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Diagnosis and treatment of sarcoidosis

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Diagnosis and treatment of sarcoidosis

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Editorial: Diagnosis and treatment of sarcoidosis

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Editorial on the Research Topic
Diagnosis and treatment of sarcoidosis

Sarcoidosis is a multisystem disease characterized by non-caseating epithelioid cell granulomas. The diagnosis of sarcoidosis is based on compatible clinical presentation, non-necrotizing granulomatous inflammation in one or more tissue samples, and the exclusion of alternative causes of granulomatous disease (1). With the development of new technology, biomarkers, CT pattern scores, and PET-CT have been applied for improved diagnosis and assessment of organ involvement. Some patients with pulmonary sarcoidosis do not require systemic treatment, given that spontaneous remission occurs at a certain rate. In the treatment of sarcoidosis, an effective individualized plan must be formulated according to the clinical manifestations, the involved organs, the severity of their involvement, and underlying conditions. The most common indication for treatment of pulmonary disease is the development of respiratory symptoms, followed by extra-pulmonary involvement of the cardiovascular, cutaneous, and nervous systems. At present, drugs for sarcoidosis include corticosteroids as first-line therapy, cytotoxic drugs as second-line treatment, and anti-tumor necrosis factor (TNF) biologics as third-line agents (2). Novel therapeutic agents such as repository corticotropin injection and rituximab have been studied for sarcoidosis and some positive results have been obtained. However, additional research efforts need to be undertaken to develop improved tools for the diagnosis and treatment of sarcoidosis. In this Research Topic, we aim to provide an overview of recent progress in the diagnosis and treatment of sarcoidosis and to present innovative solutions to existing challenges.

This Research Topic consists of one case report, three reviews, and six original research articles focusing on advances in diagnosis and treatment, together with findings in genetics and transcriptomics that might be helpful in the early diagnosis of sarcoidosis.

Multiple factors, such as environmental and genetic predisposition, have been implicated in the pathogenesis of sarcoidosis. The search for gene candidates in sarcoidosis can be conducted on the basis of gene expression data and protein profile data from genomic, transcriptomic, or proteomic studies. Xiong et al. identify sex-dependent genetic variations in two clinical sarcoidosis phenotypes, namely Löfgren's syndrome (LS) and non-Löfgren's syndrome (non-LS), in multiethnic cohorts from Sweden, Germany, and the US. The authors confirm that differences in genetic findings between the sex groups in LS are explicitly located in the extended Major Histocompatibility Complex, while genetic differences between the sex groups in non-LS are primarily located in the MHC class II subregion and *ANXA11*. Transcriptome-wide expression studies have been conducted to reveal the

mechanisms of sarcoidosis. Jiang et al. performed a systematic database search of the Gene Expression Omnibus and utilized transcriptomic data from blood and sarcoidosis-affected tissues in a meta-analysis to identify a cross-tissue, cross-platform signature. They identify 29 robustly sarcoidosis-associated genes, including the top genes *LINC01278*, *GBP5*, and *PSMB9*. They report that pathway enrichment analysis revealed activation of IFN-γ, IL-1 and IL-18, autophagy, and viral infection response. This study provides a cross-tissue meta-analysis for expression profiles and identifies a potential non-invasive diagnostic classifier for sarcoidosis. Similar efforts have been made by Duo et al., who report on a study in which transcriptomes from eleven independent sarcoidosis cohorts comprising 313 patients and 400 healthy controls were analyzed and machine learning was employed to fit a diagnostic model. A ten-gene sarcoidosis diagnosis signature consisting of *GBP1, LEF1, IFIT3, LRRN3, IFI44, LHFPL2, RTP4, CD27, EPHX2,* and *CXCL10* was constructed in the training cohorts; this signature performed well in the four independent cohorts and has been further validated in seven independent publicly available gene expression datasets. Transcriptional signatures, developed through bioinformatics analysis, could improve the accuracy of early diagnosis of sarcoidosis.

Cardiac sarcoidosis (CS) remains diagnostically challenging as the sensitivity and specificity of the diagnostic modalities are limited. Strambu reviews current knowledge of the diagnosis and decision to treat cardiac sarcoidosis, and illustrates the information with a case presentation (Strambu). The most challenging issue is the suspicion of CS with initial cardiac manifestations in a patient with no previous diagnosis of sarcoidosis, and diagnosis can be delayed. Another difficulty in diagnosis is the absence of a gold standard for diagnosis, because biopsy of the endomyocardial tissue has low sensitivity given the risk level of the procedure. The increasing use of newer imaging modalities such as cardiac magnetic resonance (CMR) and positron emission tomography (PET) may provide valuable information for accurate diagnosis and assessment in CS patients. Brazile et al. review a set of medical records for diagnostic features of CS, including late gadolinium enhancement (LGE) patterns, increased signal on T2-weighted imaging, and non-caseating granulomas. They confirm that CMR is an important tool in the non-invasive diagnosis of CS, and the presence of LGE on CMR in a pattern consistent with CS has been shown to be a predictor of mortality.

Three manuscripts published in this Research Topic relate to rare clinical features associated with or mimicking sarcoidosis. Prevel et al. describe the clinical and biological presentation, treatments, and outcomes of *cryptococcus* spp. infection of central nervous system (CINS) in patients with and without sarcoidosis. They found that 31% (5/16) of CINS patients had associated sarcoidosis. CINS symptoms, biological and CSF features, and treatments were similar for CINS patients with and without sarcoidosis, except regarding CD4 cell percentage and CD4/CD8 ratio, which were higher in blood from those with sarcoidosis. Ji et al. present a case report of pleural sarcoidosis with pleural nodules and effusion, along with a review of the literature on this rare manifestation. Another study describes the natural course and prognostic value of cancer-associated sarcoid-like reaction (SLR) (Huh et al.). During follow-up, progression of cancer-related SLR to overt sarcoidosis was not observed. Development of SLR was also not associated with overall survival or disease-free survival in patients with non-small cell lung cancer.

Two reviews of differential diagnosis and treatment approaches are presented in our Research Topic. In the review by Valeyre et al. the authors establish optimal differential diagnosis strategies tailored to each of several situations in order to help non-sarcoidosis experts. They emphasize that alternative diagnoses must be ruled out before a diagnosis of sarcoidosis can be made. First, epidemiological factors must be clarified. Subsequently, a detailed medical history and physical examination are also crucial. Chest CT is also helpful to characterize the findings as typical or atypical for sarcoidosis. Ultimately, the Sarcoidosis Diagnostic Score (Clinical and Biopsy) can be very helpful to assess sarcoidosis diagnosis before and after evidence of granuloma. Recent therapeutic drug trials and treatment approaches are reviewed by Obi et al.. Up to now, only two medications (prednisone and repository corticotropin injection) have been approved in the treatment of sarcoidosis by the United States Food and Drug Administration. This past decade has seen a renewed interest in developing new drugs and exploring novel therapeutic pathways for the treatment of sarcoidosis. The next challenge will lie in funding and in evaluating the effectiveness and safety of treatments.

For accurate diagnosis and effective treatment, further efforts should be focused on early detection, new diagnostic technologies, and multicenter clinical trials of new drugs. Moreover, the identification of new biomarkers is warranted for differential diagnosis and for individualized treatment. This will provide a powerful decision-making tool for doctors that can be used to better manage sarcoidosis patients.

Author contributions

The draft was written by YZ and edited by FJ and RB. All authors reviewed the final document.

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Central Nervous System Cryptococcosis in Patients With Sarcoidosis: Comparison With Non-sarcoidosis Patients and Review of Potential Pathophysiological Mechanisms

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Introduction: *Cryptococcus* spp. infection of the central nervous system (CINS) is a devastating opportunistic infection that was historically described in patients with acquired immunodeficiency syndrome (AIDS). *Cryptococcus* spp. infections are also associated with sarcoidosis; the impairment of cell-mediated immunity and long-term corticosteroid therapy being evoked to explain this association. Nevertheless, this assertion is debated and the underlying pathophysiological mechanisms are still unknown. The aims of this study were (i) to describe the clinical and biological presentation, treatments, and outcomes of CINS patients with and without sarcoidosis and (ii) to review the pathophysiological evidence underlying this clinical association.

Patients and Methods: Every patient with positive cerebrospinal fluid (CSF) cryptococcal antigen testing, India ink preparation, and/or culture from January 2015 to December 2020 at a tertiary university hospital were included, and patients with sarcoidosis were compared with non-sarcoidosis patients. Quantitative variables are presented as mean ± SD and are compared using the Mann-Whitney Wilcoxon rank-sum test. Categorical variables are expressed as the number of patients (percentage) and compared using the χ^2 or Fisher's tests.

Results: During the study period, 16 patients experienced CINS, of whom 5 (31%) were associated with sarcoidosis. CINS symptoms, biological, and CSF features were similar between CINS patients with and without sarcoidosis except regarding CD4 cells percentages and CD4/CD8 ratio that was higher in those with sarcoidosis (47 ± 12 vs. 22 ± 18, $p = 0.02$ and 2.24 ± 1.42 vs. 0.83 ± 1.10, $p = 0.03$, respectively). CINS patients with sarcoidosis had less often positive blood antigen testing than those without sarcoidosis (2/5 vs. 11/11, $p = 0.02$). CINS patients with and without sarcoidosis

were treated with similar drugs, but patients with sarcoidosis had a shorter length of treatment. CD4 cell levels do not seem to explain the association between sarcoidosis and cryptococcosis.

Conclusion: Sarcoidosis was the most frequently associated condition with CINS in this study. CINS patients associated with sarcoidosis had overall similar clinical and biological presentation than CINS patients associated with other conditions but exhibited a lower rate of positive blood cryptococcal antigen testing and higher CD4/CD8 T cells ratio. Pathophysiological mechanisms underlying this association remain poorly understood but B-1 cell deficiency or lack of IgM could be a part of the explanation. Another plausible mechanism is the presence of anti-granulocyte-macrophage colony-stimulating factor (GM-CSF) antibodies in a subset of patients with sarcoidosis, which could impair macrophage phagocytic function. Further studies are strongly needed to better understand those mechanisms and to identify at-risk patients.

Keywords: cryptococcal meningitis, sarcoidosis, innate immunity, humoral immune response, anti-GM-CSF autoantibodies

INTRODUCTION

Sarcoidosis is a systemic disease characterized by the formation of non-caseating epithelioid granulomas in several organs, mainly the lungs and the lymphatic system (1). It affects between 4.7 and 64 per 100,000 people and its prevalence varies from 1.0 to 64 per 100,000 per year (2). It can occur at any age of life, but with a particular proclivity for young adults. Spontaneous resolution occurs in about 60% of patients. Nevertheless, chronic and progressive forms are not rare and about 20% of patients have permanent clinical symptoms due to fibrotic lesions in the involved organs (2). Mortality is thought to be up to 5–10% (1), mainly associated with severe pulmonary fibrosis or, less usually, with cardiac or central nervous system involvement. Even if its exact origin remains unknown, the persistence of an unidentified antigen in individuals with genetic predisposition is supposed to trigger a pro-inflammatory Th1 response, leading to the formation of these granulomas. Sarcoidosis is characterized by a paradoxical immune status, i.e., an exaggerated immune response within granulomas, in contrast to various immune defects as stated by anergy to tuberculin test and the occurrence of opportunistic infections (3, 4), with corticosteroid therapy being a constant risk factor (4).

The first suspected opportunistic infections were mycobacterial ones but, except in patients receiving anti-TNFα treatments, sarcoidosis does not seem to be at particular risk for tuberculosis nor for any other mycobacterial infections. In fact, tuberculosis is less frequent, more typical, and not associated with immune reconstitution inflammatory syndrome in sarcoidosis patients compared with patients with acquired immunodeficiency syndrome (AIDS) (5). *Mycobacterium avium* complex infections were organ-specific in sarcoidosis patients and not disseminated as in patients with AIDS with no correlation with CD4 count (6). On the contrary, opportunistic infections such as progressive multifocal leukoencephalopathy (PML) and aspergillosis have been described in patients with sarcoidosis (4). Sarcoidosis is an underlying disease in 8–9% of PML cases with most patients who had not received any immunosuppressive drug (7, 8). Moreover, sarcoidosis represents a risk factor in 7–17% of chronic pulmonary aspergillosis (9, 10), and chronic pulmonary aspergillosis complicates around 2% of sarcoidosis (11, 12) with long-term corticosteroid therapy being a major risk factor for invasive aspergillosis, increasing in parallel with treatment dose and duration. Nocardiosis, histoplasmosis, and pneumocystosis are also reported in patients with sarcoidosis, but less frequently when compared with patients with rheumatological disorders who have more profound immunosuppression, especially after corticosteroid exposure (4).

Cryptococcus spp. infection of the central nervous system (CINS) is a devastating opportunistic infection that was first described in patients affected by AIDS historically representing about 70% of cryptococcal infections (13, 14). *Cryptococcus* spp. is responsible for one million infections per year among human immunodeficiency virus (HIV)-infected patients in the world; of these, approximately 625,000 die (15). *Cryptococcus neoformans* and *Cryptococcus gattii* are the most common and major pathogenic species complex in the genus *Cryptococcus* (16). *C. gattii* shares major virulence determinants with *C. neoformans* and was previously thought to be a subtype of *C. neoformans*, but genomic and transcriptomic studies revealed distinctions leading to recognize *C. gattii* as a unique species (17). Both species usually cause pulmonary or central nervous system (CNS) infections, but they differ in epidemiology, clinical features, and pathophysiology (18). *C. gattii* has traditionally been considered as a "tropical or subtropical fungus" despite the fact that, even before the North American outbreak, a large proportion of disease in Australia occurred in its southern temperate region. It has now been isolated from human and animal samples worldwide (17). Shifts in the appreciation of the clinical epidemiology of *C. gattii* in the past two decades include the recognition that it affects hosts known to be

immunocompromised (including those with HIV/AIDS) as well as hosts presumed to be immunocompetent (17). The patients affected by other immunocompromising conditions such as sarcoidosis or others (lymphoproliferative disorders, malignancy diseases, organ transplant, and/or immunosuppressive therapy) are so predisposed to CINS, possibly caused by both *C. neoformans* and *C. gattii* (19–21) and cryptococcal infections affect 0.8 per 100,000 HIV-negative inhabitants (22). Their proportion is increasing with the advances in the care of HIV-infected patients (23, 24) and the increasing number of patients receiving immunosuppressive drugs. T cell-mediated immunity is the major pathway of defense against *Cryptococcus* spp. with a key role for Th1-Th2 imbalance resulting in impaired TNFα, IL-12, and IFN-γ production (25), and patients with AIDS are known to have impaired T cell-mediated immunity. The use of corticosteroids is a well-recognized risk factor for cryptococcosis (20, 22, 26), as for other opportunistic infections, with an estimated risk of fungal infections 1.5 times greater (95% CI: 1.3–1.9) in patients taking corticosteroids compared with naïve controls (11). The risk is especially increased for dosages exceeding 20 mg/day (27). In fact, corticosteroids cause dysregulation of Th1/Th2 T helper cells to balance favoring Th2 cytokines response and decrease cooperation with B cells (28). They are also responsible for a reduction in monocyte-macrophage functions by reducing chemotaxis, phagocytosis, and production of IL-1, IL-6, and TNF-α (29). The impairment of cell-mediated immunity (low CD4 cell count and lower CD4/CD8 ratio) has also been suggested as a risk factor for opportunistic infections in patients with sarcoidosis (11, 30, 31). Inconsistent with this hypothesis, numerous patients with sarcoidosis suffering from CINS reported in the literature were not receiving long-term corticosteroids therapy (11, 32, 33). A case-control study comparing sarcoidosis patients with and without CINS further confirmed corticosteroids therapy as a risk factor for CINS (34) but no association between the risk of opportunistic infection and severe CD4 lymphocytopenia was found.

To the best of our knowledge, no case-control study compared CINS patients with and without sarcoidosis to further address the underlying mechanisms. The aims of this study were to describe the clinical and biological presentation, treatments, and outcomes of CINS patients with and without sarcoidosis and to review the pathophysiological evidence underlying this clinical association.

