Potential use of biomarkers for the clinical evaluation of sarcoidosis

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ABSTRACT

Sarcoidosis is a systemic granulomatous disease of unknown etiology and pathogenesis with a heterogeneous clinical presentation. In the appropriate clinical and radiological context and with the exclusion of other diagnoses, the disease is characterized by the pathological presence of non-caseating epithelioid cell granulomas. Sarcoidosis is postulated to be a multifactorial disease caused by chronic antigenic stimulation. The immunopathogenesis of sarcoidosis encompasses a complex interaction between the host, genetic factors and postulated environmental and infectious triggers, which result in granuloma development. The exact pathogenesis of the disease has yet to be elucidated, but some of the inflammatory pathways that play a key role in disease progression and outcomes are becoming apparent, and these may form the logical basis for selecting potential biomarkers.

Biomarkers are biological molecules that are altered pathologically. To date, there exists no single reliable biomarker for the evaluation of sarcoidosis, either diagnostically or prognostically but new candidates are emerging. A diagnosis of sarcoidosis ideally requires a biopsy confirming non-caseating granulomas, but the likelihood of progression that requires intervention remains unpredictable. These challenging aspects could be potentially resolved by incorporating biomarkers into clinical practice for both diagnosis and monitoring disease activity. This review outlines the current knowledge on sarcoidosis with an emphasis on pulmonary sarcoidosis, and delineates the understanding surrounding the implication of biomarkers for the clinical evaluation of sarcoidosis.

BACKGROUND

Epidemiology of sarcoidosis

The incidence and prevalence of sarcoidosis is reported to be highest in Nordic countries (an incidence of 5–40 per 100 000 per year and a prevalence of 0.16%) and in African-Americans (incidence of 17.8–46 per 100 000 and a prevalence of 0.14%).[1–3] The incidence is reported to be significantly lower in East Asia countries with Japan having an overall incidence of 1.01 per 100 000 and Korea having an incidence rate of 0.85 per 100 000.[4 5]

The peak age for sarcoidosis onset ranges from 30 to 55 years. Gender plays a role in manifestation of sarcoidosis, as males with sarcoidosis are diagnosed 3–10 years earlier than females who also have a higher prevalence.[6] Mortality rates in sarcoidosis ranges from 1% to 8% depending on the type and location of the disease and other health factors.[7]

Etiology of sarcoidosis

The exact etiology of sarcoidosis remains unknown with no single genetic, infectious or environmental factor being identified to have a causal link to sarcoidosis.[8]

The current pathophysiological concept suggests a model in which sarcoidosis is caused by the combination of genetic polymorphisms creating a tendency to a specific immune response, associated with exposure to environmental or infectious agents.[9] The sequence of events for the progression of sarcoidosis is depicted in figure 1.[10 11]

Genetics

A genetic tendency in the development of sarcoidosis is demonstrated by familial clustering of sarcoidosis, the varying prevalence and subtype of presentation among different ethnicities and a higher occurrence in twin studies. African-Americans have a higher prevalence of sarcoidosis (3.8-fold to 4.0-fold greater risk than European Americans), a higher rate of extrathoracic involvement and more chronic and severe disease with a lower rate of remission. Twins studies indicate an 80-fold increased risk among monozygotic twins, and a 7-fold increase among dizygotic twins.[12–14]

Case-control studies have identified that HLA alleles, responsible for the CD4+ T lymphocyte polypeptides in the HLA class I and II antigens, as being associated with specific disease subtypes and tendencies to sarcoidosis, for example, Lofgren's syndrome, a type of sarcoidosis with a good prognosis, being linked to the HLA-DR3 allele, in contrast to the HLA-DR15 allele which carries a worse prognosis.[15 16] These discoveries were further expanded by Genome Wide Association Studies and Case-Control Etiologic Study of Sarcoidosis (ACCESS) that also show the importance of genetic components in sarcoidosis.[11 17]

