inability to determine the outcome and severity of the disease. Biomarkers have been studied in both blood and breath, but none have been reliable to stage the severity and the prognosis of sarcoidosis. As such, it is difficult to apply effective therapies that will reduce the possibility of irreversible lung fibrosis and other complications. This review examines the epidemiology, possible aetiological agents (genetic predisposition, infective and non-infective agents), and current understanding of the immunopathogenesis of the disease. It also explores the existing research on exhaled breath analysis, a novel way to detect immunological biomarkers to differentiate sarcoidosis patients from healthy controls, as well as to introduce biomarkers that can be further investigated as potential diagnostic tools.

2. Introduction

Sarcoidosis is a multisystemic, inflammatory disorder of unknown aetiology, typified by non-caseating epitheloid granulomas with the disruption of normal micro-architecture [1]. It mainly affects young adults (25-40 years old), and commonly manifests in the respiratory system, although granulomatous lesions have also been found in the heart, skin, eye and central nervous system [2]. Sarcoidosis is usually first detected on a chest radiograph during a screening examination, with bilateral opacities and bilateral hilar lymphadenopathy [3]. Patients can also present with non-specific chest problems such as cough, or systemic features such as fatigue [4]. This contributes to the fact that sarcoidosis is still currently a diagnosis of exclusion.

Patients with the disease usually proceed into remission due to spontaneous resolution or corticosteroid therapy. A minority have chronic, prolonged inflammation that results in irreversible lung fibrosis and possible severe sequelae such as cardiac death, neurological disorders, and blindness [2].

This diverse range of consequential aspects of sarcoidosis has therefore directed the focus of research onto the pathogenesis and aetiology of the disease. More research effort has also been placed into diagnostic methods to determine the outcome and severity of sarcoidosis, as there is currently no gold standard of diagnosis [5].

This review will describe the current knowledge of sarcoidosis, in terms of the epidemiology, etiology, and immunopathogenesis, with reference to the analysis of exhaled breath condensate, a novel method in detecting immunological markers.

3. Epidemiology

The prevalence and incidence of sarcoidosis are determined by various methods to ascertain the diagnosis of the disease, such as mass chest radiography, use of national databases, and questionnaires with specific diagnostic criteria [6]. It is found the prevalence of sarcoidosis ranges from 0.2 per 100,000 to 64 per 100,000 in different countries [6-9], due to the different processes of collecting statistical data, as well as the possibility that the prevalence of sarcoidosis is associated with certain ethnicity [6].

Although there is no predilection in which gender will be more likely to be affected by sarcoidosis, majority of the sarcoid patients are females, with more Scandinavians and African Americans affected as compared to the other ethnic groups [10]. Two peak incidences are observed in females at 25-29 years old and 65-69 years old, with a median age of 45 years old; while males prominently display one peak incidence, with a median age of 38, differing slightly across different backgrounds. Extra-pulmonary manifestations tend to occur in the females as well, such as erythema nodosum and eye involvement [10].

As sarcoidosis has a highly variable clinical presentation, the estimates of epidemiological data are largely dependent on different standardisations of the diagnostic criteria and different methods of case detection . This therefore possibly leads to selection bias, as there is a lack of specific and sensitive diagnostic tests to correctly diagnose the disease, and a lack of systematic, standardised epidemiological investigations of the cause [1].

4. Putative aetiology

It is postulated that the development of sarcoidosis is highly dependent on the host's genetic predisposition, immune responses, and the environmental factors [11]. Genetic factors such as human leukocyte antigens (HLA) genes have been discovered through genome-wide association scans (GWAS) to affect the patient's immune response, rendering one more susceptible or less likely to have sarcoidosis [12-14]. This is further supported by several studies, , with the percentage of patients having a positive family history ranging from 2.7% to 17% in the countries studied [15-18]. This wide range is accounted by the small sample size and the low prevalence of sarcoidosis to begin with [17].

Environmental factors have also been found to play a crucial role in the development of sarcoidosis, through mounting epidemiological data showing seasonal, time and space clusters, as well as a possible relation to several occupations (e.g. firefighters) [5]. Possible organisms include mycobacterium and propionibacterium acne, as they can be detected in sarcoid granulomas, but further large-scale research is required in order to verify these associations.

Genetic predisposition

Human leukocyte antigens (HLA) genes

Many studies have reported on the association of human leukocyte antigen genes with sarcoidosis, as they are crucial in antigen presentation and immunoregulation via the major histocompatibility complex (MHC) [14, 18, 19]. The HLA class II molecules are expressed on antigen-presenting cells (APC), in a specific way that will generate an abnormal inflammatory response that results in the formation of granulomas, as seen in sarcoidosis [20]. Additionally, with the limited involvement of the T cell receptor α and β chain variable gene segments on T cells detected, it suggests that there is recognition of specific antigenic peptides on the HLA class II variants, increasing the efficiency of presentation [21, 22].

There are different genes affecting susceptibility, phenotype, and prognosis of sarcoidosis. DRB1-11,12,14,15 are risk factors, while DRB1-01,-04 are protective, indicating a good prognosis [23-25].Among the specific DR alleles investigated, HLA-DRB1-1101, -0402, -1201 and -1501 were the most closely related to sarcoidosis, with -1101 and -402 associated with extra-pulmonary involvement [5]. HLA-DRB1-0301 also promotes the expression of lung restricted AV2S3+ CD4+ T cells, which further encourages the pathogenesis of pulmonary sarcoidosis in patients [26].

However, a tight and highly variable degree of linkage disequilibrium within the MHC region has made it difficult to find a primary gene responsible for the initiation of granuloma formation. Strong linkage between certain genes, such as DRB1-1501 and DQB1-0602, has also contributed to the difficulty of determining a specific gene [24, 27, 28].

Non-HLA genes

A summary of the other non-HLA genes are listed in Table 1 [29].

Candidate gene	Location	Possible functional significance
Butyrophilin-like 2 gene	Premature splice location at exon 5 (rs 276530G -> A) [30]	Promotes exaggerated T lymphocyte activation and is related to CD80 and CD86 costimulatory factors [31] However, the independence of HLA class II alleles is controversial [13]
Annexin A11	Single nucleotide polymorphism (rs 1049550, R230C) [13]	Affects calcium signaling and apoptosis, therefore influencing the number of viable inflammatory cells [32]
Angiotensin-converting enzyme (ACE)	17q23 (insertion of 287 base pairs into intron 16)	Reflects granuloma burden, but has controversial conclusions in several studies regarding the correlation of ACE gene polymorphisms and sarcoidosis [33, 34]
Chemokine receptor 2 and 5	3p21.3	Immune cell trafficking [35] However, studies have mixed conclusions, some sharing association with disease, disease phenotypes, or no significant associations [36, 37]
Toll-like receptor (TLR) TLR 10, 1, 6 cluster	Chromosome 4	Cluster is particularly involved in activation of immune cells by acting as co-receptor for TLR2, and is linked to self-remitting Löfgren's syndrome and chronic sarcoidosis [38]

Table 1. Summary of non-HLA genes involved in the genetic predisposition of sarcoidosis.