PATIENTS AND METHODS

Study Design and Inclusion Criteria

This study was conducted at Bordeaux University Hospital including every patient with positive cerebrospinal fluid (CSF) cryptococcal antigen testing, India ink preparation, and/or culture from January 2015 to December 2020.

Cerebrospinal fluid cryptococcal antigen testing was performed using CryptoPS test (BIOSYNEX®) and blood cryptococcal antigen testing using CALAS® (Meridian Bioscience). CSF samples were processed with India ink preparation and incubated on Sabouraud Agar + Chloramphenicol + Gentamicin media (Bio-Rad). Blood samples were incubated on BACT/ALERT® FA (bioMérieux) culture bottles. Identification of growing isolates was performed using MALDI-TOF mass spectrometry (Microflex®, Bruker Daltonics).

Diagnosis of sarcoidosis was retrospectively confirmed according to the current recommendations (35): (i) clinical and paraclinical features consistent with sarcoidosis, (ii) an histopathological analysis revealing non-caseating granuloma except for patients presenting Löfgren's syndrome, and (iii) exclusion of other possible etiologies, including other granulomatous disorders. Data were retrospectively collected from the electronic medical records, and the electronic worksheet was completed by two medical intensive care residents.

Statistical Analyses

No statistical sample size calculation was performed *a priori*, and the sample size was equal to the number of patients with positive CSF cryptococcal antigen testing and/or culture during the study period. Quantitative variables are presented as mean ± SD and compared using the Mann-Whitney Wilcoxon rank-sum test. Categorical variables are expressed as the number of patients (percentage) and compared using the χ^2 or Fisher's tests. All statistical tests were 2-tailed, and statistical significance was defined as $p < 0.05$. Statistical analyses were assessed using the R version 3.6.0 statistical software (R Foundation for Statistical Computing Vienna, Austria).

Ethics

According to the French law and the French Data Protection Authority, the handling of these data for research purposes was declared to the Data Protection Officer of the Bordeaux University Hospital. Patients (or their relatives, if any) were notified about the anonymized use of their healthcare data *via* the department's booklet.

RESULTS

High Proportion of Patients With Sarcoidosis in Patients With CINS

During the study period, 16 patients presented CINS, of whom five were associated with sarcoidosis, four with an onco-hematological disease including two patients treated with ibrutinib, three patients with AIDS, two with kidney transplantation, one with autoimmune hepatitis, and one with epilepsy associated to Arnold-Chiari malformation (**Table 1**).

The median duration of sarcoidosis at cryptococcosis diagnosis was 36 ± 31 months. Patients with sarcoidosis were mostly men (4/5) with a median age of 49 ± 11.7 years, of whom three had lung involvement, two with mediastinal adenopathy, one with central and peripheral nervous system involvement, and one with muscular involvement. One of them only had liver involvement. Of these five patients, four were receiving corticosteroids therapy at the time of cryptococcosis

TABLE 1 | Characteristics of patients diagnosed with *Cryptococcus* sp. infection of the central nervous system.

	Sarcoidosis ($n = 5$)	Non-sarcoidosis ($n = 11$)	*p*-value
Age	49 ± 11.7	55 ± 18.6	0.53
Male	4 (80%)	8 (78%)	1.00
Etiology of immunosuppressive state in non-sarcoidosis patients			
Onco-hematology	-	4 (39%)	-
HIV	-	3 (30%)	-
Kidney transplant	-	2 (20%)	-
Auto-immune hepatitis	-	1 (10%)	-
Epilepsy with Arnold-Chiari malformation	-	1 (10%)	-
Characteristics of sarcoidosis			
Known sarcoidosis	5	-	-
Duration of sarcoidosis evolution (months)	36 ± 31	-	-
Lung involvement	3 (60%)	-	-
Mediastinal adenopathy	2 (40%)	-	-
Central nervous system	1 (20%)	-	-
Peripheral nervous system	1 (20%)	-	-
Muscular involvement	1 (20%)	-	-
Liver involvement	1 (20%)	-	-
Joint, eye, skin, kidney, parotid	0 (0%)	-	-
Calcemia (mmol/L) Normal values: 2.2–2.6 mmol/L	2.32 ± 0.14	-	-
Angiotensin-converting enzyme Normal values: 20–80 IU/L	39 ± 27	-	-
Immunosuppressive drugs			
Past therapy with corticosteroids	4 (80%)	6 (59%)	0.59
Past other immunosuppressive drug	3 (60%)	5 (49%)	1.00
Cyclophosphamide	-	3 (30%)	-
CHOEP	-	1 (10%)	-
RCD then R-bendamustine	-	1 (10%)	-
Azathioprine	1 (20%)	1 (10%)	-
Mycophenolate-mofetil	1 (20%)	0 (0%)	-
Methotrexate	1 (20%)	0 (0%)	-
Current therapy with corticosteroids	4 (80%)	5 (49%)	0.31
Current other immunosuppressive drug	1 (20%)	5 (49%)	0.59
Ibrutinib	-	2 (20%)	-
Tacrolimus	-	2 (20%)	-
Mycophenolate-mofetil	0 (0%)	1 (10%)	-
Azathioprine	1 (20%)	2 (20%)	-

CHOEP, cyclophosphamide, doxorubicin, vincristine, etoposide and prednisone; HIV, human immunodeficiency virus; IU, international unit; R, rituximab; R-CD, rituximab–cyclophosphamide-dexamethasone.

diagnosis and three of them had previously received another immunosuppressive drug (one with azathioprine, one with mycophenolate-mofetil, and one with methotrexate) (**Table 1**). In patients receiving immunosuppressive drugs, only azathioprine was still ongoing at the time of cryptococcosis onset. No other previous opportunistic infection was reported among those patients with sarcoidosis.

In non-sarcoidosis patients, 6/11 previously received corticosteroid therapy, still ongoing for five of them, and 5/11 previously received other immunosuppressive drugs: 3 cyclophosphamide, 1 CHOEP, 1 R-CD then R-bendamustine, and 1 azathioprine. At the time of diagnosis, five of them were receiving immunosuppressive drugs: 2 ibrutinib, 2 tacrolimus, 2 azathioprine, and 1 mycophenolate-mofetil.

CINS Patients With Sarcoidosis Have a Similar Clinical and Biological Presentation at Diagnosis Than Those Without Sarcoidosis but Lower Positive Blood Cryptococcal Antigen Testing

All of the five patients with sarcoidosis reported fever and headaches as symptoms of CINS. Two of them exhibited neck stiffness, two seizures, one behavior abnormalities, and one focal neurological deficit, being similar for all those features to non-sarcoidosis patients (**Table 2**). CSF cell count, protein, lactate, and glucose levels as a proportion of positive CSF antigen testing or culture were comparable between CINS patients with and without sarcoidosis. Each included patient had blood antigen testing that was less often positive (2/5 vs. 11/11, $p = 0.02$) in patients with sarcoidosis than in those without sarcoidosis (**Table 2**). Each cultivated strain but one was from *C. neoformans* species. Microbiological results on a patient basis are provided (**Table 3**).

CINS Patients With Sarcoidosis Exhibit a Higher CD4/CD8 T Cells Ratio Than Those Without Sarcoidosis

The blood B lymphocytes count and proportion and the blood gamma globulin levels were similar between CINS patients with and without sarcoidosis (**Table 2**).

Regarding cellular immunity, CD4 cells proportion among total lymphocyte blood count and CD4/CD8 ratio were higher in CINS patients with sarcoidosis than in those without sarcoidosis (47 ± 12 vs. 22 ± 18, $p = 0.02$ and 2.24 ± 1.42 vs. 0.83 ± 1.10, $p = 0.03$, respectively) (**Table 2**). The differences in CD4 and CD8 T cells proportion remained statistically significant after excluding patients with AIDS from non-sarcoidosis patients (**Table 2**).

CINS Patients With Sarcoidosis Have a Shorter Duration of Antifungal Treatment but Similar Outcomes Than Those Without Sarcoidosis

As stated in current therapeutic guidelines, patients with CINS mostly received a 2-week association of *intravenous* liposomal B amphotericin (3 mg/kg per day) and flucytosine (25 mg/kg every 6 h) as initial therapy (4/5 in patients with sarcoidosis vs. 8/11 in patients without sarcoidosis, $p = 1.00$) and then fluconazole *per os* (400 mg per day for 8 weeks then 200 mg per day) as maintenance therapy (4/5 in patients with sarcoidosis vs. 10/11 in patients without, $p = 1.00$) (**Table 4**). Nevertheless, patients with sarcoidosis were treated for a shorter duration (34 ± 29 days vs. 66 ± 13, $p = 0.04$) than those without sarcoidosis with a similar death rate and long-term sequelae ($p = 1.00$ for both) (**Table 4**).

DISCUSSION

In this study, sarcoidosis is the most prevalent disease associated with CINS: 5 CINS over a period of 6 years with sarcoidosis accounting for 31% of all CINS and 38% (5/13) of the HIV-negative patients. CINS patients with sarcoidosis have a similar clinical and biological presentation at diagnosis but exhibit a higher CD4/CD8 T cells ratio than those without sarcoidosis. Importantly, CINS patients with sarcoidosis exhibited a lower rate of positive blood antigen testing compared with those without sarcoidosis. In front of CINS suspicion in a patient with sarcoidosis, blood cryptococcal antigen testing should be reiterated if negative when a reasonable clinical probability is assessed. CSF antigen testing should also be performed in case of clinical suspicion even if blood antigen testing is negative. CINS patients with sarcoidosis had a shorter duration of treatment than those without sarcoidosis. Every patient was treated based on specialized team stewardship. Shorter duration could be explained by the absence of severe quantitative CD4 count defect, the rarer use of immunosuppressive drugs, and the lower proportion of blood positive antigen testing in patients with sarcoidosis than in other patients. Recommendations regarding the duration of treatment for CINS have been established in AIDS and solid-organ transplant patients, and to the best of our knowledge, no consensus exists in patients with sarcoidosis.

Contrary to our findings, only very few cases were identified in the main case-control study (cryptOsarc study) comparing sarcoidosis patients with and without cryptococcosis (34). This study included 18 sarcoidosis patients with cryptococcosis, of whom 13 had CINS, over a period of 25 years. These patients were compared with 36 sarcoidosis patients without cryptococcosis. Sarcoidosis accounted for 0.6% of all cryptococcosis patients and 2.9% of the HIV-negative cryptococcosis patients. This discrepancy could be explained by the major therapeutic advances in the care of HIV-infected patients and by increasing the awareness of practitioners regarding the risk of cryptococcosis in other immunosuppressive conditions leading to more frequent testing. Among these 18 sarcoidosis patients with cryptococcosis included in the cryptOsarc study, 4 (23%) had sarcoidosis diagnosed at the time of infection and the 14 others had a median duration of 1,005 days (0–5,876) from the onset of the sarcoidosis. Notably, 12 out of the 18 patients (67%) had previously been treated with corticosteroids with a median therapy duration of 137 days (0–5,695), and a median dose of 18 mg/day (0–55) and two of them were receiving immunosuppressive drugs (cyclophosphamide for one and methotrexate plus infliximab for the other). The median level of CD4 lymphocytes at the time of cryptococcosis diagnosis was 145/mm^3 (55–1,300). Compared with those free from cryptococcosis, patients with sarcoidosis and cryptococcosis were mostly men (72 vs. 47%, $p = 0.145$), they were younger (median age 28 vs. 42, $p = 0.0004$) and extra-pulmonary involvement was more frequent (83 vs. 56%, $p = 0.069$), including cardiac involvement, neurosarcoidosis or naso-sinusal, and/or parotid involvement. Factors associated with cryptococcosis in those patients with sarcoidosis were extra-thoracic sarcoidosis ($p = 0.055$) and possibly the intake of corticosteroid therapy or not ($p = 0.123$). Nevertheless, in this cryptOsarc study, only two-thirds of these patients received corticosteroids therapy and none of them experienced other opportunistic infections suggesting a specific susceptibility. In fact, patients with sarcoidosis are known to exhibit a decreased T cell response to cryptococcal antigen *in vitro* (36). As the

TABLE 2 | Patients' presentation and immunological assessment at diagnosis of *Cryptococcus* spp. infection of central nervous system.

	Sarcoidosis ($n = 5$)	Non-sarcoidosis ($n = 11$)	p-value
	Patients' presentation		
Fever	5 (100%)	9	1.00
Headaches	5 (100%)	9	1.00
Neck stiffness	2 (40%)	3	1.00
Seizures	2 (40%)	1	0.21
Dizziness	1 (20%)	2	1.00
Behavior abnormalities	1 (20%)	2	1.00
Focal neurologic deficit	1 (20%)	1	1.00
Confusion	0 (0%)	4	0.24
Asthenia	4 (80%)	8	1.00
Weight loss	2 (40%)	7	0.33
Neutrophils count (/mm^3)	8,070 ± 4,585	6,762 ± 4,584	0.65
Platelets count (/mm^3)	297,600 ± 154,063	282,909 ± 194,531	0.65
Lymphocytes count (/mm^3)	858 ± 567	797 ± 667	0.82
Positive blood culture	0	0	1.00
Positive blood antigen	**2**	**11**	**0.02**
CSF cells count (/mm^3)	143 ± 112	544 ± 1,087	0.57
%age of lymphocytes in CSF	42.8 ± 26.1	63.2 ± 39.6	0.30
CSF protein (/mm^3)	1.82 ± 1.61	1.06 ± 0.71	0.31
CSF lactates (mmol/L)	5.7 ± 3.8	7.2 ± 12	0.42
CSF glucose level (mmol/L)	2.37 ± 0.46	3.13 ± 1.59	0.21
Positive Ink coloration	2	5	1.00
Positive CSF culture	2	8	0.30
Positive CSF antigen	5	11	1.00
Meningitis on MRI	2/4	0/7	0.11
Encephalitis on MRI	2/4	2/7	0.58
Time from onset of symptoms to diagnosis (days)	65 ± 68	34 ± 30	0.25
Pitfalls in diagnosis	3	4	0.60
	1 neuro-sarcoidosis 1 viral meningitis 1 pneumonitis	2 sinusitis 1 bronchitis 1 pneumonitis	-
Concomitant pulmonary involvement	1	4	1.00
	Immunological assessment		
CD4 lymphocytes count (/mm^3)	424 ± 242	242 ± 340 w/o AIDS patients: 355 ± 374	0.11 0.43
CD4 lymphocytes proportion (%)	**47 ± 12**	**22 ± 18 w/o AIDS patients: 29 ± 8.8**	**0.02 0.03**
CD8 lymphocytes count (/mm^3)	202 ± 118	356 ± 191 w/o AIDS patients: 314 ± 198	0.15 0.33
CD8 lymphocytes proportion (%)	**20 ± 4.5**	**47 ± 21 w/o AIDS patients: 34 ± 12**	**0.02 0.01**
CD4/CD8 lymphocytes ratio	**2.24 ± 1.42**	**0.83 ± 1.10** w/o AIDS patients: 0.96 ± 0.69	**0.03** 0.08
B lymphocytes count (/mm^3)	92 ± 99	157 ± 290	0.79
B lymphocytes proportion (%)	13.8 ± 9.91	6.99 ± 7.72	0.25
Gamma globulins level (g/L)	6.57 ± 1.53	11.46 ± 8.11	0.48
IgG level (g/L)	8.45 ± 4.88	8.49 ± 5.09	0.89
IgA level (g/L)	0.99 ± 0.02	2.12 ± 1.80	0.67
IgM level (g/L)	1.18 ± 0.25	0.85 ± 0.59	0.50

CSF, cerebrospinal fluid; MRI, magnetic resonance imaging. Bold values mean statistically significant.

TABLE 3 | Microbiologic results on a patient basis.