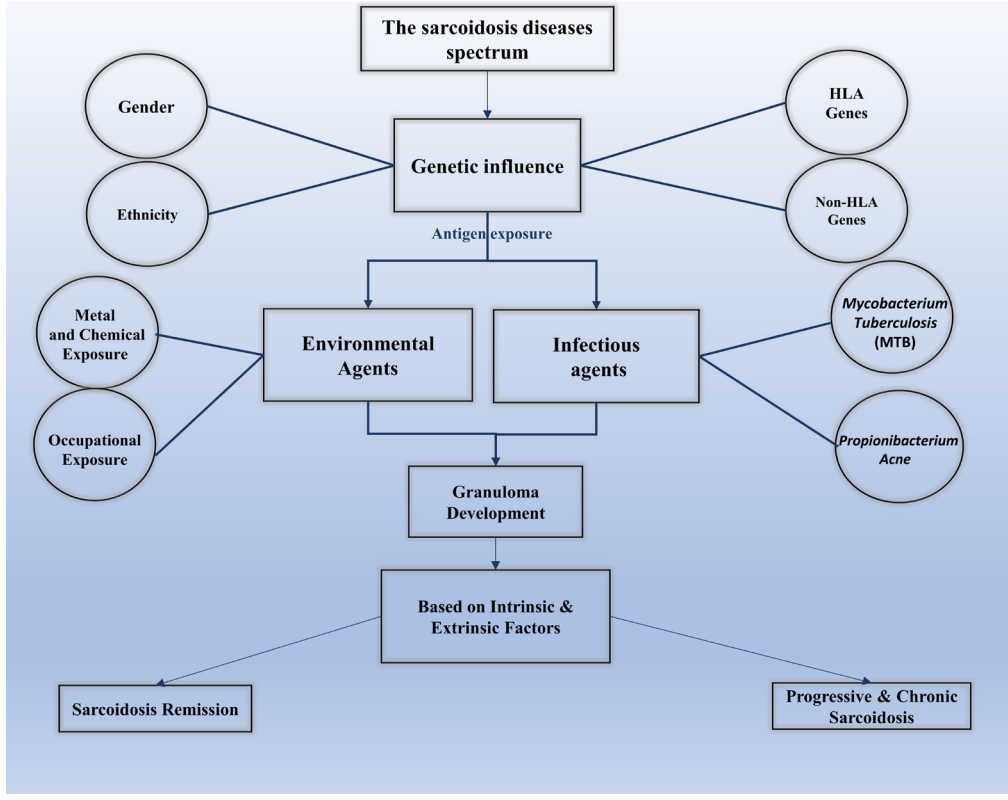

Figure 1 The sequence of events with the influence of different etiologies leading to granuloma formation.

Infectious agents

The radiological, clinical and immunological similarities between tuberculosis (TB) and sarcoidosis suggest the possible role of mycobacteria as an etiological agent.[18] The OR for the presence of mycobacterial DNA in tissue samples of patients with sarcoidosis compared with those from healthy subjects was found to be 9-fold to 19-fold and mycobacterial DNA was present in almost 30% of sarcoidosis biopsy specimens from sites such lymph nodes, lung, skin and others,[19–21] but to date, the culture of live organisms has been rare. *Propionibacterium acnes* has been isolated from culture in 78% of sarcoid lesions, which suggest a role for this organism in sarcoidosis; however, *P. acnes* was also found in 20% of non-sarcoid lymph nodes.[22] Animal models have also indicated that *P. acnes* can induce antigen-driven granulomatous inflammation.[23] Diseases with similar pathological and immunological features that resemble sarcoidosis, such as hypersensitivity pneumonitis and chronic beryllium disease, indicate that it is unclear whether sarcoidosis has an infectious etiology or not and more studies on the subject are required to investigate the exact role of these putative infectious agents.[24–26]

Environmental agents

Exposure to metals and minerals such as beryllium, chromium, aluminum, titanium, zirconium, talc and nickel has been shown to induce sarcoid-like granulomas.[27] Epidemiological studies have identified positive associations between occupations such as metal-working, fire-fighting and the handling of building supplies and sarcoidosis.[11 28 29]

Tobacco smoking decreases the risk of sarcoidosis, possibly because smoking deactivates M2 alveolar macrophages and the macrophage is thought to be pivotal in the pathogenesis of the disease.[30] The ACCESS studies have identified several environmental exposures associated with an increased risk of sarcoidosis including agricultural materials, pesticides, insecticides and microbial aerosols (mouldy and musty odours).[11] Although there are many findings implicating environmental agents as risk factors, the current evidence does not strongly favor a single environmental or occupational exposure, and may implicate a range of precipitants.[31]