More GWAS studies have been conducted recently, identifying novel chromosomal regions that may be risk loci for sarcoidosis, such as RAB23 and OSA9 [39, 40], but more genetic studies must be conducted to verify these associations.

Extrinsic factors

Extrinsic factors are generally divided into two broad categories: infectious and non-infectious (Table 2.1 and 2.2).

Infectious agents

Agent	Evidence for	Evidence against
Mycobacteria	Mycobacterial DNA and peptide fragments detected in 48% of bronchoalveolar lavage fluid (BALF) or biopsy obtained from patients freshly diagnosed with sarcoidosis (9-19 fold increase as compared to non-sarcoidosis controls) [41] Patients have had disease associated with mycobacteria before, during or after sarcoidosis [42-44] T-cells in sarcoidosis patients recognise unique epitopes of antigen 85A (virulence factors of mycobacterial species) and respond to ESAT-6, katG and SodA (proteins that are released during active mycobacterial replication) [45-47] Common disease-related blood markers have been found in both TB and sarcoidosis, underlining similar pathology [48, 49]	Inability to identify mycobacteria via staining and culture from sarcoidosis tissues (other than occasional granulomas) Anti-tuberculosis treatment does not work on sarcoidosis patients [41] Patients with sarcoidosis have tuberculin anergy [50] Highly variable percentages of presence of mycobacteria in sarcoidosis patients - due to different types of mycobacteria observed (dependent on origin on tissue and sensitivity and specificity of polymerase chain reaction) [41] The presence of mycobacterium only proves a possible association, not a causative relationship [51]
Propionibacterium acnes (P.*acnes*)	Bacterium can be cultured from 78% of sarcoidosis samples [52] P. acnes proteins have been observed in 40% of BAL samples	P. acnes can be found in 57% of healthy control tissues [51]

	40% of sarcoidosis samples have antibody response to P. acnes protein [53]	
Viruses and other infectious organisms	Serologic evidence found in patients with sarcoidosis (herpes virus) Antibody responses to these viruses have been found in sarcoidosis patients Patients with sarcoidosis are more likely to report high humidity, water damage and musty odours in their environment, which possibly contributes to the growth and presence of microbial aerosols [54]	Viruses are not known to cause the development of granulomas Patients with antibodies response have previous exposure to the organisms before Increased antibodies levels may not be due to viruses exposure, but to non-specific polyclonal hypergammaglobulinemia (a feature of sarcoidosis) [55] Levels of viruses in both healthy controls and sarcoidosis patients are similar [51]

Table 2.1. The infectious organisms that are potentially causative agents or act as a co-factor in the development of sarcoidosis.

Non-infectious agents

Agent	Evidence for	Evidence against
Transplant	Patients develop sarcoidosis after receiving transplant from donor with sarcoidosis (for haematopoetic stem cell transplant, breast cancer, testicular cancer, heart transplant) [56-59]	-

Environmental agents	Studies have shown a positive link between silica, talc, man-made fibers, chemical/metal dust (berrylium), organic agents, wood dust or smoke Sarcoidosis clusters in firefighters have been reported [60]	Extra-pulmonary involvement is often not involved in other dust exposure diseases, suggesting it may be another respiratory disease instead of sarcoidosis [60]

Table 2.2. Non-infectious factors that have been found to have a positive relation with the development of sarcoidosis.

5. Immunopathogenesis

Immune paradox

Sarcoidosis is commonly described as an 'immune paradox', due to the presence of extensive local inflammation being associated with the lack of $CD4^+$ T cells activation peripherally [61]. This peripheral anergy (lack of immune response) may be due to a disruption of balance between effector and regulatory T lymphocytes, with a high percentage of $CD4^+CD25^{bright}FoxP3^+$ T regulatory cells found in the blood, BALF and granulomas in patients with active sarcoidosis [61-63]. They have been noted to exert anti-proliferative effects on naive T cells by decreasing the production of interlekin-2 (IL-2), but only weakly downregulate other inflammatory cytokines like tumour necrosis factor-α (TNF-α), therefore still allowing granuloma formation [61, 64]. Other potential hypotheses include possible accumulation of activated T cells at the local sites of inflammation, leaving few T cells at the periphery; as well as the increase in immunosuppressive $CD8^+$ T cells peripherally as the disease progresses chronically, thus resulting in an immune paradox [65]. However this is a poorly understood area, as none of the hypotheses has been able to fully explain the immune paradox.

Granuloma formation

Well formed, non-caseating granulomas are the histological hallmarks of the disease, although necrotising granulomas can be observed in some cases of sarcoidosis [4, 66]. It must still, however, be differentiated from granulomas caused by other diseases, such as brucellosis, neoplastic disorders and reconstitution syndrome of acquired immunodeficiency syndrome, via thorough clinical and histological examination [67, 68].

The core of the granuloma is made up of epithelioid cells (activated tissue macrophages) and CD4 type 1 helper T cells (T_H1) cells in various stages of activation and differentiation, while the periphery is made up of fibroblasts, CD8 T lymphocytes, T regulatory cells and B cells [61, 65]. In a classic granuloma, the cells are arranged presumably to contain the antigen, preventing its spread and limiting the inflammation to prevent damage to the surrounding tissue [66] (Figure 1). DNA and peptide fragments of mycobacterium have been detected in sarcoid granulomas previously, but there is still much evidence against mycobacterium being a main causative agent (Table 2.1.).