	Sex	Age (years old)	Associated condition	Blood culture	Blood antigen testing	Titer (dilution)	India ink	CSF culture	Isolated specie	CSF antigen testing	Titer (dilution)
Patient 1	M	48	Sarcoidosis	Negative	Negative	-	Positive	Negative	-	Positive	1/128
Patient 2	M	40	Sarcoidosis	Negative	Negative	-	Negative	Positive	*C. neoformans*	Positive	NA
Patient 3	F	35	Sarcoidosis	Negative	Negative	-	Negative	Negative	-	Positive	NA
Patient 4	M	59	Sarcoidosis	Negative	Positive	1/512	Negative	Negative	-	Positive	1/16
Patient 5	M	62	Sarcoidosis	Negative	Positive	1/256	Positive	Positive	*C. neoformans*	Positive	1/512
Patient 6	M	85	Hematological malignancy	Negative	Positive	1/128	Negative	Positive	*C. neoformans*	Positive	1/1,024
Patient 7	M	65	Hematological malignancy	Negative	Positive	1/1,024	Positive	Positive	*C. neoformans*	Positive	1/1,024
Patient 8	F	55	Hematological malignancy	Negative	Positive	1/12	Negative	Negative	-	Positive	1/8
Patient 9	M	76	Hematological malignancy	Negative	Positive	1/128	Negative	Negative	-	Positive	1/16
Patient 10	F	40	HIV	Negative	Positive	1/64	Positive	Positive	*C. neoformans*	Positive	1/256
Patient 11	M	44	HIV	Negative	Positive	1/8,192	Positive	Positive	*C. neoformans*	Positive	1/4,096
Patient 12	M	32	HIV	Negative	Positive	1/2,048	Negative	Positive	*C. neoformans*	Positive	1/2,048
Patient 13	M	69	Kidney transplantation	Negative	Positive	1/256	Positive	Positive	-	Positive	1/512
Patient 14	M	46	Kidney transplantation	Negative	Positive	1/128	Negative	Negative	-	Positive	1/1,024
Patient 15	M	63	Auto-immune hepatitis	Negative	Positive	1/2	Negative	Positive	*C. neoformans*	Positive	1/32
Patient 16	F	27	Epilepsy associated to Arnold-Chiari malformation	Negative	Positive	1/1,024	Positive	Positive	*C. gattii*	Positive	1/2,048

CSF, cerebrospinal fluid; HIV, human-immunodeficiency virus; F, female; M, male; NA, not available.

TABLE 4 | Management and outcomes of *Cryptococcus* spp. infection of the central nervous system.

	Sarcoidosis ($n = 5$)	Non-sarcoidosis ($n = 11$)	p-value
Time from diagnosis to treatment initiation (days)	0.20 ± 0.45	0.45 ± 0.93	0.76
Initial 2-week liposomal B amphotericin and flucytosine bitherapy*	4	8	1.00
Fluconazole maintenance therapy**	4	10	1.00
Treatment total duration (days)	**34 ± 29**	**66 ± 13**	**0.04**
Death due to *Cryptococcosis*	1	1	1.00
Long-term sequelae	1/4 Cerebellar ataxia	2/10 Motor function	1.00 -

**Intravenous liposomal B amphotericin: 3 mg/kg per day. Intravenous flucytosine: 25 mg/kg every 6 h.*

***Fluconazole per os: 400 mg per day for 2 weeks, then 200 mg per day. Bold values are statistically significant.*

pathophysiology of sarcoidosis still remains poorly understood, an unexplained primary immunodeficiency could favor both the occurrence of sarcoidosis and cryptococcosis (36).

Cryptococcosis is known to develop in patients with AIDS and idiopathic CD4 lymphopenia underlining the importance of CD4 T cells in the defense against *Cryptococcus* spp. (37). Systemic CD4 cells anergy is a feature of sarcoidosis as lymphopenia is correlated with disease severity during sarcoidosis (38) due to the accumulation of CD4 T lymphocytes in active granulomas participating in the "immune paradox" described in sarcoidosis: despite an extensive local inflammation, systemic anergy may develop (39). Nevertheless, in the cryptOsarc study, CD4 lymphocytopenia was not an independent risk factor for cryptococcosis (34) consistent with our findings that in CINS patients with sarcoidosis had a higher CD4 T cell proportion and CD4/CD8 T cells ratio than non-sarcoidosis patients. The authors of the cryptOsarc study even considered that "the CD4 levels in this study did not explain cryptococcosis in sarcoidosis". If quantitative alteration of CD4 T cells function is not responsible *per se* for this association with cryptococcosis, alteration of qualitative CD4 T cell function could be involved in the pathophysiology (**Figure 1**), but T cell dysfunction in sarcoidosis is poorly understood so far. Data on peripheral $CD25^{high}$ regulatory T cells (Treg) are contradictory; some authors reported a peripheral expansion that contributed to anergy, whereas others have reported a decrease in Treg cells with an imbalance in favor of Th17 cells (3).

Besides this potential qualitative CD4 T cell deficiency, an altered CD4 T cells-macrophage crosstalk could also be involved *via* decreased macrophage ability to contain *Cryptococcus* spp. (40). This hypothesis is reinforced as patients with X-linked CD40L deficiency or interleukin 12 receptor mutations exhibit higher susceptibility for cryptococcal infections (37). $CD40L^{-/-}$ mice exhibited exacerbation of infection with a high fungal burden due to diminished interferon-γ production by CD4 and CD8 cells and decreased CD28 expression by CD4 cells (41). Moreover, nitrite production and antimicrobial activity by macrophages were impaired as was IL-12 production by splenic macrophages. Patients with auto-antibodies to interferon-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF) also exhibit higher susceptibility to cryptococcal infections (37) as discussed below. The fact that interferon-γ and GM-CSF are known to be produced by CD4+ helper T cells to activate macrophages reinforce the plausibility of an altered CD4 T cells-macrophage crosstalk as a potential mechanism of increased susceptibility to cryptococcosis in sarcoidosis patients (42) (**Figure 1**).

Interest in the role of humoral immunity against *Cryptococcus* spp. was regained after the recent report of an association between invasive fungal infections, including cryptococcosis (43–45), and ibrutinib, an irreversible inhibitor of Bruton's tyrosine kinase (BTK). This clinical finding is strengthened by the fact that X-linked immunodeficient mice, which possess a mutation leading to a defective BTK, lack B-1 cells and natural IgM. These mice exhibit very high fungal burden after challenge with *C. neoformans* and fungal dissemination to the brain (46) consistent with previous findings in C57BL/6 mice depletion of B-1 cells (47) or $sIgM^{-/-}$ (48). Conclusions from these studies were flawed by the presence of T cells in those models and by associated defects in cellular immunity (XID mice) and in B cell development ($sIgM^{-/-}$ mice). To overcome these limitations, Rag1-deficient mice, which lack both T and B cells, were used, and B cells purified from wild-type animals were transferred (49) restoring B-1a and B-1b cells but not T cells or B-2 cells (50). This transfer led to a marked reduction in the cryptococcal burden in the brain but not in the lungs of the mice. Interestingly, transfer of IgG-depleted, IgM-containing serum in Rag1-deficient mice increased in alveolar macrophage recruitment and phagocytic index suggesting that the host benefit could be mediated by IgM-induced macrophage activation rather than a direct interaction (48). Another hypothesis is that B-1 cells could migrate to the lungs and differentiate into macrophage-like cells called B-1-derived mononuclear phagocytes (BDMP) (**Figure 1**) even if mechanisms by which B-1 B cells traffic to *C. neoformans*-infected lungs remains unknown (47). Those BDMP cells have been demonstrated to phagocyte *C. neoformans via* a complement receptor 3-mediated pathway with a higher fungicidal activity than a macrophage (51).

Even if sarcoidosis and granuloma formation in sarcoidosis are normally considered T cell-mediated peripheral B cells seem to be anergic in patients with sarcoidosis (52). This observed anergy could be due to the decreased levels of NF-κB/p65 (53) or to the lack of co-stimulation from CD4+ helper T cells (54). Nevertheless, whether sarcoidosis is associated with an impaired IgM production or with deficient B-1 cells is still not known.

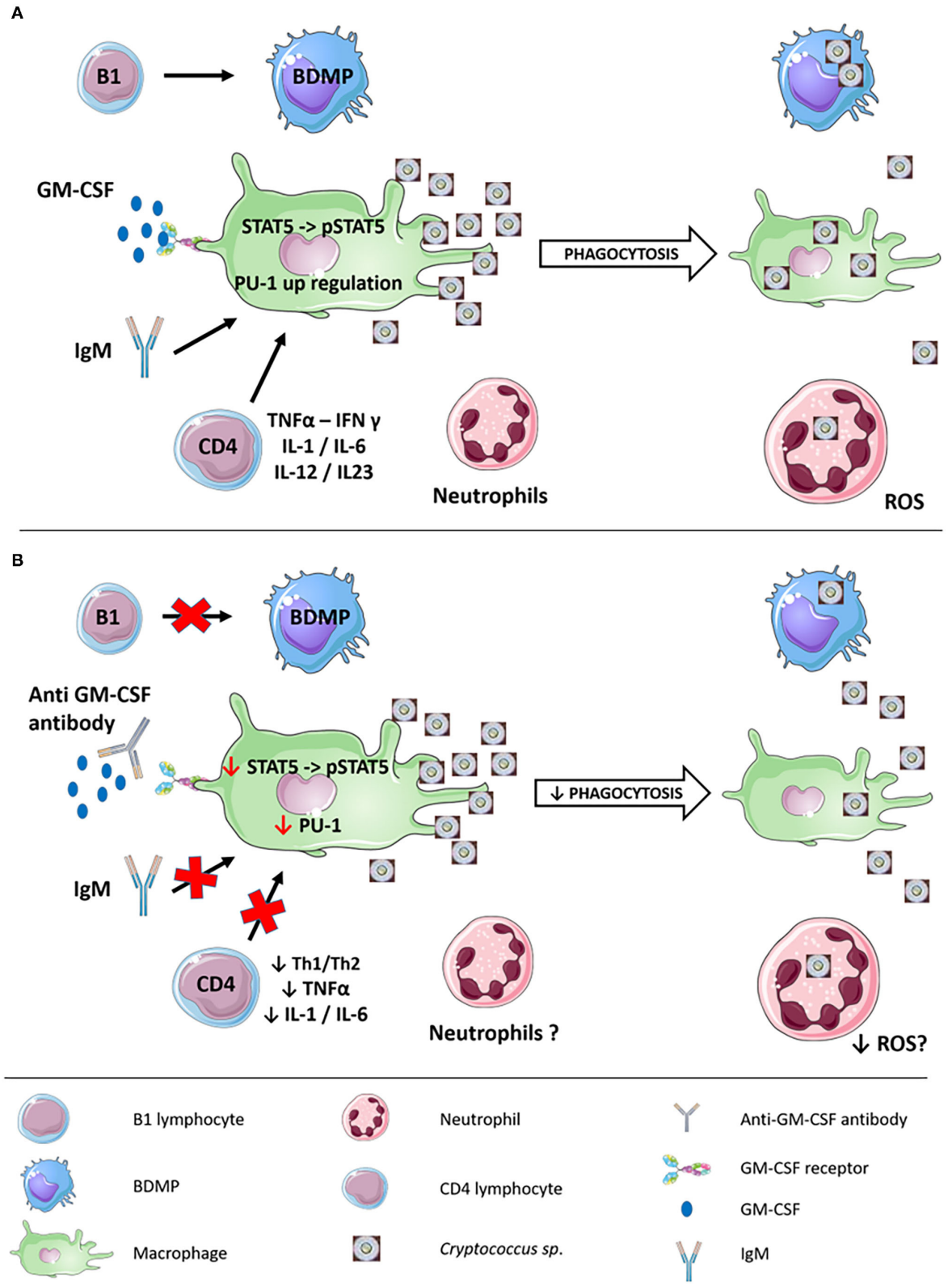

FIGURE 1 | Immune response to cryptococcal infection and potential pathophysiological mechanisms responsible for the association between sarcoidosis and cryptococcosis. **(A)** Immune response to cryptococcal infection involving both innate and adaptative immunity. B1 cells can differentiate into BDMP with phagocytic

(Continued)

FIGURE 1 | activity against *Cryptococcus* spp. GM-CSF, IgM, and CD4 activate macrophage phagocytic activity, partly *via* the phosphorylation of STAT-5 upregulating the transcription factor PU-1. Neutrophils exert a fungicidal activity through the production of ROS. **(B)** Potential pathophysiological mechanisms responsible for the association between sarcoidosis and cryptococcosis. B1 cells could have impaired differentiation leading to defective BDMP phagocytic activity. Lack of IgM and CD-4 qualitative defects could lead to a defect in macrophage phagocytic activity. The presence of anti-GM-CSF antibodies could also prevent STAT-5 phosphorylation downregulating the transcription factor PU-1. Whether ROS species neutrophils production is impaired in sarcoidosis remains unknown. B-1, B-1 lymphocytes; BDMP, B-1-derived mononuclear phagocytes; CD4, CD4 lymphocytes; GM-CSF, granulocyte-macrophage colony-stimulating factor; ROS, reactive oxygen species.

The real impact of B cells on susceptibility for cryptococcosis has also been questioned as invasive fungal infections are very rare in patients with X-linked agammaglobulinemia with BTK deficiency, probably because of residual BTK activity in myeloid cells (37). In fact, BTK is expressed in all bone marrow cell lineages except T cells and plasma cells (55). The increased susceptibility of X-linked immunodeficient mice seems to be mostly due to the inability of macrophages to phagocyte *Cryptococcus* spp. rather than impairment of humoral immunity (46).

Besides adaptive immunity deficiency previously discussed, innate immunity deficiency seems to be a key player in the susceptibility to *Cryptococcus* spp. infection. This deficiency of innate immunity could be indirectly due to the impaired crosstalk with adaptive immunity, i.e., impaired activation of macrophages by deficient CD4 T cells or by lack of IgM as previously discussed, but it can also be directly due to the intrinsic deficiency of innate immunity (**Figure 1**).

For instance, anti-GM-CSF antibodies have been isolated from the serum of apparently immunocompetent patients with cryptococcosis with or without pulmonary alveolar proteinosis (56–59). The addition of sera from the patients containing anti-GM-CSF antibodies impaired myeloid cells activation from controls in the presence of *C. gatii* (56). These antibodies are exclusively IgG and mostly IgG1 and are biologically active by inhibition of GM-CSF-induced macrophage inflammatory protein-1α expression (MIP-1 α) and signal transducer and activator of transcription-5 (STAT-5) phosphorylation in control peripheral blood mononuclear cells (58–60). This inhibition reduces myeloid cells proliferation and differentiation but also their phagocytic and bactericidal capacities *via* the inhibition of PU.1 transcription factor (59, 61) (**Figure 1**). Anti-GM-CSF antibodies are also involved in pulmonary alveolar proteinosis (PAP), but most of the cryptococcosis patients presenting with anti-GM-CSF antibodies did not suffer from PAP (62). This might be explained by the extreme heterogeneity of those antibodies regarding their avidity and the targeted GM-CSF epitopes (63). Moreover, multiple clones of anti-GM-CSF antibodies could be present in the same patient.

Anti-GM-CSF antibodies could be part of an explanation for this association between cryptococcosis and sarcoidosis as a potential association has been described between PAP and sarcoidosis (64, 65). A recent study found in 5/92 (5.4%) patients with sarcoidosis to have detectable anti-GM-CSF antibodies, two of them with clinical PAP (62). Those patients exhibited significantly higher serum levels of Krebs von den Lungen-6, surfactant protein-D, lactate dehydrogenase and required more often systemic corticosteroid therapy. It would thus be interesting to assess if anti-GM-CSF antibodies are more prevalent in patients with sarcoidosis and suspicion of cryptococcal infection. If so, the presence of those antibodies could help to identify at-risk patients. Nevertheless, whether uptake by macrophages is a conclusive readout for protecting against cryptococcal invasion is still unclear. In fact, recruited M1 polarized monocyte-derived macrophages are thought to have fungicidal activity contrary to alveolar macrophages (66). Alveolar macrophages could even be involved in the cryptococcal dissemination outside the lungs (67, 68).