Pathogenesis of sarcoidosis

The histopathological appearance of sarcoidosis is that of non-caseating granulomas formed as the result of aberrant cell-mediated immune responses to unknown antigens. Sarcoid granulomas are characterized by a central core of giant cells, epithelioid cells and helper T cells (Th).[16 32] This central area is surrounded by monocytes, mast cells, CD8+ and CD4+ T lymphocytes, B lymphocytes and fibroblasts, which in turn are surrounded by lamellar rings of hyaline collagen (figure 1). The proportions of lymphocytic infiltrate and fibrosis surrounding the central core vary depending on the patient and disease duration. Additional histopathological elements of sarcoid granulomas that may be present include Schaumann bodies, birefringent crystalline particles and asteroid bodies, which are biomolecules and mineral components incorporated into the granulomas.[33]

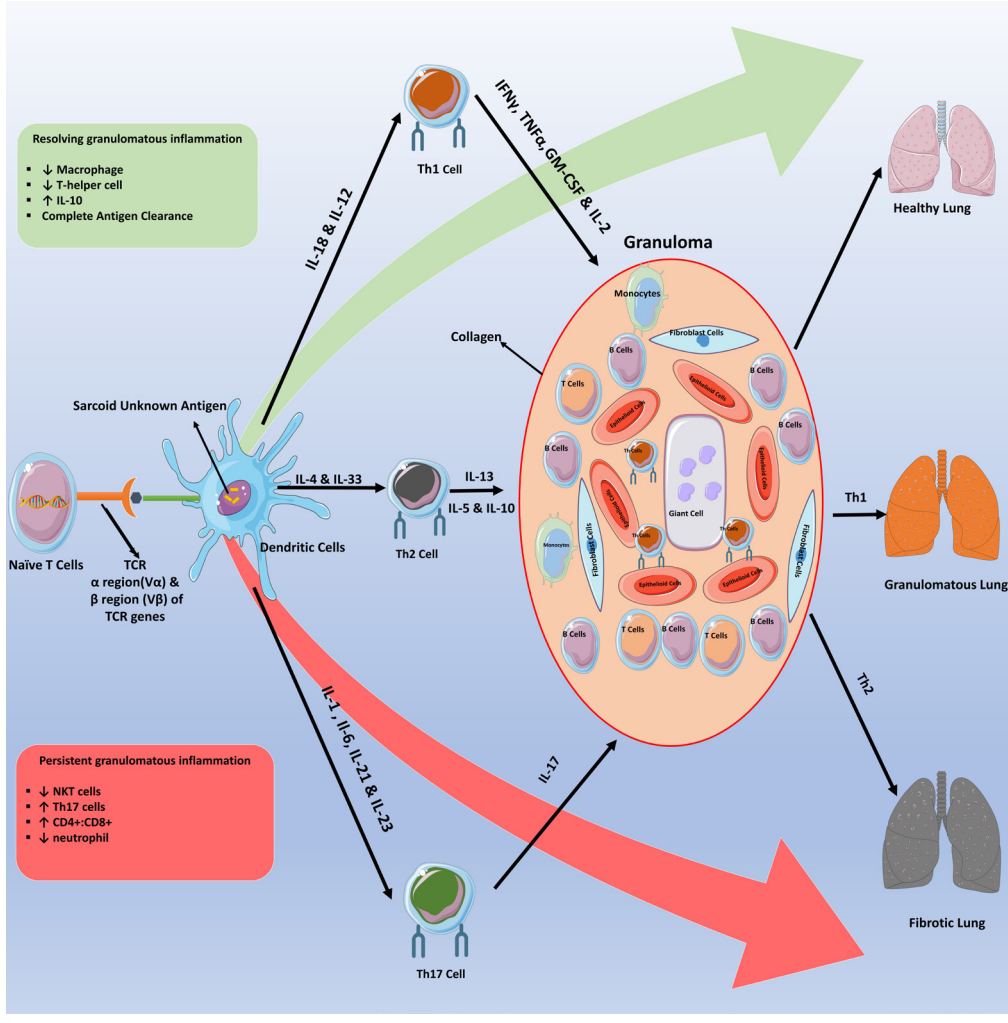

Figure 2 Overview of the postulated immunopathogenesis of sarcoidosis. The immunopathogenesis of sarcoidosis showing three different outcomes of the disease and the dominant immunological markers involved in each. GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IFN-γ, interferon gamma; NKT, natural killer cells; TCR, T-cell receptors; TNF-α, tumor necrosis factor alpha.