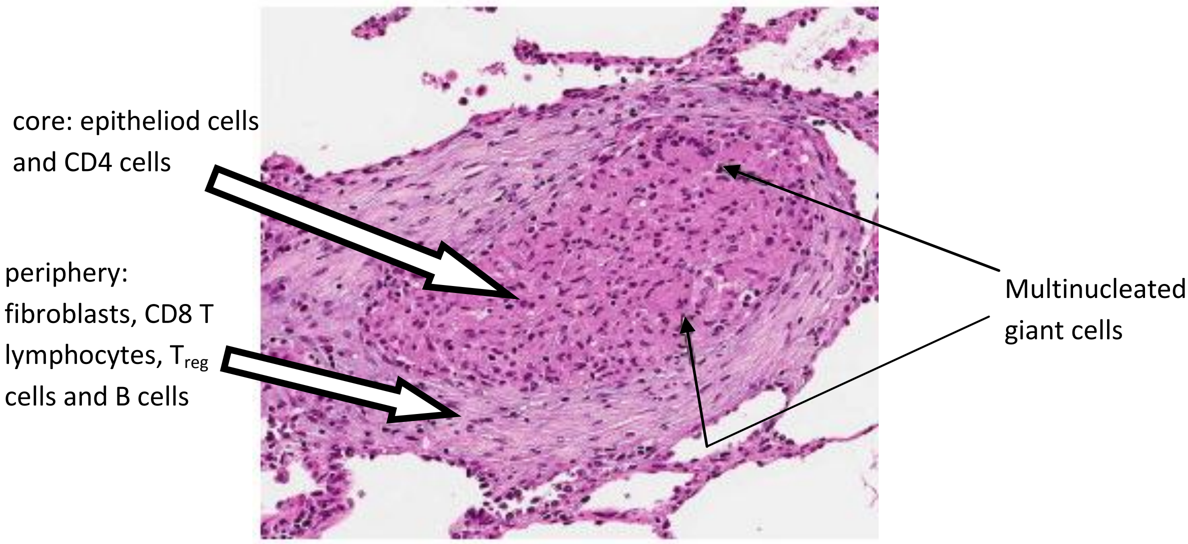

Figure 1. The histopathological appearance of a sarcoid granuloma. The center of the granuloma is formed by epithelioid cells, with the black arrows pointing to multinucleated giant cells. [11]

T_H1-driven immune response

Granuloma formation can be separated into four main stages: initiation, accumulation, effector phase, and resolution [66].

In the initiation phase, alveolar macrophages and dendritic cells arrive at the site of antigen presentation to internalise the foreign body. The APCs then migrate to local lymph nodes, where they mature and present peptides to CD4 T cells via MHC complex molecules [66, 69]. The variable portions (V-α and V-β) of the T cell receptor (TCR) on naive $CD4^+$ T cells then bind to the MHC-antigen complex, stimulating T cells and cause oligoclonal expansion of V-β-specific T cells in blood, lung and skin, which releases T_H1 cytokines [26, 70]. CD28, a co-stimulatory molecule expressed by T cells, interacts with CD80 and CD86 on APCs to make a second signal, enhancing stimulation [71, 72]. Other co-stimulatory molecules that play an important role include CD83 and HLA-DR [2].

The T cells then play the major role in the accumulation and effector phase by releasing chemokines and cytokines to recruit and organise effector cells at the site of granuloma formation [66]. IL-6 and IL-8 are released by T cells and macrophages respectively in local inflammatory sites, recruiting eosinophils, CD4 T cells and neutrophils [73]. Further chemokines, such as RANTES, MIP-1β, MCP-1, CXCL10, further attract effector cells [2, 74].

In the effector phase, IL-2 and interferon-γ (IFN-γ) are secreted spontaneously by the T_H1 cells, and act as growth factors for T_H1 lymphocytes and enhancers of the cytotoxic T cells respectively, encouraging the inflammation process [66, 75]. Alveolar macrophages also aid this process by releasing TNF-α and IL-15, which induces proliferative responses in T cells by acting on T cell IL-2R [74, 76]. They secrete IL-12 and IL-18 as well, which skew the immune response towards a T_H1-driven type by further inducing IFN-γ [77, 78]. IL-12 and IL-18 also work together in a positive feedback loop to promote granuloma formation, with IL-12 increasing IL-18 receptor expression on $CD4^+$ T cells, and IL-18 upregulating IL-12β receptor [77].

T_H17 immune response

Recently, IL-17A has been implicated in the immunopathogenesis of sarcoidosis. It is the main cytokine produced by T_H17 cells, along with IL-17F, IL-22 and IFN-γ. Differentiation into T_H17 cells is dependent on IL-1β, IL-6, TGF-β and IL-23, in which increased levels of IL-1βmRNA expression and IL-23 have been noted [79]. One study observed heightened levels of $IL\text{-}22^+$ T cells in local granulomas, which indicates that sarcoidosis may be a T_H1/T_H17 multi-system disorder, with T_H17 cells having the ability to confer protective effects during infection [80, 81] (Figure 2).

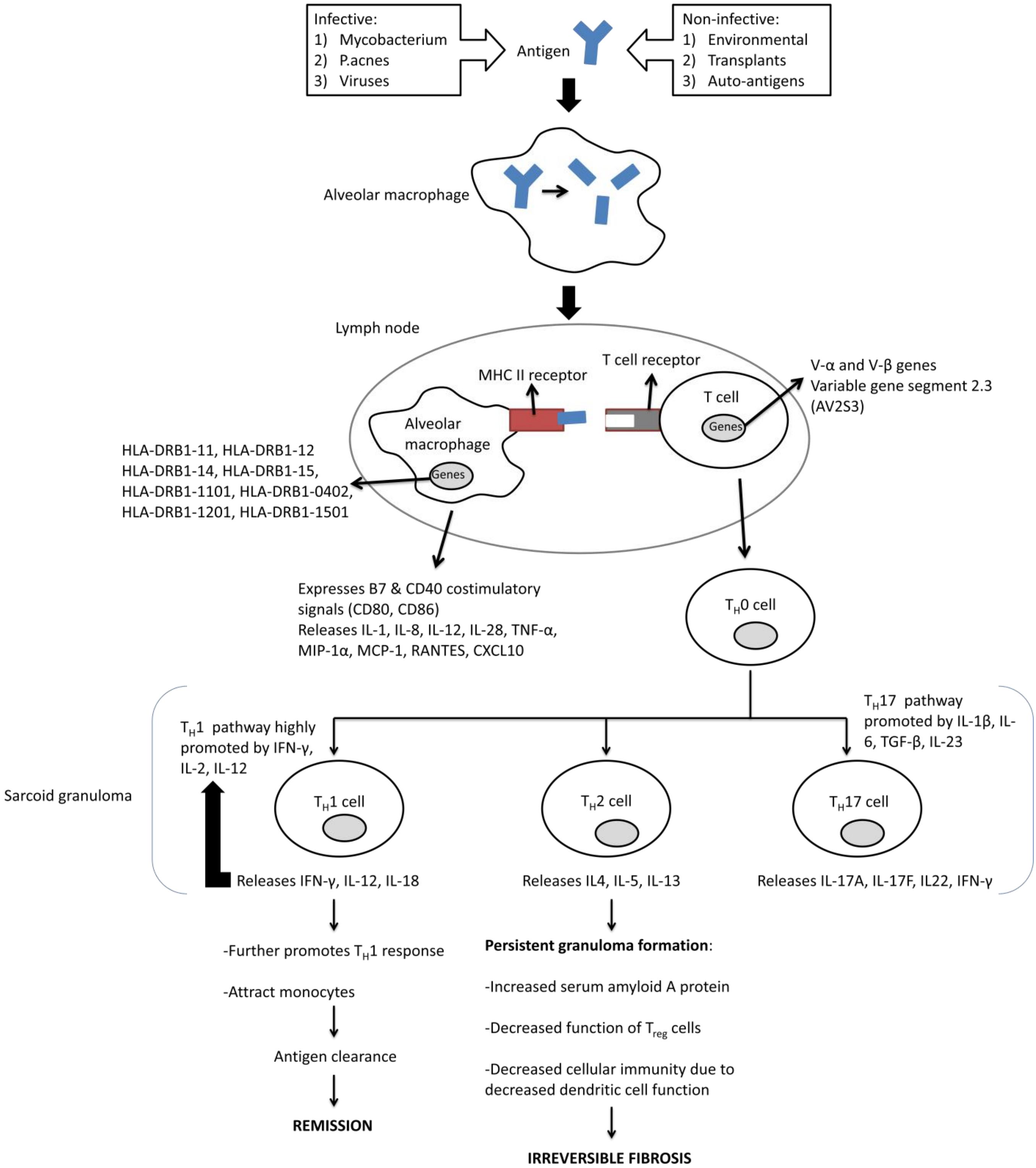

Figure 2. The immunopathogenesis of sarcoidosis. [2, 11, 67, 69, 74]