Another major effector of first-line defense against cryptococcal infection is the pool of neutrophils as they have been demonstrated to engulf and kill *Cryptococcus* spp. more efficiently than monocytes (69) and produce reactive oxygen species (ROS) that kill *Cryptococcus* spp. (70). However, the role of neutrophils in cryptococcal infections is not straightforward as, in contrast to intravascular infection, they seem to worsen the prognosis in the setting of intratracheal infection (71, 72). Absolute neutrophil count of more than 3,500 cells/mm^3 is even associated with increased mortality in HIV-infected patients with cryptococcal meningitis (73). This could be explained by the fact that neutrophils are recruited to the lungs in response to cryptococcal infection by lung parenchymal lymphocytes and that T cells (CD4 cells but also CD8 cells and γδ cells) impairment is associated with a compensatory neutrophil response requiring IL-17A, which worsens lung injury (71, 72, 74). Moreover, the following two distinct neutrophil subsets seem to be generated in response to cryptococcal infection: (i) one with an oxidative stress signature interacting directly with the fungus and generating ROS and (ii) another with enhanced cytokine gene expression which are longer-lived and that indirectly respond to cryptococcal ligands to modulate crosstalk, *via* the expression of IL-1α, TNFα, and complement C3, with dendritic cells and alveolar macrophages through CCR5 and CCR1, respectively (75). Mechanisms responsible for such a differentiation remain unknown. While in contact, *Cryptococcus* spp. phagocytosis by neutrophils is dependent on the complement C5a-C5aR axis (76).

Very less is known about neutrophil's involvement in sarcoidosis pathophysiology except for that neutrophil/lymphocyte ratio in the complete blood count can be used as an indicator of inflammation in sarcoidosis (77). Although neutrophil accumulation-inducing chemokines like IL-8 are increased in sarcoidosis, the percentage of neutrophils in the bronchoalveolar lavage remains low and is not correlated to CXCL8 or CXCL5 as in idiopathic pulmonary fibrosis (78–80). Nevertheless, the percentage of neutrophils in bronchoalveolar lavage is associated with progressing sarcoidosis and an increased

risk for corticosteroid therapy (81, 82). The role of neutrophils in the association between sarcoidosis and cryptococcosis thus remains to be demonstrated (**Figure 1**).

CONCLUSION

Sarcoidosis was the most frequently associated condition with CINS in this study. CINS patients with associated sarcoidosis had overall similar clinical and biological presentation than CINS patients associated with other conditions but exhibited a lower rate of positive blood cryptococcal antigen testing and higher CD4/CD8 T cells ratio. Pathophysiological mechanisms underlying this association remain poorly understood, but B-1 cell deficiency or lack of IgM could be a part of the explanation. Another plausible mechanism is the presence of anti-GM-CSF antibodies in a subset of patients with sarcoidosis that could impair macrophage phagocytic function. Further studies are strongly needed to better understand those mechanisms and to identify at-risk patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

RP, LD, PB, and PD contributed to the conception and design of the study. RP, SI, and VG contributed to the data collection. RP and PD wrote the manuscript. Each author drafted or provided critical revision of the article and provided final approval of the version submitted for publication.

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Sarcoid-like reaction in patients with malignant tumors: Long-term clinical course and outcomes

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Background: The development of non-caseating epithelioid cell granulomas in cancer patients who do not fulfill the systemic sarcoidosis criteria is termed sarcoid-like reaction (SLR). Little is known about this condition's natural course and impact on the prognosis of malignancy. We aimed to investigate the natural course and prognostic value of cancer-associated SLR.

Methods: Clinical data were retrospectively analyzed in 32 patients with biopsy-proven cancer-associated SLR. Among patients with non-small cell lung cancer (NSCLC), SLR cases ($n = 8$) were matched with non-SLR cases ($n = 78$) for survival analysis.

Results: Among the included patients, the mean age was 59.7 years, and 68.8% were female. The median follow-up period was 35.6 months [interquartile range (IQR): 14.0–61.4 months]. Of all the included malignancies ($n = 32$), breast cancer (25.0%) and NSCLC (25.0%) were the most common, with stage I being the most frequent tumor stage (59.4%). During follow-up, SLR progression to overt sarcoidosis was not observed. In the 28 patients with available follow-up computed tomography images (median interval: 24.9 months; IQR: 14.4–41.7), 4 patients received corticosteroids ($n = 4$), resulting to a decrease of SLR lesions. Meanwhile, among those who did not receive treatment ($n = 24$), the extent of SLR decreased or did not change in 85.7% of them, whereas 3.6% had increased SLR extent. Furthermore, among patients with NSCLC, SLR was not associated with overall survival [hazard ratio (HR) = 1.28, 95% confidence interval (CI): 0.02–67.71, $P = 0.882$] and recurrence of malignancy (HR = 1.27, 95% CI 0.21–7.51, $P = 0.793$) in the Cox proportional hazard regression model.

Conclusions: During the follow-up of cancer-related SLR, we found no further evidence for systemic sarcoidosis, and most of the lesions decreased or did not change. Development of SLR was also not associated with overall survival or disease-free survival in patients with NSCLC.

KEYWORDS

sarcoidosis, neoplasms, granuloma, mortality, progression-free survival

Introduction

Sarcoid-like reaction (SLR) is defined as the development of non-caseating epithelioid cell granulomas in patients who do not meet the criteria for systemic sarcoidosis (1). Its occurrence is known to be linked with diverse conditions, including malignancies (2–4), infections (5, 6), and exposures to inorganic substances or certain drugs (7–9). Studies have suggested that the pathogenesis of SLR is attributed to diverse antigens that are coupled with genetic susceptibility, consequently triggering T cell mediated immune responses, which then leads to the formation of non-necrotizing epithelioid cell granulomas (10).

Although various types of malignancies, including lymphoma (11, 12), breast cancer (13, 14), stomach cancer (15, 16), colon cancer (17, 18), and lung cancer (19, 20), have been reported to be associated with SLR development, its clinical significance in cancer patients remains unclear. In previous studies (14, 21), SLR has been associated with better cancer outcomes. In particular, Chen et al.'s study in five breast cancer patients with SLR showed that all patients were disease-free during the median follow-up of 6 years following curative resection (14). Similarly, Steinfort et al. reported that among 24 patients with early-stage (stage I) non-small cell lung cancer (NSCLC), no recurrence after lobectomy was observed in those with SLR ($n = 8$), whereas a recurrence rate of 44% was found in those without SLR ($n = 16$) (21). However, contradicting findings have also been reported; Tomimaru et al., for one, reported that among 1,733 lung cancer patients who underwent surgical resection, no significant difference in the overall survival (77.7 vs. 75.2%, $P = 0.8227$) was found between patients with ($n = 22$) and without SLR ($n = 1,711$) (19). Given all these findings, there are still uncertainties regarding impact of SLR on the prognosis of malignancy, and notably, the clinical course of SLR has not been addressed in previous studies. Therefore, we aimed to investigate the clinical course and prognostic value of SLR in patients with malignancies.

Materials and methods

Study population

Adult patients with ICD-10 codes for malignant neoplasm (C00-C97) identified between 2008 and 2018 at the Asan Medical Center, Seoul, Republic of Korea ($n = 243,320$) were screened for this study. Among them, 2,455 cases with medical records containing the following keywords were selected: "sarcoid-like reaction," "non-caseating epithelial cell granuloma," or "non-necrotizing epithelial cell granuloma." After reviewing the pathologic reports of these cases, 2,344 patients were excluded since no previous diagnoses or evidence of malignancy were provided at the time of biopsy. From the remaining 107 patients, those diagnosed with overt sarcoidosis ($n = 75$) were excluded. Overt sarcoidosis was defined by evidence of two or more organ involvements at the time of biopsy (22). Patients' past medical history, ophthalmologic tests, electrocardiograms, echocardiography, Holter monitoring, laboratory examinations, including serum creatinine, serum alkaline phosphatase and complete blood cell count, and urine calcium levels were reviewed (Supplementary Table 1). Finally, a total of 32 patients with malignancy associated SLR were included in this study (Figure 1).

The study protocol was approved by the Institutional Review Board of the Asan Medical Center (IRB No. 2019-1015), and the requirement for informed consent was waived due to the retrospective nature of the analysis.

Data collection

The clinical and survival data of all the included patients were retrospectively collected from their medical records and/or the National Health Insurance records of Korea. Spirometry, diffusing capacity of the lung for carbon monoxide, and total lung capacity were measured according to the ERS/ATS recommendations (16, 17), and the results were expressed as percentages of the normal predicted values.

Data from follow-up visits or hospitalizations were reviewed to determine malignancy recurrence. To evaluate the clinical course of SLR, baseline and the follow-up computed tomography (CT) images [median interval: 24.9 months, interquartile range (IQR): 14.4–41.7 months] were compared side by side by two readers (JYH and DSM), wherein disagreements were resolved *via* consensus. For each subsequent CT scan reading, findings were categorized into "improved," "unchanged," or "aggravated." Specifically, an SLR lesion was categorized as "improved" if the sum of lengths of the lesions decreased by 30% or more, whereas an "aggravated" SLR lesion was defined if the sum of lengths of the lesions increased by 20%, which is based on the modified RECIST criteria (23). Lesions without changes or with changes that did not belong to either the "improved" or "aggravated" categories were defined as "unchanged" SLR lesions.

Case-control matching in NSCLC

For the evaluation of the impact of SLR on the prognosis of patients with NSCLC, overall survival and disease-free survival were compared between patients with and without SLR. NSCLC patients without SLR were selected from the Center for Cancer Data Management at Asan Medical Center. Following selection, case-control matching (1:10) was performed to adjust for differences in baseline characteristics, including age, sex, T stage, N stage, the time of cancer diagnosis, and treatment modality.

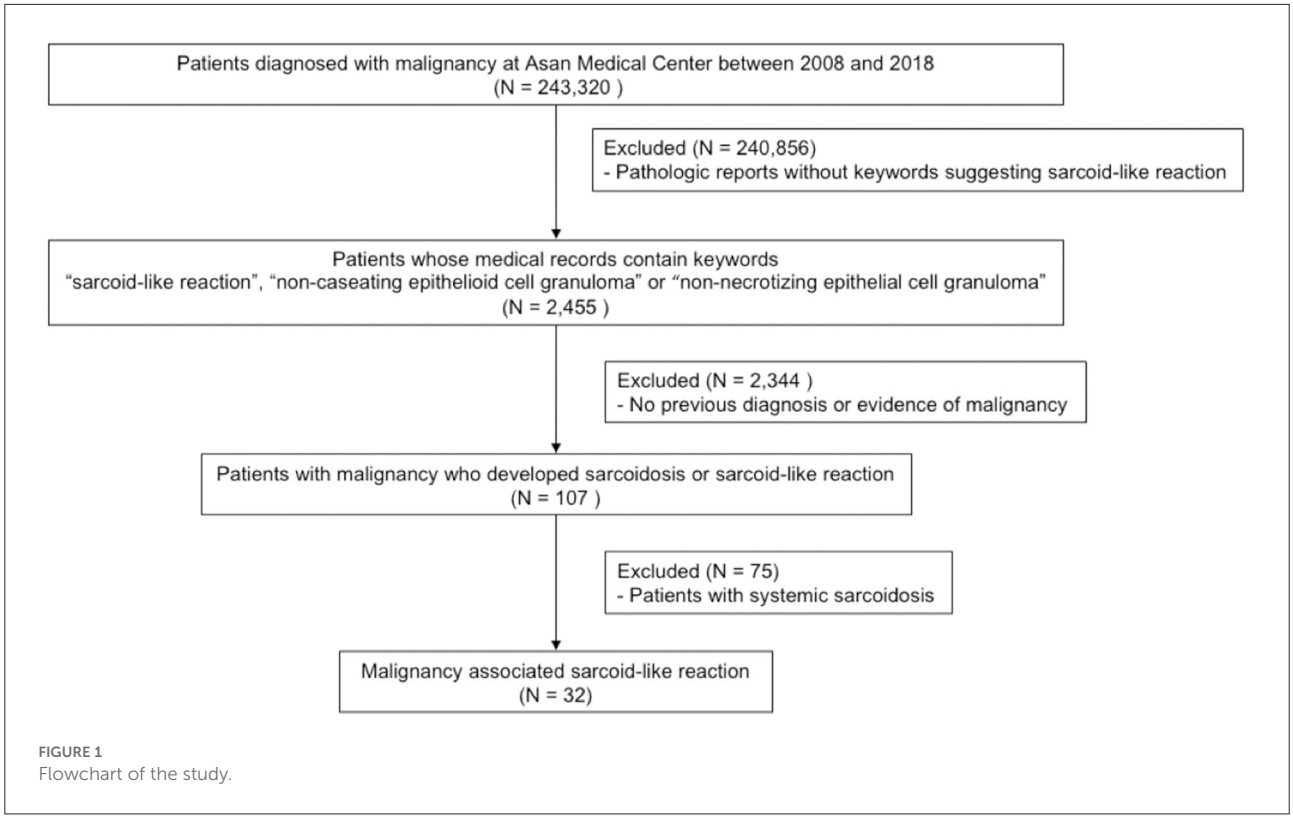

FIGURE 1
Flowchart of the study.

Statistical analysis

All values are presented as means ± standard deviations for continuous variables or as percentages for categorical variables. Survival was assessed using the Kaplan–Meier survival analysis, and differences between groups were evaluated using the log-rank test. Furthermore, the Cox proportional-hazards model was applied to evaluate impact of SLR on overall survival and disease-free survival. All statistical analyses were performed using the R Statistical Software (version 4.0.3; R Foundation for Statistical Computing, Vienna Austria), and a P-value <0.05 was considered to be statistically significant (two-tailed).

Results

Baseline characteristics

Among the included patients in this study, the mean age was 59.7 years, and 68.8% were female (Table 1). The median follow-up period from the date of SLR diagnosis was 35.6 months (IQR: 14.0–61.4 months). Notably, the mean angiotensin-converting enzyme (ACE) level was 38.4 U/L ($n = 14$), which was elevated (normal range: 7.5–53.0 U/L) in 28.6% (4/14) of them.

For all patients, SLR developed in various types of cancer. Among them, breast cancer (25.0%) and NSCLC (25.0%) were the most common, which was followed by colon cancer (18.8%) (Table 2). The median interval between cancer and SLR diagnoses was 3.8 months (IQR: 0.7–30.5 months), with most patients (81.3%) reporting SLR occurrence within 3 years after cancer diagnosis (Supplementary Figure 1). Regarding tumor staging at the time of SLR diagnosis, stage I was the most frequently diagnosed stage (59.4%, 19/32), while only one patient had stage IV disease (Table 2). Also, in terms of the treatment of malignancy, 26 (81.3%) underwent surgical resection, 4 (12.5%) received chemotherapy, 1 (3.1%) had concurrent chemoradiotherapy, and 1 (3.1%) was treated with radiotherapy. No patients were treated with immune check point inhibitors. Furthermore, 93.8% of the patients presented with lymphadenopathy (mediastinum: 65.6%, neck: 21.9%, axilla: 3.1%, intrabdomen: 3.1%), and 6.3% had lung lesions (Table 1). For mediastinal lymph nodes, endobronchial ultrasound-guided transbronchial needle aspiration was performed and, for lymph nodes at other sites, ultrasound-guided needle biopsy was done. The samples of the lung parenchymal lesions were acquired with percutaneous lung biopsy.

Clinical outcomes of malignancy

During follow-up, two patients with SLR (6.3% of total patients) died. Specifically, one patient with Hodgkin lymphoma died due to an acute exacerbation of combined idiopathic

TABLE 1 Baseline characteristics of the study population.

Characteristics	
Patient number	32
Age, years	59.7 ± 11.7
Female sex	22 (68.8)
Smoking status	
Current smoker	2 (6.2)
Ever-smoker	8 (25.0)
Never-smoker	22 (68.8)
Angiotensin-converting enzyme, U/L	38.4 ± 21.8
Pulmonary function test	
FVC, % predicted	84.7 ± 17.8
DLCO, % predicted	77.4 ± 16.7
TLC, % predicted	93.6 ± 20.8
6-minute walk test	
Distance, meter	490.1 ± 49.8
Lowest $SpO_{2,\%}$	95.6 ± 2.4
BAL fluid analysis	
WBC, /mm^3	120.8 ± 112.3
Neutrophil, %	1.8 ± 1.6
Lymphocyte, %	21.6 ± 18.9
Site of sarcoid-like reaction	
Mediastinal lymph node	21 (65.6)
Neck lymph node	7 (21.9)
Lung parenchyma	2 (6.3)
Axillary lymph node	1 (3.1)
Intraabdominal lymph node	1 (3.1)

Data are presented as means ± standard deviations, or as numbers (%), unless otherwise indicated. FVC, forced vital capacity; DLCO, diffusing capacity of the lung for carbon monoxide; TLC, total lung capacity; SpO_2, saturation of oxygen; BAL, bronchoalveolar lavage; WBC, white blood cell.