As a result of antigen-presentation by dendritic cells and subsequent T cell activation, monocytes are recruited to the site of inflammation. In the case of incomplete antigenic clearance, the monocytes build up and eventually mature into epithelioid cells. Following the cell-mediated immune responses, T lymphocytes are also recruited and infiltrate the tissue, and a granuloma develops (figure 2). A sarcoid granuloma is thought to be a dynamic structure recruiting newly formed monocytes, which gradually penetrate the core where they mature into epithelioid cells.[34 35]

Th1 cells

Interleukin-12 and interleukin-18 (IL-12 and IL-18 as cytokines) are activated and released by dendritic cells to promote Th1 proliferation and interferon gamma (IFN-γ) production.[36] Subsequently, IL-18 amplifies the expression of tumour necrosis factor alpha (TNF-α), IL-2 and granulocyte-macrophage colony-stimulating factor (GM-CSF) that are produced from activated dendritic cells which in turn enhance the CD4+T cells and formation of granulomatous inflammation in a synergistic manner.[37 38]

IFN-γ promotes the production of T-bet and the chemokine receptor CXCR3 that result in a positive feedback-loop which further increases IFN-γ through Th1 mechanisms. Increased levels of IFN-γ from the feedback-loop decreases the expression of Th2 cytokine transcription and the production of Th2 cytokines such as IL-4 and IL-13.[39 40]

Despite the dominant role of Th1 cells in sarcoidosis, recent studies show that Th17 cells also play a role. Th17 cells produce IL-17A which is vital for formation of mature granulomas. Additionally, Th17 cells appear to be an important Th cell subtype in bronchoalveolar lavage fluid (BALF) of patients with sarcoidosis and have elevated levels of IFN-γ.[41–44]

Th2 cells

Fibrosis in sarcoidosis is thought to be caused by the shift in the immune response from Th1 to Th2 dominance.[45] IL-13, a Th2 cytokine, stimulates transforming growth factor beta (TGF-β) activity which induce the transformation of fibroblasts into myofibroblasts. Fibrosis in sarcoidosis may also be related to the role of elevated chemokine ligand

2 (CCL2, a Th2 chemokine) as it prevents fibroblast cell death through IL-6/STAT3 signaling.[46 47] A summary of the contribution of Th1, Th17 and Th2 cells to the pathogenesis of sarcoidosis is depicted in figure 2.

Clinical diagnosis

Currently, most of the diagnostic and monitoring tests available require an invasive biopsy. Hence, development of a less invasive test for sarcoidosis is a goal of ongoing research. To date, there exist no 'gold standard' diagnostic criteria. However, a presumptive diagnosis of sarcoidosis can be made following radiological evidence of sarcoidosis such as bilateral symmetrical hilar lymphadenopathy, clinical evidence of sarcoidosis such as erythema nodosum and pulmonary involvement or histological finding of non-caseating granuloma.[48] The latest American Thoracic Society clinical practice guidelines do not make a definitive statement in relation to biopsy. Endobronchial ultrasound and biopsy has a high yield, but depends on the presence of proximal mediastinal or hilar lymphadenopathy.[49]

The presentation of sarcoidosis may be an incidental asymptomatic finding on imaging, such as intrathoracic lymphadenopathy on chest radiograph in 87% of cases or by clinical presentation with cough or breathlessness in symptomatic individuals. Pulmonary disease is present in up to 97% of cases, together with cutaneous, hepatosplenomegaly, central nervous system (CNS), ocular and cardiac sarcoidosis.[3 50]

Spontaneous remission occurs in most cases of sarcoidosis, but 25%–40% of patients will have a chronic and protracted course and it can be the cause of death in approximately 5% of cases.[51]

BIOMARKERS FOR CLINICAL EVALUATION

Biomarkers can be considered as indicators of normal or pathological processes, and the use of potential biomarkers to provide a diagnosis and to monitor disease activity remain the focus of ongoing research. Several approaches have been adopted to detect novel biomarkers in BALF, exhaled breath condensate (EBC) and serum using proteomic analysis, ELISA of specific candidate meditators and genome-based approaches.[52–54] Many of these reports require to be conducted in large-scale studies for better external validity and to be able to distinguish the different phases of the disease such as radiological staging, lung function and extrathoracic involvement.[52 55]

Furthermore, studies identifying novel biomarkers may guide approaches to expand our understanding of this puzzling disease. Despite many potential biomarkers being recognized, a lack of sensitivity and specificity have hampered clinical usage, therefore necessitating further research in this area.[56]