Persistent, chronic inflammation

Serum amyloid A protein

An abundance of serum amyloid A protein has been found in the sarcoid granulomas, and is thought to provide a continuous stimulus as a ligand for TLR 2 on APCs. This therefore causes an unremitting T_H1 response to pathogenic tissue antigens, resulting in chronic sarcoidosis [2, 82].

T_{reg} cells

Instead of being normally involved with the regulation and dampening of immune response, T_{reg} cells are found to be lacking in this functional aspect in the sarcoid granulomas after having undergone extensive amplification processes. They also produce pro-inflammatory cytokines, such as IL-4, which contribute to lung fibrosis [83].

Antigenic stimuli

Dendritic cells have been found to have decreased responses in patients with active sarcoidosis. This may possibly be linked to numerous reports describing how microbial pathogens (such as mycobacterium) infecting dendritic cells can downregulate the inflammatory process, and therefore contribute to the persistence of the disease as the cellular immunity is unable to get rid of the antigenic stimuli [84]. More research is required regarding the causative agents of sarcoidosis in order to validate this theory.

Remission or progress to fibrosis

The timing, pace, and balance between a T_H1 and T_H2 response determine if the disease and granulomas will resolve or progress to fibrosis. Disease remission usually occurs when the antigen is completely eliminated by the T_H1 lymphocytic immune response [5]. Additionally, IFN-γ, which is produced by T_H1 cells, inhibits fibroblast proliferation and is therefore less likely to progress to irreversible fibrosis [65].

A switch to T_H2 cytokine predominance has been observed in patients with chronic sarcoidosis with irreversible fibrosis, as the production of cytokines like IL-4, IL-5, IL-7 and IL-13, encourages collagen production and fibrogenic processes [85]. CCL2 is another potential mediator, and it promotes the recruitment and propagation of fibroblasts together with IL-13 via the transforming growth factor-β (TGF-β) . However, studies have noted that an increase in TGF-β release has been linked to spontaneous resolution by downregulating T-cell activation and TNF-α release, therefore prompting the need for further research in this area [5, 86].

Another explanation for the initiation of fibrosis involves alternative M2 phenotype of alveolar macrophages among the T_H2 cytokines (IL-10 and/or IL-13). The cytokines promote the switch to a more profibrotic macrophage phenotype, which produces TGF-β and various chemokines, such as CCL18. This further activates fibroblasts and enhances collagen synthesis [87].

6. Exhaled breath condensate

The diagnosis of sarcoidosis presently remains as a disease of exclusion, for the clinical features are not specific to the disease. The current way to diagnose sarcoidosis consists of a compatible clinical and imaging presentation, along with histological evidence of non-necrotising granulomas on biopsy [88]. Biopsies are normally conducted via fine-needle aspiration on the most accessible organ, which may include intrathoracic lymph nodes [89]. This makes the diagnostic methodology of sarcoidosis an invasive one. Induced sputum and bronchoalveolar lavage have also been considered as alternatives, but still pose problems as they are invasive procedures, with the latter requiring general anaesthesia [90].

Exhaled breath has recently been studied as a potential diagnostic tool for sarcoidosis, due to its non-invasive nature and ability to decrease the amount of damage done to surrounding structures [91]. Biomarkers may be identified from the exhaled breath condensate (EBC), which may therefore aid in assessment and therapy of a variety of lung conditions [92-94]. It consists of two phases, a gaseous state of water vapour with volatile compounds (nitric oxide, carbon monoxide and hydrocarbons), and a liquid state, containing several nonvolatile compounds (urea, leukotrienes, prostanoids and cytokines) from the airway lining fluid [95]. In EBC collection, the gas exhaled from the lungs is cooled by a refrigeration device in a cooling unit, therefore condensing the gaseous state and volatiles into liquid state [92] (Figure 3).

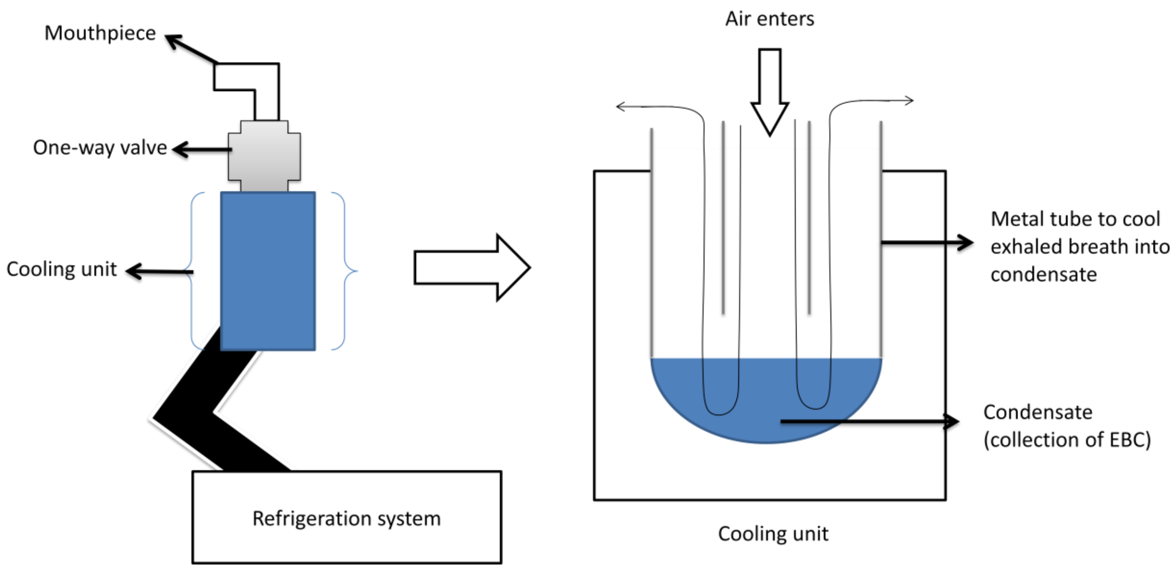

Figure 3. EBC collecting system. Breath exhaled from the subject will enter cooling unit(-20°C) via the mouthpiece and the one-way valve, and subsequently condense in the sample collection vial as the temperature is below freezing point [92].