TABLE 2 Types and stage of the malignancy in patients with sarcoid-like reaction.

Characteristics	
Patient number	32
Type of cancer	
Breast cancer	8 (25.0)
Non-small cell lung cancer	8 (25.0)
Colon cancer	6 (18.8)
Non-Hodgkin's lymphoma	3 (9.4)
Gastric cancer	2 (6.3)
Thyroid cancer	2 (6.2)
Hodgkin's lymphoma	1 (3.1)
Prostate cancer	1 (3.1)
Cervix cancer	1 (3.1)
Stage of cancer	
I	19 (59.4)
II	6 (18.8)
III	6 (18.8)
IV	1 (3.1)

Data are presented as numbers (%), unless otherwise indicated.

pulmonary fibrosis, whereas the other patient with NK/T cell lymphoma died from hemophagocytic lymphohistiocytosis progression. Moreover, malignancy recurrence following curative treatment was observed in one patient with SLR (3.1%). In this case, the patient with NSCLC (adenocarcinoma, stage IB) underwent surgical resection for curative treatment; however, brain metastasis occurred 35 months after SLR diagnosis. On the other hand, no evidence of disease progression or recurrence was observed in the remaining 29 patients with SLR.

To compare survival between NSCLC patients with (n = 8) and without SLR, a control cohort was selected from the Asan Medical Center database (n = 79). Baseline clinical characteristics and outcomes between the two groups are shown in Table 3. No deaths were reported in the SLR group, while two patients (2.5%) died in the control group ($P > 0.99$). One patient (12.5%) in the SLR group had recurrence of NSCLC, whereas 11 patients (13.9%) in the control group reported disease recurrence $P > 0.99$ as presented in Table 2. No significant differences were observed in the overall survival [100.0% (SLR group) vs. 94.6 (control group), $P = 0.633$] (Figure 2A) and disease-free survival (75.0 vs. 77.8%, $P = 0.899$) between both groups (Figure 2B). In a multivariate Cox proportional-hazards model adjusted for age, sex, cell type and treatment modality, SLR was not associated with overall survival (hazard ratio = 1.28; 95% confidence interval, 0.02–67.71; $P = 0.882$) and disease-free survival (hazard ratio = 1.27; 95% confidence interval, 0.21–7.51; $P = 0.793$) (Supplementary Table 2).

Clinical outcomes of SLR

Among the 32 included patients, follow-up CT images were available in 28 patients, which were analyzed to evaluate the clinical course of SLR. Notably, four patients 14.3% were treated with corticosteroids [initial mean dose: 28.8 ± 2.5 mg of prednisolone, the median treatment period: 20.1 months (IQR: 15.9–23.7 months)] at the attending physician's discretion; one patient with co-existing chronic obstructive pulmonary disease received corticosteroid due to progressive dyspnea, and the other three patients were treated with corticosteroid to further confirm that the lesions were due to SLR and not metastasis. All four patients had an improvement in all SLR lesions (100%). Meanwhile, in the untreated group (n = 24), the outcomes were categorized as "improved" in 58.3%, "unchanged" in 37.5%, and "aggravated" in 4.2% (Figure 3). There was no further evidence of systemic sarcoidosis during the follow-up.

TABLE 3 Comparison of baseline characteristics and clinical courses between SLR and non-SLR groups among NSCLC patients.

Characteristics	SLR	No-SLR	*P*-value
Patient number	8	79	
Age, years	68.6 ± 6.0	69.0 ± 6.1	0.877
Male sex	4 (50.0)	39 (49.4)	>0.99
Cell type			>0.99
Adenocarcinoma	7 (87.5)	69 (87.3)	
Squamous cell carcinoma	1 (12.5)	10 (12.7)	
Treatment			>0.99
Surgical resection	7 (87.5)	69 (87.3)	
Radiotherapy	1 (12.5)	10 (12.7)	
Chemotherapy	0 (0.0)	0 (0.0)	
Median follow-up period	33.2 (11.6–64.8)	18.5 (11.6–62.7)	0.831
Recurrence	1 (12.5)	11 (13.9)	>0.99
Death	0 (0.0)	2 (2.5)	>0.99
Disease-free survival, months	26.3 (11.2–41.5)	17.4 (10.3–38.0)	0.814

Data are presented as means ± standard deviations, numbers (%), or as medians (interquartile range), unless otherwise indicated.
SLR, sarcoid-like reaction; NSCLC, non-small cell lung cancer.

Discussion

In this study, we identified 32 biopsy-confirmed SLR patients with various types of malignancies. At the time of SLR diagnosis, the most common underlying cancers were breast cancer and NSCLC, with stage I as the most common tumor staging. During follow-up, most of SLR lesions improved or did not progress in treatment-naïve cases, whereas SLR lesions improved in all treatment cases. We found no further evidence of systemic sarcoidosis during the follow-up. Furthermore, overall survival and disease-free survival were similar between the SLR and non-SLR groups of NSCLC patients.

The patients included in this study were mostly in the early-stage of their malignancy (stage I in 59.4%). This was similar to the observations in Steinfort et al.'s study of 187 NSCLC patients, wherein they reported that all eight cases (4.3%) with SLR were stage I (20). Murthi et al.'s multicenter study including 133 cancer-associated SLR patients also reported that stage I was the most common tumor staging (38.3% of 131 patients with available data for cancer stages) (2). The pathogenesis of SLR in malignancy has been postulated by previous studies to be caused by immune responses to malignancy-related antigens involving dendritic cells and T cells (2, 10). Vimentin, a type III intermediate filament that forms the cytoskeletal microtubules and microfilaments (24), has been a suspected culprit antigen for SLR in cancer patients (2). While this is plausible, vimentin is not expressed universally and its expression has been associated with worse prognosis or advanced disease (25, 26). Diversity of cancer associated with SLR, and findings of more frequent early-stage cancers suggest further investigation for the causative antigen is required and we may even find that not a single antigen is responsible for all cases of cancer-related SLR. In fact, the T cell response to the antigen may be the key to impact of SLR on the clinical course. The presence of tumor-infiltrating T cells has been known to be associated with better prognosis (27), and it has been suggested that SLR might be associated with potential antitumor response, leading to cancer progression inhibition (21).

In the comparison between NSCLC patients with and without SLR, no association was found between SLR and the prognosis of NSCLC patients. Our results showed that no deaths occurred in the SLR group, whereas two deaths were found in the non-SLR group (mortality rate: 0.0 vs. 2.5%, $P > 0.99$). Tomimaru et al. showed similar results in their study of lung cancer patients who underwent surgical resection, reporting that the 3-year (85.2 vs. 82.1%) and 5-year survival rates (77.7 vs. 75.2%) were similar between the SLR ($n = 22$) and non-SLR ($n = 1,711$) groups (19). However, in other previous studies, SLR was associated with better outcomes in NSCLC patients (2, 19, 28). Dagaonkor et al.'s study, among 127 lung cancer patients who underwent surgery, reported that the survival rate was higher (3-year survival rate: 21.1 vs. 6.5%, $P = 0.06$) in those with SLR ($n = 19$) than those without SLR ($n = 108$) (28). Murthi et al.'s study of 133 patients with various cancer-related SLRs (lung cancer, $n = 30$) also showed that survival was significantly better (odds ratio, 0.223; 95% confidence interval, 0.079–0.632; $P = 0.005$) in the SLR group ($n = 46$) than in the non-SLR group ($n = 134$) (2). Furthermore, Steinfort et al. showed that among NSCLC patients, disease-free survival was better in the SLR group ($n = 8$), as compared to the non-SLR group ($n = 16$) (100 vs. 56%, $P = 0.044$) (21). This discrepancy between our study and previous investigations may be due to small number of patients in our study's SLR group. Additionally, our controls were matched based on age, sex, T stage, N stage, and the time of NSCLC diagnosis, whereas in other studies, the controls were either unmatched (2, 28) or matched regardless of the time of diagnosis (21).

Regarding the clinical course, most of the SLR lesions either improved or did not progress during follow-up; this was consistent with the results of previous studies (29, 30). Lau et al.'s study of 11 patients with SLR (associated with malignancy, hepatitis C, or medication) showed that the extent of SLR lesions decreased in 81.8%, whereas 18.2% reported no changes during the median follow-up of 10.6 months (IQR: 6.5–32.6 months) (29). Kaneko et al. also reported that among 14 patients who developed SLR following malignant tumor treatment, SLR regressed in 78.6%, remained unchanged in 7.1%, and worsened in 14.3% of the patients (30). In our study, the only patient whose CT findings were "aggravated" presented with mediastinal lymph node enlargements as a manifestation of SLR. In addition, at the time of the patient's last chest CT scan, pneumonia was also reported, possibly contributing to the

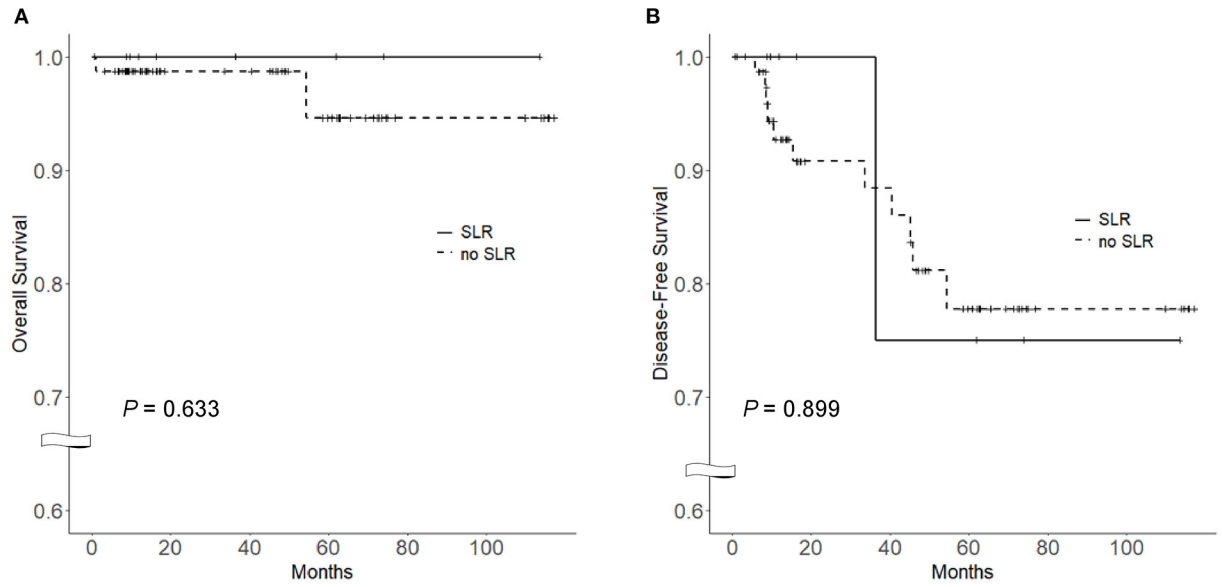

FIGURE 2
Comparison of the Kaplan–Meier survival curves between SLR and non-SLR groups among NSCLC patients. **(A)** Overall survival curve, **(B)** Disease-free survival curve. Log-rank test was used for the analysis. SLR, sarcoid-like reaction; NSCLC, non-small cell lung cancer.

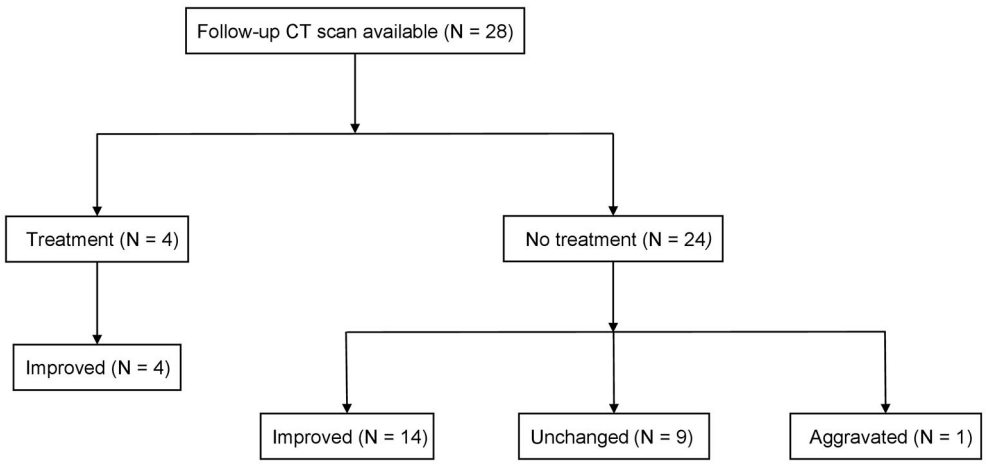

FIGURE 3
Clinical course of the patients with SLR. Patients who received treatment were given prednisolone with a mean dose of 28.8 mg. Improvements were defined as a decrease in the sum of the lengths of SLR lesions by 30% or more. Aggravation was defined as an increase in the sum of the lengths of SLR lesions by 20% or more. SLR, sarcoid-like reaction.

enlargement of the lymph nodes. Given all these findings, our study and previous studies suggest a favorable clinical course in patients with SLR. Furthermore, all patients treated with prednisolone in our study showed significant improvements, suggesting the effectiveness of corticosteroid therapy for SLR.

Interestingly, the level of ACE was not elevated in most of our patients, with only 28.6% of patients reporting an elevated ACE level. The ACE is secreted by epithelioid and giant cells, which becomes elevated among patients with epithelioid and giant cell-containing granulomas (31). In particular, a non-caseating granuloma with epithelioid and giant cells is a pathologic hallmark of sarcoidosis (32), and SLR is also characterized by epithelioid granulomas (21). Despite this pathologic linkage, previous studies have shown that the mean level of ACE was not elevated in most patients with SLR (2, 3). Murthi et al., for one, reported on the ACE levels of 54 patients

from 133 cancer-related SLR patients, showing elevated ACE levels in 15.7% of them (2). Another case-control study by Pastre et al. regarding cancer patients with SLR reported ACE level elevation in 50.0% (16/32) of them (3). Given all these findings, the studies suggest that ACE levels can be variable in SLR.

Several limitations of this study should be noted. First, as we restricted patients to those who underwent biopsy at a single center, selection bias might have affected the results. However, the clinical features of our patients were comparable to those in previous studies (2, 28). Second, the number of patients included in our study was small, consequently confounding statistical analysis and data interpretation. However, our results suggested a favorable clinical course of SLR in cancer patients despite the small sample size. Third, prognosis was only evaluated in NSCLC, and the included number of patients was very small. Nevertheless, this is one of the only few reports in the literature of the prognostic value of SLR among NSCLC patients and the relatively low incidence can make it difficult to conduct large-scale prospective studies of this subject. Lastly, chemotherapy for cancer has been associated with granulomas in previous reports (8, 9), and some of the patients in our study were exposed. Nonetheless, only about 15% were treated with chemotherapy before the diagnosis of SLR and the medications did not include immune check point inhibitors, which are commonly related. Despite these limitations, investigations on the long-term clinical course and prognostic impact of SLR were strengths of the study.