Serum

The traditional approach to investigate the pathogenesis of a disease is by examining the serum biomarkers. Initially, it was believed that analyzing serological biomarkers would be beneficial in sarcoidosis as sarcoidosis is a multiorgan disease and these might better represent the underlying systemic inflammation, but local inflammation can be assessed, for example, in the breath.[57 58] However, despite many such biomarkers being discovered to be associated with sarcoidosis, none has been shown to have the adequate accuracy to be used for routine diagnosis, with the possible exception of ACE. Some serum biomarker levels may be altered in single organ disease, but not in others, for example, in pulmonary sarcoidosis the systemic depletion of peripheral lymphocytes (peripheral anergy) may lead to serum biomarkers not being representative of the disease state in pulmonary sarcoidosis, whereas in, for example, solitary sarcoid uveitis, systemic markers may not be altered at all.[59]

Potential serum biomarkers in patients with sarcoidosis have included angiotensin converting enzyme (ACE), serum amyloid A, cytokines, chemokines, microRNAs (miRNAs), chitotriosidase and lysosomes as summarized in table 1.[60–77] Serum chitotriosidase was shown to have relatively accurate diagnostic ability with very high sensitivity and specificity, however, another study with different cut-off values indicated substantially lower diagnostic values (sensitivity and specificity of 88.6% and 92.8% for cut-off of 48.8 nmol/hour/mL and 82.5% and 70% for cut-off of 100 nmol/hour/mL, respectively).[62 78] Further research should be conducted to correctly delineate the cut-off value that provides optimal diagnostic accuracy, and with more sample size and clinical context, however, it is possible to have different cut-offs for various types of patients or clinical backgrounds.

To date, only ACE been adopted for clinical usage due to the low sensitivity/specificity and lack of reproducibility of other potential novel biomarkers, although chitotriosidase may be useful for disease monitoring, progression and response to treatment. A study assessing the ACE levels of 3277 patients with sarcoidosis concluded that despite the common practice, ACE should not be used in clinical practice for the diagnosis of sarcoidosis.[79]

Serum biomarkers could be valuable in distinguishing between granulomatous diseases such as TB and sarcoidosis. Serum leptin and intercellular adhesion molecule 1 levels were shown to be significantly elevated in patients with sarcoidosis rather than in TB.[80] Another serum biomarker, adiponectin, which is an anti-inflammatory protein was differentially expressed in patients with sarcoidosis compared with healthy individuals.[81] Soluble CD163 was also significantly elevated in patients with sarcoidosis and its level associated with serum ACE and soluble IL-2 receptor levels, an important marker of macrophage activity involved in sarcoidosis, hence CD163 could provide insight on important aspects of disease activity.[82] Biomarkers could be used to identify the organ involved in sarcoidosis. Serum ACE and soluble IL-2R levels were significantly lower in isolated cardiac sarcoidosis than systemic sarcoidosis, whereas B-type natriuretic peptide (BNP) was elevated in cardiac sarcoidosis, thus BNP might be a useful marker for detecting cardiac involvement in conjunction with other clinical tests.[83] Advanced imaging for cardiac sarcoidosis such as cardiac magnetic resonance and ^{18F}FDG PET have proven valuable in establishing clinical assessment of the disease, and these techniques coupled with specific organ biomarkers could provide a possible diagnostic approach when biopsy is difficult to perform in extrathoracic organ involvement.[84] Similarly, attempts have been made to identify serum neurosarcoidosis biomarkers, and serum S100B

Table 1 Serum biomarkers in patients with sarcoidosis

Serum biomarkers	Change in patients with sarcoidosis	Clinical significance
ACE (produced by epithelioid cells derived from activated macrophages)	↑	↑ serum ACE →↑ in granuloma formation, extrathoracic involvement and disease activity[69 76] Sensitivity ranges from 41% to 100% and specificity ranges from 83% to 99% for diagnosis of sarcoidosis[67]
Soluble interleukin (IL)-2 receptor (sIL-2R) (marker for T-cell activation) Soluble CD163 (sCD163)	↑ ↑	↑ sIL-2R → ↑ extrathoracic organs involvement and disease severity[66 75] The sensitivity and specificity of ACE are 62% and 76%, respectively[64] ↑ sCD163→ ↑ serum ACE and soluble ↑ sIL-2 ↑ sCD163 correlates with disease activity
Chemokines (induced by interferon (IFN)-γ)	↑ (An inverse relationship with pulmonary function)	↑ CXCL-9 and CXCL-10 → More chronic and severe form of sarcoidosis[73 74]
Chitotriosidase (CTO) (serum marker of macrophage activation)	↑	↑ CTO correlates with disease severity[62] The sensitivity and specificity of CTO are 89% and 93%, respectively with a cut-off of 48.8 nmol/hour/mL[62]
Serum amyloid A (an acute phase protein stimulated by IL-1 and IL-6)	↑	↑ SAA in patients with sarcoidosis[63] ↑↑ SAA in active sarcoidosis[77]
Serum lysozyme (bacteriolytic enzyme present in macrophages)	↑	Serum lysozyme correlates with radiographic stage[70]
MicroRNAs (miRNAs) (regulation of gene expression)	↑	↑ hsa-miRNA-128-3p, hsa-miRNA-22-5p, hsa-miRNA-30e-3p, hsa-miRNA-4306, hsa-miRNA-92a-1-5p, hsa-miRNA-150-3p, hsa-miRNA-6729-5p and hsa-miRNA-342-5p → Potential initial diagnostic biomarker[61] sensitivity of 74.8% and positive predicted value of 88.24%[61]
Interleukins (IL) (synthesized by CD4+ T cells and macrophages)	↑	↑ IL-18 and IL-12 → ↑ IFN-γ correlates with sarcoidosis activity[65 68 71 72]