EBC holds several advantages over BAL and the induction of sputum, as it is a non-invasive (no introduction of foreign substances and no induction of inflammation), simple and low cost technique, allowing the use of this methodology in large studies. Patients who are unable to be tested via other methods due to the severity of their illness can successfully participate in this technique as well [92, 96].

However, there are still uncertainties regarding the use of EBC, such as low detectable biomarkers the lack of knowledge regarding the origin of the biomarkers [97, 98]. Suggestions have been made to overcome the former problem, such as collecting larger amounts of EBC to concentrate the cytokines, coating sampling equipment surfaces to prevent absorbance of proteins and using high sensitivity assay kits [99].

The second uncertainty still requires further research, as current literature suggests that EBC includes fluid from alveoli and larger airways due to its collection from the mouth, as compared to BAL, which samples the lining fluid of the lower respiratory tract [95]. There may also be components from the oral cavity, but this can be countered with the use of a salivary trap [96]. Nevertheless, inflammatory biomarkers, DNA and epithelial cells from the lower respiratory tract have been detected, suggesting that this is a potential novel procedure to identify new biomarkers for sarcoidosis.

Currently, a few biomarkers have been studied in EBC so as to further understand the pathogenesis and possibility of these biomarkers being diagnostic tools of sarcoidosis. In one study, neopterin, ACE and active TGF-β_1 levels were measured in EBC. Neopterin and TGF-β_1 were revealed to be significantly higher in sarcoid patients as compared to normal subjects, but there was no difference detected in EBC ACE activity [100]. Another study also detected 8-isoprostane (8-IP), cysteinyl leukotrienes and leukotriene B4 in EBC and found them to be significantly higher than that in healthy controls [101-103]. Larger sample sizes and further research regarding their origins will be required in order to validate the possibility of these biomarkers being diagnostic markers [102].

In addition, mediators such as IL-6, TNF-α, insulin-like growth factor -1 and plasminogen activator inhibitor-1 have been evaluated in EBC and compared to BALF. Highly significant correlations were observed in EBC and BALF, although a negative association has been noticed in IL-6, possibly due to its tendency to form complex molecules that do not transfer to exhaled breath condensate [104].

Although there have been no biomarkers suitable to diagnose sarcoidosis to date, these studies encourage researchers to delve further into the potential of using EBC as a diagnostic tool, especially with its non-invasive advantage over BALF.

There are several T_H1 and T_H17 cytokines that have yet to be studied in EBC, such as IL-12p40, IL-18 and IL-23 [90]. They have been detected in blood and BALF in previous studies, and have been found to be significantly higher in sarcoidosis patients compared to healthy control subjects [105-107]. As most of the patients have pulmonary involvement, research in this aspect may thus yield positive correlations between IL-12p40, IL-18 and IL-23 with sarcoidosis, without having to resort to use of invasive and high cost methods like biopsies and BALF. With a current unclear picture of the aetiology, pathogenesis and prognosis of sarcoidosis, new diagnostic methodologies such as EBC and the respective novel biomarkers detected may be valuable in shedding light on to these uncertain concepts.

7. Conclusion

Sarcoidosis is a multisystemic inflammatory disorder typified by non-caseating epitheloid granulomas. Despite the extensive research that has been conducted, the aetiology and pathology of sarcoidosis remain unclear. This contributes to the difficulty of diagnosing and staging the patients, especially with the non-specific symptoms patients with sarcoidosis present with. As majority of the patients have pulmonary involvement, EBC may be valuable as a non-invasive diagnostic method in detecting novel biomarkers present in patients with sarcoidosis. Further research is therefore required to identify new significant biomarkers involved in the pathogenesis of sarcoidosis in breath, which may also aid in shedding light regarding the undetermined aetiology and pathology of the disease.

8. References

1. U. Costabel and G.W. Hunninghake," ATS/ERS/WASOG statement on sarcoidosis. Sarcoidosis Statement Committee. American Thoracic Society. European Respiratory Society. World Association for Sarcoidosis and Other Granulomatous Disorders," Eur Respir J, vol. 14, no. 4, 1999, pp. 735-737.
2. M. Iannuzzi and J. Fontana," Sarcoidosis," Journal of American Medical Association, vol. 305, no. 4, 2011, pp. 391-399.
3. O.A. El-Zammar and A.L.A. Katzenstein," Pathological diagnosis of granulomatous lung disease: a review," Histopathology, vol. 50, no. 3, 2007, pp. 289-310.
4. M.C. Iannuzzi, B.A. Rybicki, and A.S. Teirstein," Sarcoidosis," New England Journal of Medicine, vol. 357, no. 21, 2007, pp. 2153-2165.
5. J. Müller-Quernheim, A. Prasse, and G. Zissel," Pathogenesis of sarcoidosis," Press Med, vol. 41, no. 6, 2012, pp. e275-287.
6. M. Thomeer, M. Demedts, and W. Wuyts," Epidemiology of sarcoidosis," European Respiratory Monograph, vol. 32, no. 2005, pp. 13-22.
7. T. Morimoto, et al.," Epidemiology of sarcoidosis in Japan," Eur Respir J, vol. 31, no. 2008, pp. 372-279.
8. A. Gillman and C. Steinfort," Sarcoidosis in Australia," Internal Medicine Journal, vol. 37, no. 6, 2007, pp. 356-359.
9. D. Anantham, et al.," Sarcoidosis in Singapore: Epidemiology, clinical presentation and ethnic differences," Respirology, vol. 12, no. 3, 2007, pp. 355-360.
10. R.P. Baughman, et al.," Clinical Characteristics of Patients in a Case Control Study of Sarcoidosis," American Journal of Respiratory and Critical Care Medicine, vol. 164, no. 10, 2001, pp. 1885-1889.
11. D. Culver," Sarcoidosis," Immunology and allergy clinics of North Amercia, vol. 32, no. 4, 2012, pp. 487-511.
12. S. Hofmann, et al.," Genome-wide association study identifies ANXA11 as a new susceptibility locus for sarcoidosis," Nature Genetics, vol. 40, no. 9, 2008, pp. 1103-1106.
13. I. Adrianto, et al.," Genome-Wide Association Study of African and European Americans Implicates Multiple Shared and Ethnic Specific Loci in Sarcoidosis Susceptibility," PloS one, vol. 7, no. 8, 2012, pp. p.e43907.