In conclusion, among cancer-associated SLR patients, early-stage breast cancer and NSCLC were the most common underlying malignancies. Our findings showed no further evidence of systemic sarcoidosis after initial evaluation, wherein most of the SLR lesions decreased or did not change during follow-up. Furthermore, SLR development may not be associated with the prognosis of NSCLC patients.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board of Asan Medical Center. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

JWS, DM, and J-YH contributed to conception and design of the study. DM organized the database. DM and J-YH performed the statistical analysis. J-YH wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.884386/full#supplementary-material

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A transcriptomics-based meta-analysis identifies a cross-tissue signature for sarcoidosis

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Sarcoidosis is a granulomatous disease of unknown etiology, immunologically characterized by a Th1 immune response. Transcriptome-wide expression studies in various types of sarcoid tissues contributed to better understanding of disease mechanisms. We performed a systematic database search on Gene Expression Omnibus (GEO) and utilized transcriptomic data from blood and sarcoidosis-affected tissues in a meta-analysis to identify a cross-tissue, cross-platform signature. Datasets were further separated into training and testing sets for development of a diagnostic classifier for sarcoidosis. A total of 690 differentially expressed genes were identified in the analysis among various tissues. 29 of the genes were robustly associated with sarcoidosis in the meta-analysis both in blood and in lung-associated tissues. Top genes included *LINC01278* ($P = 3.11 \times 10^{-13}$), *GBP5* ($P = 5.56 \times 10^{-07}$), and *PSMB9* ($P = 1.11 \times 10^{-06}$). Pathway enrichment analysis revealed activated IFN-γ, IL-1, and IL-18, autophagy, and viral infection response. IL-17 was observed to be enriched in peripheral blood specific signature genes. A 16-gene classifier achieved excellent performance in the independent validation data (AUC 0.711–0.964). This study provides a cross-tissue meta-analysis for expression profiles of sarcoidosis and identifies a diagnostic classifier that potentially can complement more invasive procedures.

KEYWORDS

sarcoidosis, transcriptome, interferon, IL-17, machine learning

Introduction

Sarcoidosis is a systemic disorder featured by the presence of non-caseating granuloma. The incidence varies considerably depending on sex, age, ethnicity and geographic regions, indicating that both genetic predisposition, and environmental factors play essential roles in the pathogenesis. The etiology of sarcoidosis remains uncertain despite decades of effort. Multiple genome-wide expression studies have been performed on sarcoidosis in order to understand underlying molecular mechanisms, including directly affected tissues such as lung and skin, fluids in contact with granulomas like bronchoalveolar lavage (BAL), and peripheral blood.

Expression profiles of circulatory blood and sarcoid tissues are quite different, but can be implicated in pathways involved in innate and adaptive immunity, granuloma formation, and fibroproliferation (1–3). Th1 associated molecules, especially INF-γ response transcription factor STAT1 as well as STAT1 regulated chemokines (IL-5, IL-7, IL-15, CCR5, CXCL9, CXCL10, and CXCL11) have been recognized as key inflammatory factors in sarcoidosis in transcriptome-wide analysis of lung, lymph nodes and peripheral blood (4, 5).

Aside from tissue-independent common pathways, genes enriched in IFN signaling (type I and II) and the Th17 pathway, including *IL-23*, *IL-23R,* and *IL-21*, are dysregulated in skin tissue of active cutaneous sarcoidosis (6). Upregulated genes in orbital tissues further validated the role of IFN-γ and type I IFNs, including *CXCL10, GBP5, STAT1, AIM2, ICAM1,* and *JAK2* (7). Enrichment analysis of transcription factor binding sites revealed that interferon-response factor 1 and 2 (IRF-1 and IRF-2), and nuclear factor κB, are involved in the transcriptional modulation in sarcoidosis.

In addition to pathways associated with adaptive immune system and T-cell signaling, differentially expressed genes in BAL identified a novel gene network linkage between immunoproteasome subunits (*PSMB-8, −9, −10*), and found *TWIST1*, a biomarker of M1-activation, to be up-regulated in sarcoidosis patients compared to controls (8, 9). Comparison of BAL cells from patients with severe and stable sarcoidosis demonstrated increased expression of protein kinase *TYK2* and cell cycle inhibitor *p21Waf1/Cip1*, as well as reduced expression of Cathelicidin (CAMP), confirming the involvement of Th1 and INF-γ immune responses (10, 11). *MMP12* and *ADAMDEC1* were newly identified to be significantly associated with sarcoidosis severity in lung tissue and BAL (5).

Peripheral blood has also been extensively examined for sarcoidosis specific gene identification. Whole blood gene expression signature distinguished sarcoidosis from healthy controls with an error rate of 12.1% (12). The genes belonged to Th1-type inflammation, such as INF signaling pathway (*IFN*, *STAT1*), and to T-cell homeostasis and survival (*IL-15* and *IL-7R*). A 20 gene signature was identified in peripheral blood mononuclear cells (PBMC) with an accuracy of 86.0% to distinguish healthy subjects from those with sarcoidosis, but performed less well when applied to replication datasets (13). Unlike the prior model, the cohort-specific 20 gene signature was not composed of genes belonging to T-cell, JAK/STAT, or cytokines pathways.

The diagnosis of sarcoidosis relies on histopathologic examination and compatible clinical presentation, with other causes of granulomatous inflammation excluded (14). A definitive diagnosis is challenging because several other diseases can show similar histopathologic changes (15). The prognosis is less favorable among patients with more advanced stage at diagnosis, emphasizing the significance to develop auxiliary approaches to help early diagnosis of such potentially hazardous disease. However, the clinical value of sarcoid tissue-based diagnostic gene markers remains unclear owing to the limitations of obtaining biopsy samples.

In this study, a full meta-analysis was performed utilizing all available genome-wide expression datasets for sarcoidosis vs. healthy subjects from public database to explore robust gene markers across different tissues and to propose an expression-oriented diagnostic panel. To our knowledge, this is to date the largest cross-tissue transcriptomic meta-analysis of sarcoidosis.

Materials and methods

Dataset identification

A systematic database search was performed on Gene Expression Omnibus (GEO). A total of 1,757 records were found with sarcoidosis as search term and organism confined to Homo sapiens (Feb 5 2021). We excluded 1,712 duplicated and 7 irrelevant results. Nine cell lines were excluded since they do not depict transcriptional features and functions *in vivo*. 3 methylation datasets, 1 single-cell dataset, 3 non-coding RNA datasets, 1 dataset with less than two sarcoidosis subjects, and 6 datasets without compatible control were subsequently excluded. Two array-based studies without transcriptome-wide data were removed, and 13 datasets were finally included in our meta-analysis (**Figure 1**). We checked definition of sarcoidosis and control in each of the included study. Diagnosis of sarcoidosis was made by a sarcoidosis specialist, biopsy evidence, compatible clinical, and radiological findings according to the WASOG guidelines (15) in GSE19314, GSE42834, GSE16538, and GSE37912. Similar diagnosis criteria were applied in GSE83456 and GSE75023. The other studies used pathology-confirmed biopsy displaying typical non-necrotizing epithelioid granuloma as definition. Controls were defined as recruited healthy volunteers or disease-uninvolved tissues.

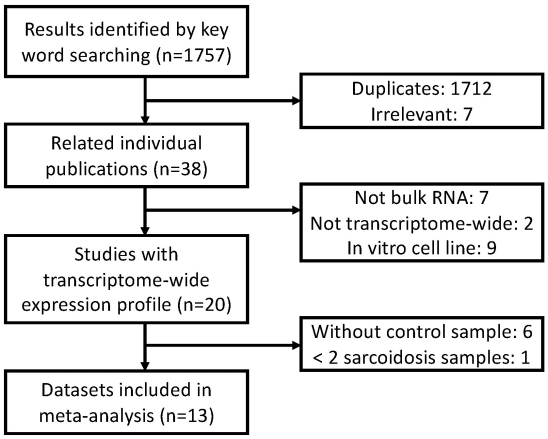

FIGURE 1
Overview of the meta-analysis approach for development and validation of sarcoidosis transcriptome panel.

Quality control and pre-processing

Selected datasets downloaded from the GEO repository[1] consist of different forms of expression measurements and probe annotation files. R package limma and affy were utilized to concordantly process the datasets to enable comparison (16, 17). For datasets with available raw CEL files for download, expression intensities were extracted and normalized using robust multi-array average (RMA) based on the corresponding custom chip definition file (CDF). Only genes estimated to be present in more than 10% samples were included in subsequent analysis. Data generated from Affymetrix HGU133 Plus 2.0, Affymetrix Human Gene/Exon 1.0 ST Array, and Illumina HumanHT-12 V4.0 expression beadchip underwent procedures described above. For RNA sequencing data generated from Illumina HiSeq 3000 platform, raw counts were downloaded and normalization factors were calculated with edgeR (18). Probes of different arrays were subsequently replaced by official gene symbols, and multiple expression measurements were collapsed by maximum value when one gene has replicative measurements.

Differential gene expression analysis for generating meta-signature

We performed differential expression analysis on individual datasets by comparing sarcoidosis vs. normal samples using a linear model-based R package LIMMA (16). Considering limited and incomplete demographic and clinical variables available in public database, we used permutated unwanted variation estimation instead of including available but incomplete variables into the model to test difference between cases and controls. To identify, estimate and remove unwanted sources of variation to compensate for incomplete information of samples provided by public datasets, surrogate variable analysis was applied to each dataset using the "leek" method (19). The estimated surrogate variables were used as covariates in the formula of differential expression analysis. The probes with Benjamini-Hochberg corrected P-value < 0.05, with multiple-testing adjusted, were considered as significant (20). Since inconsistence in terms of study design, cohorts, measurements, etc., meta-analysis was performed with metafor package using residual maximum likelihood (REML) model (21). We performed the analysis in blood sets, lung-associated sets, and all available sets, respectively. Pathway enrichment analysis was further conducted with ClueGO based on GO biological process databases (08/05/2020) (22). For differentially expressed genes (DEGs) in the meta-analysis of blood and lung-associated tissues, significant genes in one meta-analysis but not in the other were identified as tissue-specific. Top genes of these tissue-specific genes were also enriched for biological processes to unveil potential involved pathways of tissue-relevance.

Training and validation of sarcoidosis classifier

To build a diagnostic model for sarcoidosis across tissues, the datasets were further divided into training and testing sets, each containing expression profiles from both blood and lung-associated tissue. Datasets GSE19314, GSE18781, GSE42834, GSE83456, GSE16538, GSE73394, and GSE148036 were used as training set to build the prediction model, while datasets GSE37912, GSE34608, GSE75023, GSE105149, GSE32887, and GSE119136 were included as testing sets. Candidate predictors were filtered by the criteria that the gene should be significantly differentially expressed in more than 3 of the 4 blood sets and in more than 2 of the 3 lung-tissue-associated sets. And those candidates with consistent regulatory directions across the discovery sets will be selected as predictors. The gene with log-transformed fold change (logFC) > 0 is regarded as a positive regulatory factor, and that less than 0 is a negative regulator. Classifier was generated by random forest (RF), Lasso and Elastic-Net Regularized Generalized Linear Models (GLMNET), and Gradient Boosting Machine (GBM) implemented in R package caret. The models were tuned using 10-fold cross-validation. Predictor selection and model training were performed only in training sets, and thus the other test sets could be used as external validation sets. The performance of classifiers was measured using threshold-dependent (sensitivity, specificity, accuracy) and threshold-independent ROC analysis (AUC). The prediction model with the highest performance in the training sets was chosen for assessment of predictive power

1 http://www.ncbi.nlm.nih.gov/geo

in six independent testing datasets. To address the problem of systemic difference between cases and controls, randomly selected genes of identical size were compared to ensure the prediction power of model.

Results

Differential expression analysis and meta-signature identification

In total 317 sarcoidosis patients and 339 healthy controls from the 13 GEO datasets were included in the final meta-analysis (Table 1 and Supplementary Table 1). Sarcoidosis patients included cutaneous, pulmonary, and lacrimal gland involvement. Random-effect models were applied to the 6 blood sets, the 4 lung-associated sets, and all 13 sets combined, respectively, to identify genes associated with sarcoidosis within blood, within lung, and between various tissues. Of the 12,968 genes available in at least 3 blood datasets and 3 lung tissue associated sets, 856 were significant at FDR < 0.05 in meta-analysis of peripheral blood, and 690 were significant in lung associated tissues, while 290 genes were differentially expressed when all 13 datasets from various tissues were combined in the meta-analysis (Figure 2A). Despite elevated significance and robustness of the meta-analysis, only 69 DEGs are commonly observed to be differentially expressed in blood and lung tissue, and 29 of them remain significant when more heterogenous sarcoid tissue are included (Table 2).

Top up-regulated genes in sarcoidosis include interferon signature genes such as *GBP5* and *IFITM1*, indicating active regulation of the interferon signaling. Chemokine genes induced by interferon such as *CXCL10* are also upregulated. Intriguingly, we identified genes significantly associated with sarcoidosis that caught limited attention in previous studies. These genes include the long non-coding gene *LINC01278* and some other genes known to be involved in the interferon network but lacking understanding of their role in pulmonary diseases, such as *IDO1* and *BTN3A2*.

Down-regulated genes that achieved significance in the meta-analysis of blood but failed in lung-associated tissues include *TRABD2A* and *NLRC3*. The former encodes for a metalloprotease and negative regulator or Wnt signaling, while the latter is characterized as a negative regulator of the type I IFN pathway. Up-regulated blood-specific genes include *MYD88*. The gene ranking top in lung-specific DEGs is the lncRNA gene *HCP5* and *IL10RA*. Most of the genes lack sufficient exploration as to their roles in pulmonary sarcoidosis, but more or less are associated with inflammatory pathways potentially of influence in the pathogenesis of the disease.

Pathway enrichment analysis of sarcoidosis signature genes

Pathway enrichment analysis of the top 200 significant genes in meta-analysis revealed particular pathways that may be associated with sarcoidosis in blood and sarcoid tissues. 55 significant biological processes are functionally grouped into 10 critical pathways as shown in Figure 2B, In addition to the well-known IFN-γ response, we found activation of the cytokines IL-1 and IL-18. Host defense response to biotic stimuli, especially virus infection, is also significantly involved.

Top DEGs in meta-analysis of lung-associated tissue unveiled 16 groups of 36 pathways, most of them also observed in cross-tissue analysis (Supplementary Table 2). Although sarcoidosis is widely recognized as a Th1 disease, NK cells may also play a role in the pathogenesis. The

TABLE 1 Datasets used for cross-tissue meta-analysis and sarcoidosis classifier development.

GEO dataset	Platform	Tissue	Control samples	Sarcoid samples	ID
GSE19314	Affy U133 Plus 2.0 Array	Blood	20	38	Train1
GSE18781	Affy U133 Plus 2.0 Array	Blood	25	12	Train2
GSE42834	Illumina beadchip	Blood	113	61	Train3
GSE83456	Illumina beadchip	Blood	61	49	Train4
GSE16538	Affy U133 Plus 2.0 Array	Lung	6	6	Train5
GSE73394	Affy Gene 1.0 ST array	BAL	20	26	Train6
GSE148036	Illumina HiSeq 3000	Lung	5	5	Train7
GSE37912	Affy Exon 1.0 ST array	Blood	35	39	Test1
GSE34608	Agilent microarray	Blood	18	18	Test2
GSE75023	Affy U133A 2.0 array	AM	12	15	Test3
GSE105149	Affy U133 plus 2.0 array	Lacrimal gland	7	8	Test4
GSE32887	Affy U133 plus 2.0 array	Skin	5	26	Test5
GSE119136	Affy gene 1.0 ST array	Nasal brushing	12	14	Test6

Affy, Affymetrix Human Genome; AM, alveolar macrophage; Illumina beadchip, Illumina HumanHT-12 V4.0 expression beadchip; Agilent Microarray, Agilent-014850 Whole Human Genome Microarray 4 × 44K G4112F.