which is a calcium-binding protein was found to be elevated and correlated with CNS injury in neurosarcoidosis.[85]

Neurosarcoidosis and cardiac sarcoidosis remain difficult to diagnose and remain based on a combined clinical picture with MRI/PET and other features such as CSF ACE and oligoclonal bands, or the presence of more accessible disease to biopsy.

Bronchoalveolar lavage fluid

Since pulmonary disease is common, sampling the lung could provide valuable insight about sarcoidosis activity and clinical evaluation. Changes in the inflammatory profile in BALF or breath could reflect the activity of components of sarcoidosis-related inflammation, that is, macrophages and lymphocytes.[86] Sarcoidosis is characterized by an increase in BALF lymphocytes and the CD4/CD8 ratio (1.7±1 in healthy individuals vs 9.3±5.0 in symptomatic individuals).[86] A meta-analysis demonstrated that the BALF CD4/CD8 ratio provides a sensitivity of 70% and specificity of 83% for sarcoidosis, a better accuracy than ACE, a marker commonly used in clinical practice. No clear cut-off value was found as the values ranged from 2 to 4 between studies, therefore it is crucial to find a value to provide optimal diagnostic accuracy. The value, CD4/CD8 ratio ≥3.5, was useful for patients who presented with typical clinical and radiological manifestation of the disease, but, the ratio is not selective enough to be employed on its own and must be integrated with other established diagnostic methods.[87]

Furthermore, matrix metalloproteinase 12 (MMP12), an elastase enzyme produced by macrophages is known to have elevated gene and protein expression in BALF of patients with sarcoidosis and the levels were correlated with disease severity.[88] The altered MMP12 expression in BALF resulted in animal studies being conducted, which demonstrated that MMP12 knockout mice had a significant reduction in granuloma formation and reduced expression of IFN-γ, an important mediator in sarcoidosis pathogenicity. These findings suggest a critical role for MMP12 in the chronicity of granulomatous inflammation.[89] Another BALF biomarker is the T-cell subset CD4+ Vα2.3+, which is associated with a better prognosis and potentially may be used as a surrogate prognostic marker.[90]

As in serum, a range of chemokines, cytokines, ILs and lymphocytes are altered in the BALF of patients with sarcoidosis, as summarized in table 2. For instance, patients with Löfgren's syndrome, who have a better prognosis, were established to have lower IFN-γ and TNF-α messenger RNA (mRNA) levels in their BALF, pro-inflammatory mediators involved in sarcoidosis pathology, than HLA subtypes with worse prognosis.[91]

Exhaled breath condensate

An alternative method to assess the BALF is by collecting EBC which is a non-invasive method of collecting exhaled breath containing the airway lining fluid (ALF) and soluble exhaled gases. The ALF can be analyzed by collecting BALF, however collection requires bronchoscopy and therefore is not a feasible approach for routine monitoring.[92] Moreover, bronchoscopy is an invasive technique that has complications such as pneumothorax (0%–4%), desaturation (0.7%–76.3%), bleeding (2.5%–89.9%), arrhythmia (8%–25.7%) and patient discomfort (55.4%–96.3%), making it impractical as a repeated routine test and would likely have low acceptance as a frequent test.[93]

EBC samples the ALF, and therefore can assess airway inflammation and disease activity[94 95] with potential EBC biomarkers linked to the underlying pathophysiology of respiratory conditions, including asthma, cystic fibrosis,