14. M. Schurmann, et al.," Results from a Genome-wide Search for Predisposing Genes in Sarcoidosis," American Journal of Respiratory and Critical Care Medicine, vol. 164, no. 5, 2001, pp. 840-846.
15. B.A. Rybicki, et al.," Familial Aggregation of Sarcoidosis," American Journal of Respiratory and Critical Care Medicine, vol. 164, no. 11, 2001, pp. 2085-2091.
16. M. Schürmann, et al.," Angiotensin-converting enzyme (ACE) gene polymorphisms and familial occurrence of sarcoidosis," Journal of Internal Medicine, vol. 249, no. 1, 2001, pp. 77-83.
17. E. Fité, et al.," Sarcoidosis: Family Contact Study," Respiration, vol. 65, no. 1, 1998, pp. 34-39.
18. B.A. Rybicki, et al.," Genetic linkage analaysis of sarcoidosis phenotyps: the sarcoidosis genetic analysis (SAGA) study," Genes and Immunity, vol. 8, no. 5, 2007, pp. 379-386.
19. A. Fischer, et al.," A genome-wide linkage analysis in 181 German sarcoidosis families using clustered biallelic markers," Chest, vol. 138, no. 1, 2010, pp. 151-157.
20. P. Spagnolo and J. Grunewald," Recent advances in the genetics of sarcoidosis," Journal of Medical Genetics, vol. 50, no. 5, 2013, pp. 290-297.
21. J. Grunewald, et al.," Restricted Vα2. 3 gene usage by CD4 T lymphocytes in bronchoalveolar lavage fluid from sarcoidosis patients correlates with HLA‐DR3," European journal of immunology, vol. 22, no. 1, 1992, pp. 129-135.
22. J. Forman, et al.," Selective Activation and Accumulation of Oligoclonal V beta-Specific T cells in Active Pulmonary Sarcoidosi," The journal of clinical investigation, vol. 94, no. 4, 1994, pp. 1533-1542.
23. M. Schürmann, et al.," HLA-DQB1 and HLA-DPB1 genotypes in familial sarcoidosis," Respiratory Medicine, vol. 92, no. 4, 1998, pp. 649-652.
24. M. Berlin, et al.," HLA-DR Predicts the Prognosis in Scandinavian Patients with Pulmonary Sarcoidosis," American Journal of Respiratory and Critical Care Medicine, vol. 156, no. 5, 1997, pp. 1601-1605.
25. J.C. Grutters, et al.," The importance of sarcoidosis genotype to lung phenotype," American Journal of Respiratory Cell and Molecular Biology, vol. 29, no. 3, 2003, pp. S59-62.
26. J. Grunewald, et al.," Lung restricted T cell receptor AV2S3+ CD4+ T cell expansions in sarcoidosis patients with a shared HLA-DRβ chain conformation," Thorax, vol. 57, no. 4, 2002, pp. 348-352.
27. C.E.M. Voorter, M. Drent, and E.M. van den Berg-Loonen," Severe Pulmonary Sarcoidosis Is Strongly Associated With the Haplotype HLA-DQB1*0602–DRB1*150101," Human Immunology, vol. 66, no. 7, 2005, pp. 826-835.

28. H. Sato, et al.," HLA-DQB1*0201: a marker for good prognosis in British and Dutch patients with sarcoidosis," American Journal of Respiratory Cell and Molecular Biology, vol. 27, no. 4, 2002, pp. 406-412.
29. B.P. Sah and M.C. Iannuzzi, *Genetic Factors Involved in Sarcoidosis*. Sarcoidosis. 2013.
30. Y. Li, et al.," BTNL2 gene variant and sarcoidosis," Thorax, vol. 61, no. 3, 2006, pp. 273-274.
31. B.A. Rybicki, et al.," The BTNL2 Gene and Sarcoidosis Susceptibility in African Americans and Whites," The American Journal of Human Genetics, vol. 77, no. 3, 2005, pp. 491-499.
32. C.S. Jùrgensen, et al.," Determination of autoantibodies to annexin XI in systemic autoimmune diseases," Lupus, vol. 9, no. 7, 2000, pp. 515-520.
33. A. Planck, et al.," Angiotensin-converting enzyme gene polymorphism in relation to HLA-DR in sarcoidosis," Journal of Internal Medicine, vol. 251, no. 3, 2002, pp. 217-222.
34. M.J. Maliarik, et al.," Angiotensin-converting Enzyme Gene Polymorphism and Risk of Sarcoidosis," American Journal of Respiratory and Critical Care Medicine, vol. 158, no. 5, 1998, pp. 1566-1570.
35. R. Valentonyte, et al.," Study of C-C Chemokine Receptor 2 Alleles in Sarcoidosis, with Emphasis on Family-based Analysis," American Journal of Respiratory and Critical Care Medicine, vol. 171, no. 10, 2005, pp. 1136-1141.
36. A. Fischer, et al.," Female-specific association of C-C chemokine receptor 5 gene polymorphisms with Löfgren's syndrome," Journal of molecular medicine (Berlin, Germany), vol. 86, no. 5, 2008, pp. 553-561.
37. P. Spagnolo, et al.," C-C Chemokine Receptor 5 Gene Variants in Relation to Lung Disease in Sarcoidosis," American Journal of Respiratory and Critical Care Medicine, vol. 172, no. 6, 2005, pp. 721-728.
38. M. Veltkamp, et al.," Genetic variation in the Toll-like receptor gene cluster (TLR10-TLR1-TLR6) influences disease course in sarcoidosis," Tissue Antigens, vol. 79, no. 1, 2011, pp. 25-32.
39. S. Hofmann, et al.," Genome-wide association analysis reveals 12q13.3–q14.1 as new risk locus for sarcoidosis," European Respiratory Jounral, vol. 41, no. 4, 2013, pp. 888-900.
40. S. Hofmann, et al.," A genome-wide association study reveals evidence of association with sarcoidosis at 6p12.1," European Respiratory Jounral, vol. 38, no. 5, 2011, pp. 1127-1135.
41. D. Gupta, et al.," Molecular evidence for the role of mycobacteria in sarcoidosis: a meta-analysis," Eur Respir J, vol. 30, no. 3, 2007, pp. 508-516.
42. K. Hatzakis, N. Siafakas, and D. Bouros," Miliary sarcoidosis following miliary tuberculosis," Respiration, vol. 67, no. 2, 2000, pp. 219-222.