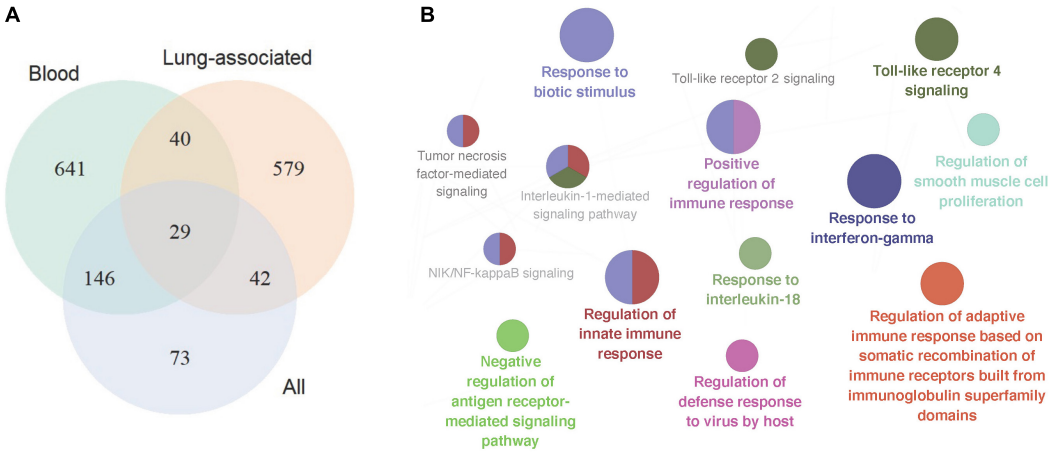

FIGURE 2
Signature gene identification in blood, lung-associated tissues, and all available tissue types. **(A)** Venn plot depicted differentially expressed genes in the three sets of meta-analyses, in peripheral blood samples, lung-associated tissues, and in all 13 cross-tissue samples combined, respectively. **(B)** Pathway enrichment analysis of the top signature genes in the meta-analysis across various tissues. The size of the nodes reflects the enrichment significance of the terms, and the colors denote groups of biological process.

biological process of cell-cell adhesion mediated by integrin is also significantly enriched in lung-associated tissues. With deepened understanding of integrin functions such as roles in cell survival, migration, and proliferation, potentials as therapeutic targets especially in respiratory disease may be exhibited. Pathway enrichment analysis in top genes of peripheral blood showed limited results with 18 biological processes divided into 4 groups, including mitosis, mRNA stabilization, IFN-γ signaling, and viral infection response (**Supplementary Table 3**). Unsurprisingly, interferon-gamma-mediated signaling pathways are consistently involved in all three meta-analyses.

Biological processes enriched in tissue-specific differentially expressed genes in blood and lung

To identify tissue-specific sarcoidosis signature in peripheral blood and sarcoid lung tissue, we further performed pathway enrichment based on the top 200 genes ranked by *P*-value selected from those DEGs that achieved significance in blood but not in lung-associated tissues or vice versa (**Figure 3**). Positive regulation of interleukin-17 (IL-17) production was significantly involved in blood-specific DEGs, concordant with previous understanding of this critical signaling pathway. Activation of autophagy was significantly enriched in both blood and lung-specific genes. The gene AIM2, functioning as a key factor of pyroptosis, was among the identified signatures for sarcoidosis, further emphasizing the role of cell death in sarcoidosis. NK cell mediated immune response to tumor cells was evident in DEGs in lung-associated tissues.

Predictor selection and classifier establishment

Sixteen genes met the requirement in the training sets based on the variable selection criteria with consistent directions of fold change across all discovery sets (n = 447), indicating robust dysregulation across datasets and thus were used for training of classifier (**Figure 4A**). All of these 16 genes function as protein-coding genes, and 8 were up-regulated while the other half were down-regulated. GBM and RF outperformed GLMNET in respect of both AUC and accuracy (GBM: AUC = 0.985, Accuracy = 0.937; RF: AUC = 0.998, Accuracy = 0.978; GLMNET: AUC = 0.949, Accuracy = 0.895) (**Figure 4B** and **Supplementary Figure 1**). The importance of each gene as a predictor variable was evaluated in the model (**Figure 4C**). *STAT1* provides the heaviest weight in all three models amongst the 16 predictors. Other predictors making major contributions include *TMEM140*, *AQP3*, and *SOD2*.

The independent performance of GBM and RF classifiers was evaluated in the 6 external testing sets (n = 209). The GBM model performed more reliable than the RF model in both blood and sarcoid tissues (AUC 0.711–0.964), possibly owing to problem of over-fitting in RF model despite cross-validation (**Figure 4D** and **Supplementary Table 4**). The lacrimal tissue set achieved the best AUC with 0.964 (accuracy = 0.933). We also randomly selected gene sets of identical size from the candidates that were available across all 13 datasets. With identical training and testing parameters, the model built from 16 randomly selected genes achieved an AUC of 0.464, 0.596, and 0.528 using the algorithms of GLMNET, GBM, and RF, respectively

TABLE 2 Triple-significant differentially expressed genes (DEGs) in cross-tissue, blood, and lung-associated tissue meta-analyses.

Gene	Cross-tissue		Blood		Lung		Significance label*
	logFC	P-adjust	logFC	P-adjust	logFC	P-adjust	
LINC01278	−0.38	3.11×10^{-13}	−0.35	8.72×10^{-06}	−0.32	2.65×10^{-03}	//++/?/??/++?
GBP5	1.55	5.56×10^{-07}	1.47	4.58×10^{-03}	1.57	3.21×10^{-07}	+++++++/+?+//
PSMB9	0.55	1.11×10^{-06}	0.45	3.80×10^{-03}	0.56	3.18×10^{-15}	++++++//++++/
TAP2	0.60	1.35×10^{-06}	0.44	1.20×10^{-03}	0.74	8.43×10^{-06}	//++++//++++/
PSTPIP2	0.86	2.11×10^{-06}	0.78	1.31×10^{-02}	1.14	9.65×10^{-22}	++++/±+++//
CCNB1	0.20	2.39×10^{-06}	0.17	1.35×10^{-05}	0.28	1.75×10^{-03}	/////+/+//+//
HDAC4	0.13	1.69×10^{-05}	0.16	6.52×10^{-03}	0.13	1.90×10^{-02}	//+//+///////
SQOR	0.48	5.99×10^{-05}	0.54	2.11×10^{-05}	0.34	5.85×10^{-09}	++++/+??++++/
FKBP14	−0.22	8.74×10^{-05}	−0.22	1.76×10^{-02}	−0.24	8.38×10^{-05}	/+///+//++///
LINC00667	−0.33	9.40×10^{-05}	−0.37	1.96×10^{-03}	−0.38	4.60×10^{-03}	/+++/?/??///?
CD38	0.56	1.01×10^{-04}	0.51	3.50×10^{-02}	0.70	1.48×10^{-02}	//++/+//+++//
TAP1	0.78	1.13×10^{-04}	0.54	1.50×10^{-02}	0.96	1.77×10^{-05}	+++++?//++++?
IFITM1	0.49	1.77×10^{-04}	0.38	1.25×10^{-02}	0.77	1.80×10^{-03}	//++/+/++++//
PSME2	0.42	2.67×10^{-04}	0.46	7.30×10^{-03}	0.31	8.73×10^{-09}	/+++++///+++/
THOC5	0.13	3.09×10^{-04}	0.16	3.60×10^{-03}	0.14	5.61×10^{-04}	///+/+///+///
ANKRD22	1.45	5.61×10^{-04}	1.72	2.36×10^{-03}	1.44	1.63×10^{-04}	+++++++/+?+//
CXCL10	1.24	5.61×10^{-04}	0.99	1.79×10^{-02}	1.34	1.17×10^{-06}	+++++++/+++//
IDO1	1.09	6.24×10^{-04}	1.14	1.52×10^{-02}	0.93	3.24×10^{-02}	/+++++//+++//
KCNJ2	0.64	9.30×10^{-04}	0.68	7.96×10^{-03}	0.79	7.32×10^{-03}	+++++///+++//
CD300A	0.41	1.06×10^{-03}	0.26	4.99×10^{-02}	0.59	2.01×10^{-03}	/+++/++/+++//
ETV7	0.55	1.35×10^{-03}	0.79	8.78×10^{-03}	0.25	1.44×10^{-06}	++++/+//++/+/
BTN3A2	0.35	1.58×10^{-03}	0.20	1.61×10^{-02}	0.47	2.89×10^{-07}	//+/++///++//
BLOC1S1	0.20	1.85×10^{-03}	0.25	3.72×10^{-02}	0.17	2.79×10^{-02}	//+/////+////
RAB32	0.25	2.58×10^{-03}	0.26	1.67×10^{-02}	0.15	3.22×10^{-02}	//++////+/++/
KIF1B	0.19	7.84×10^{-03}	0.31	4.11×10^{-14}	0.17	2.10×10^{-02}	//+//+//+////
TPX2	0.22	1.49×10^{-02}	0.14	2.82×10^{-02}	0.34	2.73×10^{-02}	//++?/////+//
RNASE6	0.57	1.61×10^{-02}	0.26	2.82×10^{-02}	0.97	8.30×10^{-04}	//++/+//+++//
TSEN2	−0.29	1.70×10^{-02}	−0.39	1.94×10^{-02}	−0.36	1.14×10^{-02}	+++++//-++///
ENTPD1	0.35	2.69×10^{-02}	0.36	1.83×10^{-08}	0.46	1.44×10^{-02}	+/+++//??++//

LogFC, log-transformed fold change; P-adjust, FDR-adjusted *P*-value; *: label of significance in each of the 13 GEO dataset, consistent with the order listed in Table 1.+: *P*-value < 0.05, fold change direction consistent with cross-tissue meta-analysis; −: *P*-value < 0.05, fold change direction contradictory with cross-tissue meta-analysis;/: *P*-value ≥ 0.05; ?: not available.

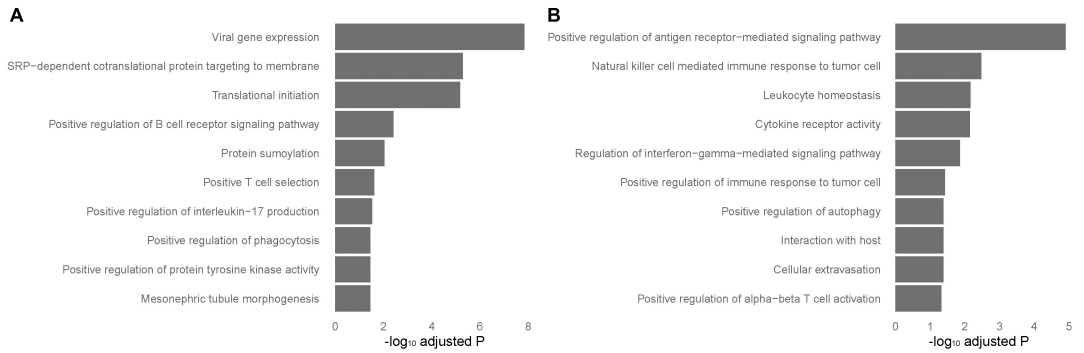

FIGURE 3
Tissue-specific pathways enriched in blood and lung-associated tissues. Enriched pathways specific to peripheral blood and lung-associated tissues. The analysis was performed based on the top differentially expressed genes specific to peripheral blood **(A)** and lung-associated tissues **(B)** identified in the results of meta-analyses.

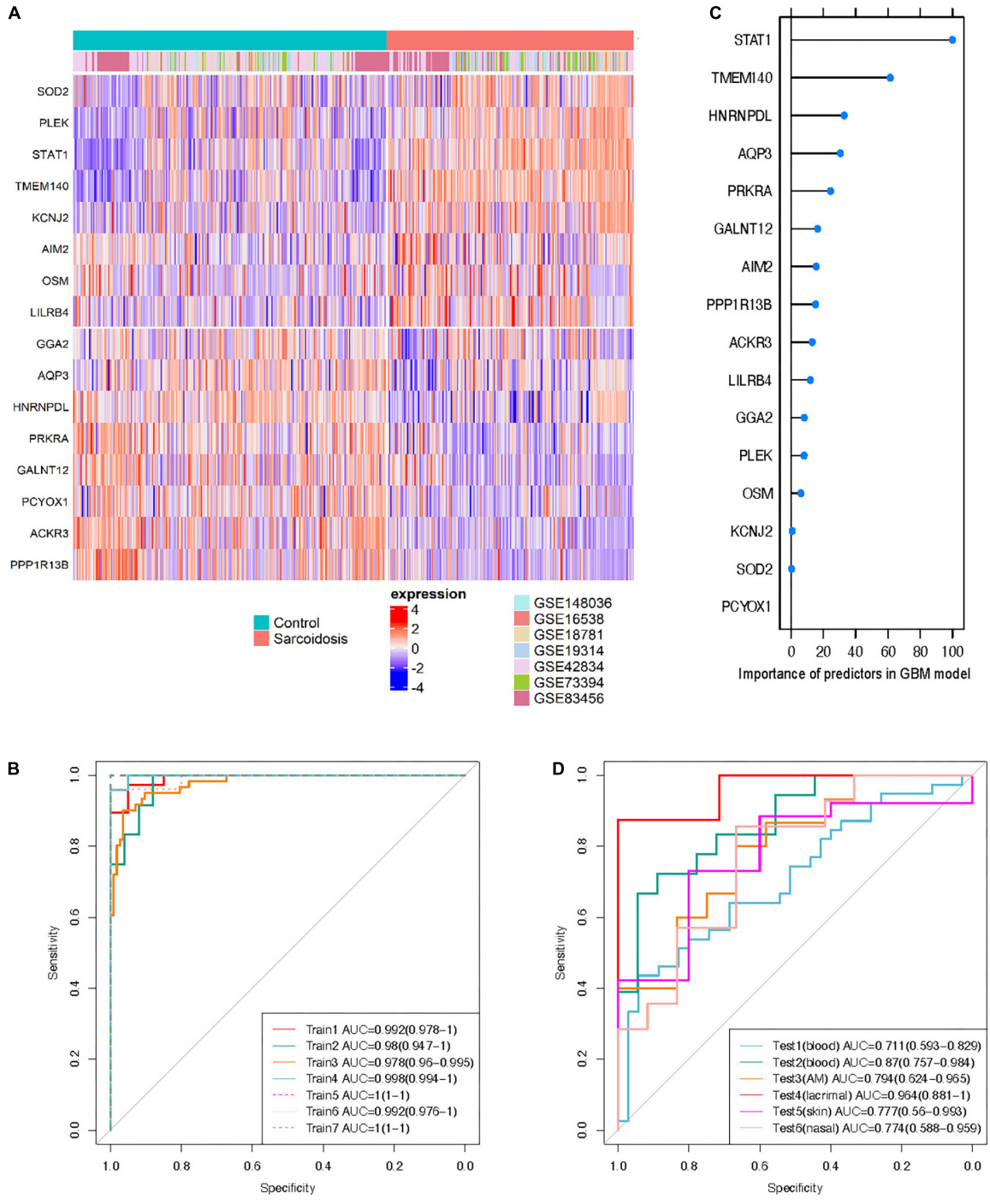

FIGURE 4
Cross-tissue classification model for sarcoidosis. **(A)** Heatmap shows expression profile of the 16 predictors in training sets. Half of the predictors were up-regulated while the others were down-regulated. **(B)** Importance of the predictors in GBM model. ROC curve of the classifier was shown in training sets **(C)**, and test sets **(D)**.

(**Supplementary Figure 1**), further emphasizing that the performance of the 16-gene set can be attributed to the biological significance of these consistently differentiated genes.

Discussion

A meta-analysis of transcriptome-wide association studies was conducted between sarcoidosis and normal subjects across

tissues by means of mining public datasets. We identified robust and novel genes potentially associated with sarcoidosis. A disease prediction classifier was subsequently built using machine learning and validated in independent datasets to discover candidate cross-tissue sarcoidosis biomarkers.

The expression profile of 29 genes was significantly associated with sarcoidosis the in meta-analysis of blood, lung, and cross-tissue datasets. Intriguingly, the long intergenic non-protein coding RNA *LINC01278* was found to be down-regulated in sarcoid tissues. *LINC01278* has been proposed to negatively regulate accumulation of β-catenin and ultimately inhibit the transcription of downstream target genes activated by Wnt/β-catenin signaling (23). Evidence of increased pulmonary Wnt-activation has been reported in sarcoidosis, potentially regulating myofibroblast differentiation of lung resident mesenchymal stem cells (24). Although *LINC01278* had never been observed to play an explicit role in sarcoidosis, it might contribute to the disease in critical biological pathways.

The role of the MHC genes in presenting antigen and triggering activation of T cells makes them good candidates for involvement in sarcoidosis. *PSMB9*, a gene downstream of *STAT1*, which is known to integrate with *IFNG* and to play a proteolytic role in MHC1 antigen presentation, has been reported to be upregulated in sarcoidosis (25). Non-MHC genes, *TAP1,* and *TAP2*, encoding the transporter associated with antigen processing, which participate in the antigen processing pathways prior to its presentation, are also interesting candidates and have been observed to be upregulated in sarcoidosis. A polymorphism of *TAP2* detected in patients with sarcoidosis further validated this point (26).