Table 2 BALF biomarkers in patients with sarcoidosis

BALF biomarkers	Change in sarcoidosis	Significance
MicroRNAs (miRNAs)	↑ and ↓	↑ miRNA-146a and miRNA-150 (extracellular) and miR-21 (cellular) in CXR-II compared with CXR-I (chest-X-ray-II stage more advanced than I)[105] ↑ miRNA-04, miRNA-146a, miRNA-150 and miRNA-222 and ↓ miR-202 and miR-204 in sarcoidosis disease[133] ↑ miRNA-27b, miRNA-192 and miRNA-221(involved in angiogenesis) in acute sarcoidosis[134]
Cytokines	↑	↑ IL-18 correlates with sarcoidosis activity[68 135] ↑ IL-33 correlates with diffusion lung capacity for carbon monoxide[136] ↑INF-γ and TNF-α (Th1 cytokines)[91] ↑ IL-2, IL-4, IL-10, IL-12 and IL-13 (Th2 cytokines)[137]
Lymphocytes	↑	↑ Lymphocyte T CD4+/CD8+ correlates with active disease state and ↑ Lymphocyte T IL-17+/CD4+ correlates with active disease state[43] CD4+/CD8+ cut-off value not clear
Chemokines	↑	↑ CCL2 and CCL5 (chemokine ligands recruiting monocytes) in all stages of disease[101]
Matrix metalloproteinase 12 (MMP12)	↑	↑ MMP12 →↑ granuloma formation and ↑ IFN-γ expression ↑ MMP12 correlates with disease severity

IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

chronic obstructive pulmonary disease and sarcoidosis.[72 96–99]

The concentration of total protein and some inflammatory modulators are significantly elevated in EBC from sarcoidosis compared with healthy subjects and this elevation is in agreement with studies of BALF. A subanalysis of these proteins and inflammatory markers is required to specifically identify the important cytokines and signaling proteins involved in sarcoidosis and are differentially expressed.[100–103] Original work from our laboratory has demonstrated that EBC TGF-β1, an inflammatory mediator involved in sarcoidosis, and neopterin are elevated in patients with sarcoidosis compared with healthy individuals.[102]

Another advantage of evaluating EBC biomarkers is that the technique is totally non-invasive. Such a measurement has potential application in serial monitoring of disease activity which lends itself to being able to indicate regression or relapse. Potential biomarkers in BALF and EBC such as TNF-α, miRNAs, cytokines, IFN-γ and extracellular vesicles (EV) (exosomes) have been found to differ between patients with sarcoidosis and healthy controls and hence identified as possible diagnostic and prognostic tools which need to be tested in prospective studies.[60 100 104–107] In order to further develop the EBC technique in clinical care, much work has been focused on standardisation of EBC collection for use in clinical practice.[100]

A summary of key studies which demonstrate the BALF biomarkers that are differentially expressed in patients with sarcoidosis compared with healthy controls is demonstrated in table 2 but more work is required to better characterize EBC findings in sarcoidosis and establish those that are most reliable.

Limitation of biomarkers

The use of biomarkers for sarcoidosis may be challenging for several reasons. First, the criteria for the diagnosis for the diagnosis of sarcoidosis rely on the clinical presentation, biopsy result with non-caseating granulomata and the exclusion of other diagnoses, which can affect the certainty of a diagnosis when assessing the accuracy of a newly developed diagnostic biomarker.[108] Furthermore, sarcoidosis is a systemic disease in which multiple organs may be affected to a varying extent. Therefore, unidimensional biomarkers are unlikely to encapsulate the whole spectrum of the disease. Techniques to overcome this problem are to use a combination of several biomarkers or to study those related to specific organ involvement.[109] In addition, it is likely that some biomarkers will reflect active macrophage and lymphocytic inflammation, while other may reflect the healing process, and yet others represent a pathway to ongoing fibrosis.[43]

Despite some research, no novel biomarkers have been adopted into clinical practice due to their relatively low accuracy, sensitivity and specificity.[60] Therefore, biomarkers remain an area that requires more study.

DISCOVERY AND VALIDATION OF NOVEL BIOMARKERS

While many advances have been made in understanding the pathogenesis and the identification of biomarkers that are plausible indicators of sarcoidosis, novel aspects of the basic mechanisms are becoming apparent as being involved in disease progression and resolution and may offer opportunities for novel biomarkers. Some of these novel biomarkers that have shown initial success and require more research include miRNAs and exosomes.