43. S. Ganguly and D. Ganguly," Sarcoidosis following sputum positive pulmonary tuberculosis: A rare entity," Indian Journal of Dermatology, vol. 57, no. 1, 2012, pp. 76-78.
44. I. Skoie, K. Wildhagen, and R. Omdal," Development of sarcoidosis following etanercept treatmt: a report of three cases," Rheumatology International, vol. 32, no. 4, 2012, pp. 1049.
45. W. Drake, et al.," Cellular recognition of Mycobacterium tuberculosis ESAT-6 and KatG peptides in systemic sarcoidosis," Infection and Immunity, vol. 75, no. 1, 2007, pp. 527-530.
46. J. Carlisle, et al.," Multiple Mycobacterium antigens induce interferon-γ production from sarcoidosis peripheral blood mononuclear cells," Clinical and experimental immunology, vol. 150, no. 3, 2007, pp. 460-468.
47. K. Oswald-Richter, et al.," Multiple mycobacterial antigens are targets of the adaptive immune response in pulmonary sarcoidosis," Respiratory Research, vol. 11, no. 1, 2010, pp. 161.
48. J. Maertzdorf, et al.," Common patterns and disease-related signatures in tuberculosis and sarcoidosis," Proc Natl. Acad. Sci. USA, vol. 109, no. 20, 2012, pp. 7853-7858.
49. L.L. Koth, et al.," Sarcoidosis Blood Transcriptome Reflects Lung Inflammation and Overlaps with Tuberculosis," American Journal of Respiratory and Critical Care Medicine, vol. 184, no. 10, 2011, pp. 1153-1163.
50. D. Gupta, et al.," Anergy to tuberculin in sarcoidosis is not influenced by high prevalence of tuberculin sensitivity in the population," Sarcoidosis Vasc Diffuse Lung Dis, vol. 20, no. 1, 2003, pp. 40-45.
51. S. Saidha, E. Sotirchos, and C. Eckstein," Etiology of Sarcoidosis: Does Infection Play a Role?," Yale Journal of Biology and Medicine, vol. 85, no. 1, 2012, pp. 133-141.
52. Y. Eishi, et al.," Quantitative Analysis of Mycobacterial and Propionibacterial DNA in Lymph Nodes of Japanese and European Patients with Sarcoidosis," J. Clin. Microbiol., vol. 40, no. 1, 2002, pp. 198-204.
53. M. Negi, et al.," Localization of Propionibacterium acnes in granulomas supports a possible etiologic link between sarcoidosis and the bacterium," Modern Pathology, vol. 25, no. 9, 2012, pp. 1284-1297.
54. L.S. Newman, et al.," A Case Control Etiologic Study of Sarcoidosis," American Journal of Respiratory and Critical Care Medicine, vol. 170, no. 12, 2004, pp. 1324-1330.
55. E. Chen and D. Moller," Etiology of Sarcoidosis," Clin Chest Med, vol. 29, no. 3, 2008, pp. 365-377.
56. R. Bhagat, et al.," Pulmonary Sarcoidosis following stem cell transplantation; Is it more than a chance occurrence?," Chest, vol. 162, no. 2, 2004, pp. 642-643.

57. A. Schattenberg, et al.," A mediastinal mass after donor lymphocyte infusion for relapse of chronic myeloid leukemia after allogenic stem cell transplantation," Leukemia & Lymphoma, vol. 47, no. 6, 2006, pp. 1188-1190.
58. M. Teo, et al.," A case of sarcoidosis in a patient with testicular cancer post stem cell transplant.," Acta oncologica, vol. 52, no. 4, 2013, pp. 869-871.
59. J. Yager, et al.," Recurrence of cardiac sarcoidosis in a heart transplant recipient," The journal of heart and lung transplantation, vol. 24, no. 11, 2005, pp. 1988-1990.
60. G. Izbicki, et al.," World Trade Center “Sarcoid-Like” Granulomatous Pulmonary Disease in New York City Fire Department Rescue Workers," Chest, vol. 131, no. 5, 2007, pp. 1414-1423.
61. M. Miyara, et al.," The immune paradox of sarcoidosis and regulatory T cells," Journal of Experimental Medicine, vol. 203, no. 2, 2006, pp. 359-370.
62. T. Fujimura, Y. Kambayashi, and S. Aiba," Expression of CD39/Entpd1 on granuloma-composing cells and induction of Foxp3-positive regulatory T cells in sarcoidosis," Clinical and Experimental Dermatology, vol. 38, no. 8, 2013, pp. 883-889.
63. H. Huang, et al.," Imbalance between Th17 and Regulatory T-Cells in Sarcoidosis," International Journal of Molecular Sciences, vol. 14, no. 11, 2013, pp. 21463-21473.
64. J. Grunewald and A. Eklund," Role of CD4+ T Cells in Sarcoidosis," American Thoracic Society, vol. 4, no. 5, 2007, pp. 461-464.
65. A. Gerke and G. Hunninghake," THe Immunology of Sarcoidosis," Clinics in Chest Medicine, vol. 29, no. 3, 2008, pp. 379-390.
66. D.O. Co., et al.," T cell contributions to the different phases of granuloma formation," Immunology Letters, vol. 92, no. 1-2, 2004, pp. 135-142.
67. J.M. Reich," On the nature of sarcoidosis," European Journal of Internal Medicine, vol. 23, no. 2, 2012, pp. 105-109.
68. J.M. Reich," Neoplasia in the etiology of sarcoidosis," European Journal of Internal Medicine, vol. 17, no. 2, 2006, pp. 81-87.
69. L. Zaba, et al.," Dendritic cells in the pathogenesis of Sarcoidosis," Am J Respir Cell Mol Biol, vol. 42, no. 1, 2010, pp. 32-39.
70. G. Zissel, et al.," TCR V β Families in T Cell Clones from Sarcoid Lung Parenchyma, BAL, and Blood," American Journal of Respiratory and Critical Care Medicine, vol. 156, no. 5, 1997, pp. 1593-1600.
71. H. Bour-Jordan and J.A. Bluestone," CD28 function: A balance of costimulatory and regulatory signals," Journal of Clinical Immunology, vol. 22, no. 1, 2002, pp. 1-7.

72. H. Ahmadzai, et al.," Peripheral blood responses to specific antigens and CD28 in sarcoidosis," Respiratory Medicine, vol. 106, no. 5, 2012, pp. 701-709.
73. D. Gupta, et al.," Immune responses to mycobacterial antigens in sarcoidosis: A systematic review," Indian J Chest Dis Allied Sci, vol. 53, no. 2011, pp. 41-49.
74. M. Ziegenhagen and J. Muller-Quernheim," The cytokine network in sarcoidosis and its clinical relevance," Journal of Internal Medicine, vol. 253, no. 1, 2003, pp. 18-30.
75. T.A. Hill, et al.," Intracellular cytokine profiles and T cell activation in pulmonary sarcoidosis," Cytokine, vol. 42, no. 3, 2008, pp. 289-292.
76. S.A. Antoniu," Targeting the TNF-α pathway in sarcoidosis," Expert Opinion on Therapeutic Targets, vol. 14, no. 1, 2010, pp. 21-29.
77. T. Yoshimoto, et al.," IL-12 Up-Regulates IL-18 Receptor Expression on T Cells, Th1 Cells, and B Cells: Synergism with IL-18 for IFN-γ Production," The Journal of Immunology, vol. 161, no. 7, 1998, pp. 3400-3407.
78. K. Shigehara, et al.," IL-12 and IL-18 Are Increased and Stimulate IFN-γ Production in Sarcoid Lungs," The Journal of Immunology, vol. 166, no. 1, 2001, pp. 642-649.
79. B. ten Berge, et al.," Increased IL-17A expression in granulomas and in circulating memory T cells in sarcoidosis," Rheumatology, vol. 51, no. 1, 2012, pp. 37-46.
80. B. Berge, et al.," Increased IL-17A expression in granulomas and in circulating memory T cells in sarcoidosis," Rheumatology, vol. 51, no. 1, 2012, pp. 37-46.
81. M. Facco, et al.," Sarcoidosis is a Th1/Th17 multisystem disorder," Thorax, vol. 66, no. 2, 2011, pp. 144-150.
82. E. Chen, et al.," Serum amyloid A regulates granulomatous inflammation in sarcoidosis through toll-like receptor-2," Am J Respir Crit Care Med, vol. 181, no. 4, 2010, pp. 360-373.
83. G. Rappl, et al.," Regulatory T cells with reduced repressor capacities are extensively amplified in pulmonary sarcoid lesions and sustain granuloma formation," Clinical Immunology, vol. 140, no. 1, 2011, pp. 71-83.
84. S. Mathew, et al.," The Anergic State in Sarcoidosis Is Associated with Diminished Dendritic Cell Function," The journal of immunology, vol. 181, no. 1, 2008, pp. 746-755.
85. K.C. Patterson, et al.," Circulating cytokines in sarcoidosis: Phenotype-specific alterations for fibrotic and non-fibrotic pulmonary disease," Cytokine, vol. 61, no. 3, 2013, pp. 906-911.
86. G. Zissel, et al.," Anti-inflammatory cytokine release by alveolar macrophages in pulmonary sarcoidosis," Am J Respir Crit Care Med, vol. 154, no. 1996, pp. 713-719.

87. A. Prasse, et al.," A vicious circle of alveolar macrophages and fibroblasts perpetuates pulmonary fibrosis via CCL18," American Journal of Respiratory and Critical Care Medicine, vol. 173, no. 7, 2006, pp. 781-792.

88. G. Hong, et al.," Usefulness of Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration for Diagnosis of Sarcoidosis," Yonsei Med J., vol. 54, no. 6, 2013, pp. 1416-1421.

89. R. Agarwal, et al.," Efficacy and safety of convex probe EBUS-TBNA in sarcoidosis: A systematic review and meta-analysis," Respiratory Medicine, vol. 106, no. 6, 2012, pp. 883-892.

90. H. Ahmadzai, D. Wakefield, and P. Thomas," The potential of the immunological markers of sarcoidosis in exhaled breath and peripheral blood as future diagnostic and monitoring techniques," Inflammopharmacol, vol. 19, no. 2, 2011, pp. 55-68.

91. A.W. Proceedings," ATS Workshop Proceedings: Exhaled nitric oxide and nitric oxide oxidative metabolism in exhaled breath condensate," American Journal of Respiratory and Critical Care Medicine, vol. 173, no. 7, 2006, pp. 811-813.

92. N. Grob, M. Aytekin, and R. Dweik," Biomarkers in exhaled breath condensate: a review of collection, processing and analysis," J Breath Res, vol. 2, no. 3, 2008, pp. 037004.

93. K. Paschke, A. Mashir, and R. Dweik," Clinical applications of breath testing," F1000 Med Rep, vol. 2, no. 2010, pp. 56.

94. S. Kharitonov and B. P.," Exhaled Biomarkers," Chest, vol. 130, no. 5, 2006, pp. 1541-1546.

95. I. Horváth, et al.," Exhaled breath condensate: methodological recommendations and unresolved questions," European Respiratory Journal, vol. 26, no. 3, 2005, pp. 523-548.

96. A.S. Jackson, et al.," Comparison of Biomarkers in Exhaled Breath Condensate and Bronchoalveolar Lavage," American Journal of Respiratory and Critical Care Medicine, vol. 175, no. 3, 2007, pp. 222-227.

97. R. Effros, et al.," A Simple Method for Estimating Respiratory Solute Dilution in Exhaled Breath Condensates," American Journal of Respiratory and Critical Care Medicine, vol. 168, no. 12, 2003, pp. 1500-1505.

98. P. Montuschi," Analysis of exhaled breath condensate in respiratory medicine: methodological aspects and potential clinical applications," Ther Adv Respir Dis, vol. 1, no. 1, 2007, pp. 5-23.

99. E. Tufvesson and L. Bjermer," Methodological improvements for measuring eicosanoids and cytokines in exhaled breath condensate," Respiratory Medicine, vol. 100, no. 1, 2006, pp. 34-38.

100. H. Ahmadzai, et al.," Measurement of neopterin, TGF-β1 and ACE in the exhaled breath condensate of patients with sarcoidosis," Journal of Breath Research, vol. 7, no. 4, 2013, pp. 046003.

101. K. Psathakis, et al.," 8-Isoprostane, a Marker of Oxidative Stress, Is Increased in the Expired Breath Condensate of Patients With Pulmonary Sarcoidosis," Chest, vol. 125, no. 3, 2004, pp. 1005-1011.

102. W. Piotrowski, et al.," Eicosanoids in Exhaled Breath Condensate and BAL Fluid of Patients With Sarcoidosis," Chest, vol. 132, no. 2, 2007, pp. 589-596.

103. W. Piotrowski, et al.," Exhaled 8-isoprostane as a prognostic marker in sarcoidosis. A short term follow-up," BMC Pulmonary Medicine, vol. 10, no. 1, 2010, pp. 23.

104. A. Rozy, et al.," Inflammatory markers in the exhaled breath condensate of patients with pulmonary sarcoidosis," Journal of physiology and pharmacology, vol. 57, no. 4, 2006, pp. 335-340.

105. D. Liu, et al.," The association between interleukin-18 and pulmonary sarcoidosis: A meta-analysis," Scandinavian Journal of Clinical & Laboratory Investigation, vol. 70, no. 6, 2010, pp. 428-432.

106. K.M. Antoniou, et al.," Upregulation of Th1 Cytokine Profile (IL-12, IL-18) in Bronchoalveolar Lavage Fluid in Patients with Pulmonary Sarcoidosis," Journal of Interferon & Cytokine Research, vol. 26, no. 6, 2006, pp. 400-405.

107. R. Mroz, et al.," Increased levels of interleukin-12 and interleukin-18 in bronchoalveolar lavage fluid of patients with pulmonary sarcoidosis," Journal of physiology and pharmacology an official journal of the Polish Physiological Society, vol. 59, no. SUPPL. 6, 2008, pp. 507-513.