MicroRNAs

miRNAs are a class of regulatory molecules suggested to be as prospective biomarkers with the possible involvement in sarcoidosis pathophysiology.[104 110] miRNAs are non-coding, single-stranded RNAs composed of 18–25 nucleotides, and the importance of miRNA altered regulation in lung disease is becoming increasingly evident.[111] miRNAs likely play a role in both inflammation and granuloma formation, hence a significant component of sarcoidosis pathophysiology.[112] miRNAs are able to change the function of various inflammatory and apoptosis signaling pathways by inhibiting the post-transcriptional gene expression via mRNA or altering protein translation.[111 113] The inflammatory regulation related to sarcoidosis includes modulation of T-cell differentiation, IFN-γ expression, Th1 cells and IL-2R.[114]

Multiple studies demonstrate the relationship between dysregulation of several miRNAs and sarcoidosis.[115 116] As highlighted in BALF and serum, many miRNAs have altered expression in sarcoidosis, and some are associated with disease severity. It is unclear whether these miRNAs would be elevated in sarcoidosis EBC samples, complicated by the fact that extracellular miRNAs are mostly encapsulated inside EV.[117]

Exosomes

Exosomes are small, 30–120 nm in diameter, cell-derived EV and are secreted from most cell types.[117] Exosomes mainly serve as a means of protection to transfer cell surface molecules, proteins, miRNAs and DNAs to specific location for intercellular communication and to protect them from enzymatic breakdown.[118]

Exosomes are biologically active EV that are tightly regulated thorough various mediators and pathways. miRNAs are mostly encapsulated within exosomes and therefore exosome isolation improves the sensitivity of miRNA expression and identification,[119] although not all studies have confirmed this view with miRNAs being also present outside exosomes and bound to proteins such as Argonaute 2.[120 121]

The number and the contents of exosomes in body fluids increase in cancer and sarcoidosis, however exosomal function in sarcoidosis pathophysiology remains poorly understood.[107 122] Sarcoidosis BALF exosomal miRNA and cytokines were elevated, for example, miRNA-146a.[107 123] BALF exosomes from patients with sarcoidosis induced higher levels of IFN-γ and IL-13 production by epithelial cells therefore illustrating an association with the underlying immunopathogenesis of sarcoidosis.[107] We have shown that EBC exosomes are able to be isolated and exosome-induced production of TNF-α was greater in monocytes from patients than from controls and miRNAs and mRNA involved in sarcoidosis pathology was expressed higher in serum exosomes of patients.[124] A number of studies have investigated miRNA expression in pulmonary sarcoidosis.[114] Several investigated miRNA expression in peripheral blood, blood cells bronchoalveolar cells, exosomal/EV miRNA in BALF but none studied exosomal miRNA expression in EBC.[61 115 125–131] Thus, there is a need for more studies to be conducted with larger numbers of subjects, different sample sources such as EBC and to target known mediators of sarcoidosis.

Moreover, the exosomal miRNAs changes were not limited to pulmonary sarcoidosis as a study indicated several exososomal miRNAs levels were higher in patients with cardiac sarcoidosis, hence further illustrating the potential of miRNAs as biomarkers and the possibility of using specific biomarkers for detecting vital organ involvement.[132]

CONCLUSION AND FUTURE RESEARCH

Evaluating exosomal and non-exosomal miRNAs and cytokine expression in EBC samples has remained an unexplored area in the literature and thus requires further investigation. Furthermore, our preliminary data indicate that exosomes and miRNAs regulating key sarcoidosis cytokines that are differentially expressed in EBC, hence more research is required to identify and validate sarcoidosis biomarkers that have the advantage of being non-invasive, sensitive and amenable to repeated sampling.[124] Many recent studies have demonstrated a variety of mediators involved in the immunopathogenesis of sarcoidosis and the levels were shown to be differentially expressed in sarcoidosis, indicating that they may be potential biomarkers. However, uncertainty remains as to which aspect of clinical assessment, diagnosis, prognosis or disease activity, these biomarkers could be applied. The pattern in which biomarkers were expressed in organ-specific sarcoidosis is not always consistent with other forms of the diseases, hence more focused studies are mandated to better characterize the nature of biomarkers in different disease forms. Likewise, long-term studies with a baseline profile of the potential markers would be helpful in indicating prognosis, but the analysis will be complex with many variables including gender, ethnicity, MHC/genomic and other variables requiring assessment, and the results will need to be validated carefully.

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