Multifunctional membrane surface glycoprotein (*CD38*) is considered as a marker of immune activation and involved in the regulation of lymphocyte adhesion to endothelial cells. Both $CD3^{+}CD4^{+}CD38^{+}$ and CD38+ B cell subsets were found to be elevated in BAL as markers of an acute immune response in sarcoidosis patients (27, 28). In addition to lymphocytes, *PSTPIP2*, a gene supposed to be associated with autoinflammatory processes of macrophages in a mouse model, was found to be upregulated in progressive fibrotic pulmonary sarcoidosis (12).

Aberrant HDAC enzyme activities are evident in fibrotic diseases, of which *HDAC4* is important in lung fibrosis by modulating the production of ECM in lung myofibroblasts (29). Although widely accepted as a key factor in IPF, no HDAC inhibitors (HDACIs) have been investigated in sarcoidosis. Some dysregulated genes discovered in our meta-analysis have not been found to be associated with sarcoidosis previously, but variations of these genes such as *CCNB1*, *BLOC1S1,* and *KIF1B* are associated to some extent with fibrotic diseases, including complication of sarcoidosis and tuberculosis.

Enrichment of biological processes was performed in the top 200 genes ranked by *P*-value in the meta-analysis of blood, lung, and all datasets, respectively. Biological regulation of NK cells and myeloid cells seem to play a role in sarcoidosis. Increased cells of NK lineage were observed in our single-cell dataset of BAL. It is known that a subpopulation of CD56+ NK cells is activated and produces IFN-γ and TNF-α in sarcoidosis patients, implying involvement of these cells in granuloma formation (30). A strong Th2-M2 polarization was identified in both pulmonary and muscular sarcoidosis (31). Biological processes enriched from blood-specific DEGs revealed positive regulation of IL-17 and tyrosine kinase activity, while both blood and lung-specific genes showed activation of autophagy. Lung-specific genes otherwise were enriched for response of tumor, especially mediated by NK cells. The occurrence of a sarcoid-like localized or distant granulomatous reaction in cancer has been widely realized. In fact, sarcoidosis can occur before, during, or after the onset of solid or hematological malignancies.

To ensure the independence between training and testing sets, predictors of the cross-tissue classifier were selected based on the 7 training sets, and thus moderately different from DEGs in our meta-analysis. Interestingly, *KCNJ2*, one of the identified cross-tissue signature genes, is included as a predictor, which is activated in IPF but lacks exploration in sarcoidosis (32).

In the past few years, multiple prediction models based on transcriptomic signature have been developed in order to assist in the diagnosis of sarcoidosis, but none of them is currently used in clinical management (13, 33, 34). Intriguingly, microRNAs are frequently used in such models and perform well in diagnosis. Current models are built based on gene signature in peripheral blood or PBMC. The linear signature score is the most commonly used method to build classification models by assigning weights to selected gene markers. Two 8-microRNA diagnostic models achieved accuracy of 0.86 and 1, respectively in development datasets, suggesting that microRNAs might act as a crucial regulator in the pathogenesis (33, 34). The other two models were built using 20 and 17 genes, while long non-coding RNA genes were repeatedly identified as predictors (13, 34). *STAT4* and other factors of interferon signaling, as well as cytokine-related genes like *IL6ST* are major gene markers. These two models also performed well with an accuracy of 0.86 and AUC of 0.87, respectively. Compared with these diagnostic models, our classifier is the first cross-tissue model to predict diagnosis of sarcoidosis, indicating a scheme of systematic transcriptomic alteration over the body. However, whether the diagnostic model based on transcriptome can get universally applied assisting clinical decision still need further studies to validate.

We have to acknowledge some limitations of this study. First, meta-analysis suffers from inherent statistical limitations

since the datasets lack concordance being derived from different batches, techniques, and platforms, although surrogate variables were estimated in our study. Also, the definitions of sarcoidosis and controls are slightly different among the datasets, but largely consistent and broadly acceptable. Second, whereas sarcoidosis is a heterogenous and complicated disease, clinical characteristics and disease status were not provided in most public datasets, limiting the clinical interpretation of significant genes. Third, particularly differentially expressed genes were not validated biologically in our study. Further experiments are needed to identify exact role they play in sarcoidosis. Lastly, our classifier tested only sarcoidosis vs. "healthy" controls but not vs. other granulomatous or interstitial lung diseases. The diagnostic power of this classifier to discriminate sarcoidosis from other diseases remains to be investigated.

This transcriptomics-based meta-analysis identified gene expression profiles and shared pathways associated with sarcoidosis across various tissues. This allowed to construct a 16-gene diagnostic classifier for sarcoidosis that potentially can complement more invasive procedures. Its precise diagnostic power needs to be validated in more abundant datasets of various tissues also from patients with other diseases.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary material**.

Ethics statement

This was a meta-analysis using previously publicated data on public database. The used data were approved previously individually. The patients/participants provided their written informed consent to participate in this study.

Author contributions

HD and CW contributed to conception and design of the study. YJ conducted the analysis. YJ, DJ, and HD drafted the manuscript. UC contributed to the revision of the manuscripts. All authors contributed to manuscript revision and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementarymaterial

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.960266/full#supplementary-material

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Sarcoidosis: Updates on therapeutic drug trials and novel treatment approaches

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Sarcoidosis is a systemic granulomatous inflammatory disease of unknown etiology. It affects the lungs in over 90% of patients yet extra-pulmonary and multi-organ involvement is common. Spontaneous remission of disease occurs commonly, nonetheless, over 50% of patients will require treatment and up to 30% of patients will develop a chronic progressive non-remitting disease with marked pulmonary fibrosis leading to significant morbidity and death. Guidelines outlining an immunosuppressive treatment approach to sarcoidosis were recently published, however, the strength of evidence behind many of the guideline recommended drugs is weak. None of the drugs currently used for the treatment of sarcoidosis have been rigorously studied and prescription of these drugs is often based on off-label" indications informed by experience with other diseases. Indeed, only two medications [prednisone and repository corticotropin (RCI) injection] currently used in the treatment of sarcoidosis are approved by the United States Food and Drug Administration. This situation results in significant reimbursement challenges especially for the more advanced (and often more effective) drugs that are favored for severe and refractory forms of disease causing an over-reliance on corticosteroids known to be associated with significant dose and duration dependent toxicities. This past decade has seen a renewed interest in developing new drugs and exploring novel therapeutic pathways for the treatment of sarcoidosis. Several of these trials are active randomized controlled trials (RCTs) designed to recruit relatively large numbers of patients with a goal to determine the safety, efficacy, and tolerability of these new molecules and therapeutic approaches. While it is an exciting time, it is also necessary to exercise caution. Resources including research dollars and most importantly, patient populations available for trials are limited and thus necessitate that several of the challenges facing drug trials and drug

development in sarcoidosis are addressed. This will ensure that currently available resources are judiciously utilized. Our paper reviews the ongoing and anticipated drug trials in sarcoidosis and addresses the challenges facing these and future trials. We also review several recently completed trials and draw lessons that should be applied in future.

KEYWORDS

pulmonary sarcoidosis, progressive pulmonary fibrosis, fibrotic pulmonary sarcoidosis, patient centered and patient partners in research, interstitial lung disease, therapeutic pathways, clinical trials and clinical trial design, novel therapies

Introduction

Sarcoidosis is a systemic inflammatory disease of unknown etiology characterized by the presence of non-caseating granulomas in affected organs (1). The lungs are affected in over 90% of patients yet extra-pulmonary and multi-organ involvement occurs commonly (2, 3). The clinical presentation, disease course, and severity of sarcoidosis is highly variable, impacting treatment, prognosis, and patient outcomes (1, 4, 5). A good proportion of patients will have spontaneous disease remission, up to 50% of patients will require treatment, and 10–30% of patients will develop a chronic unremitting disease with in some cases marked pulmonary fibrosis and varying degrees of respiratory failure (5–9). Approximately 5% of patients with sarcoidosis die from their disease with higher mortality reported in the population of patients with respiratory failure, fibrotic pulmonary disease, pulmonary hypertension, and cardiac sarcoidosis (CS) (10–16).

None of the drugs currently used for sarcoidosis treatment have been rigorously studied in large randomized controlled trials (RCTs) (5, 17). Most drugs used in sarcoidosis treatment are prescribed on "off-label" indications informed by experience with other diseases. Indeed, sarcoidosis treatment is based on results from trials whose design and methods suffer from inherent trial design flaws of rarer conditions including sample size, selection for active disease, and clinically meaningful endpoints that include validated patient-reported outcome measures (PROMs). Where large studies exist, they have been focused on pulmonary sarcoidosis to the neglect of other organ-threatening extra-pulmonary disease manifestations (17). This presents several challenges to drug acquisition especially for the more advanced drugs that are favored for severe/refractory forms of sarcoidosis (5, 18, 19) and are frequently denied by reimbursement agencies because sarcoidosis is not listed as an FDA approved indication for use. The reasons for this are many. First, sarcoidosis is considered a rare disease with relatively few people affected and even fewer (50–80% of those affected) potentially needing treatment (3, 8, 20). This impacts disease awareness, research funding and severely strains the pool of patients eligible for clinical trials especially in non-pulmonary disease manifestations. Secondly, there is no widely accepted biological model of disease thus limiting the scope and rate of pre-clinical drug development. Thirdly, sarcoidosis is a very heterogenous disease with a highly variable disease course and a lack/scarcity of available validated active disease measures. Therefore, great challenges in sarcoidosis trial design are to adequately define a target study group and the availability of standardized outcome measures that accurately measure disease responsiveness while maintaining a patient-centered focus (5, 21, 22).

The recently published European Respiratory Society (ERS) clinical practice guidelines help to address these concerns by outlining tentative treatment approaches for various organ manifestations of sarcoidosis (5). Very importantly, the guidelines reaffirmed two major reasons to initiate treatment in sarcoidosis patients: to lower the morbidity and mortality risk associated with sarcoidosis or to improve quality of life (QoL) largely related to symptom burden and decline in physical function due to disease (HRQoL) (5). Although a major step in the right direction, the ERS guidelines were developed as a general guidance in response to presumed historical clinical practice and all 12 treatment recommendations were associated with a level of evidence deemed very low to low quality (5). Furthermore, the guidelines do not address all the concerns surrounding medication prescription, and do not eliminate the barriers surrounding medication acquisition. The guidelines also do not address an increasingly common practice of "hit hard and early" whereby more and more sarcoidosis physicians are combining steroids and steroid-sparing medications up front in severe manifestations of disease (5).

Current advancements in personalized and precision medicine as well as the introduction of compounds developed specifically for sarcoidosis treatment, underscore the imperative of standardized elements of clinical trial design in either collective or organ-specific sarcoidosis. Medications used in sarcoidosis warrant rigorous, methodical studies targeted to the patients for whom their use is intended. Nearly 50% of patients requiring therapy for severe forms of sarcoidosis may experience

a therapeutic failure (toxicity, intolerability, or inefficacy) (23), making it crucial that a pipeline of rigorously evaluated drugs can continue to be deployed.

This manuscript will review the current and anticipated drug trials in sarcoidosis with a focus on studies evaluating novel molecules and novel therapeutic pathways. Trials advocating for a "hit hard and early approach" (24) and re-evaluating the current paradigm of "prednisone first followed by stepwise addition of steroid sparing agents" (25) will also be discussed. We will highlight several challenges that affect future and ongoing sarcoidosis drug trials and offer potential solutions to the most pressing needs. It is hoped that this manuscript will appeal to a wide readership audience that includes clinicians caring for patients, researchers, regulators, pharmaceutical industries sponsoring drug trials, and patients for whom these drugs are intended.

Brief review of the currently available medications and treatment considerations in sarcoidosis

Current landscape of systemic treatment

The treatment of sarcoidosis is not clear cut and demands rigorous ongoing attention. As noted above, the ERS guidelines reaffirmed two major reasons to initiate treatment (5). Unfortunately, the morbidity and mortality risk, and HRQoL impact associated with disease vary from one organ manifestation to another, and perhaps from one patient to another. Consequently, though well intended, the specifics of these concepts are subject to interpretation. Furthermore, there remains an inconsistency in the risk parameters and PROMs used to quantify these concepts (26). For patients with pulmonary sarcoidosis, initiation of systemic treatment is reserved for patients who communicate symptomatic disease impacting HRQoL (4, 5); and/or whether the patient's disease can lead to progressive lung function decline or significant morbidity or mortality (4, 5, 27). For patients with extra-pulmonary involvement, the decision to treat is similarly dependent on the presence of clinically significant disease activity in the affected organ (presumed to impair HRQoL and/or threaten organ function) and is left to the clinicians judgment (5). Presence of clinically significant cardiac, neurologic, ocular, or renal involvement is often associated with significant morbidity and mortality, and treatment is usually indicated (5).

Although the guidelines and a previously published consensus study advocate for early introduction of steroid-sparing agents, there is no definition of how "early" these can be added (5, 7). Therefore, the treatment of sarcoidosis is fraught with myriad complexities along with diversity of comfort with the use of steroid-sparing immunosuppression which acculturates prescribing habits and necessitates shared decision-making (SDM) (28).

Corticosteroids have historically been the prototypical treatment in sarcoidosis, yet they are associated with a reduced HRQoL and significantly high morbidity and organ-threatening toxicities that are dependent on dose, duration and in some cases, genetic make-up (29–37). Patients with sarcoidosis on prolonged or high-dose steroids are more likely to be obese or overweight and have several endocrinological and cardiovascular adverse events (30, 32, 33). As prolonged use of corticosteroids is associated with significant organ-based and systemic toxicities without commensurable benefit to improved lung function (29–35), a dynamic treatment trial of corticosteroids for 3–6 months with proactive dose reduction to the minimal effective dose [of which the goal is < 10 mg daily (38, 39)] was mentioned as an acceptable rationale to limit the continuation and overuse of corticosteroids (5). It is hoped that this will ensure ongoing clinical evaluation to discriminate for the need to introduce alternate steroid-sparing therapy. Lack of vigilance of glucocorticoid use is common and is reported by both patients and researchers to be associated with adverse outcomes especially in non-whites and those of lower socioeconomic status (28, 40–45). In some sarcoidosis centers glucocorticoids are used as a bridge with concomitant weaning until steroid-sparing agents reach efficacious doses (28).

Approaches to systemic treatment

The ERS guidelines and a recently published Delphi consensus statement from a large group of worldwide sarcoidosis experts advocate an approach to therapy that balances the use of reduced doses of corticosteroids with the (early) stepwise addition of steroid sparing anti-inflammatory non-biologic and biologic agents (5, 7).

The proposed approach to corticosteroid use is to limit continuation to a 3–6 month period to allow for demonstration of therapeutic response (7). During that time period, attempts are made to taper to the minimal effective dose with a goal maintenance dose of < 10 mg/day of prednisone/prednisone equivalent (7). If the patient's disease remains uncontrolled on minimal steroid doses or significant steroid side effects develop, therapy is then stepped up to steroid-sparing non-biologic immunosuppressive therapy (IST) with further attempts made to wean corticosteroids to a prednisone equivalent dose of < 10 mg/day (5, 7). The guidelines make for early/concomitant initiation of steroid-sparing non-biologic agents (so-called "second-line agents") for patients with CS or other forms of severe or multi-organ disease where prolonged therapy is anticipated, or where there is a high risk of

steroid-induced toxicity (5, 7). For patients with symptomatic pulmonary sarcoidosis believed to be at higher risk of future mortality or permanent disability from sarcoidosis who have been treated with glucocorticoids and have continued disease or unacceptable side effects from glucocorticoids, the guidelines recommend the addition of methotrexate (5). Methotrexate is considered the preferred "second-line agent" with the most data supporting its use in sarcoidosis (5, 7, 46–48). Other commonly used "second-line agents" include: Azathioprine, Leflunomide, and Mycophenolate Mofetil, however, the evidence behind these latter medication recommendations is very weak (5).

For patients with symptomatic pulmonary sarcoidosis believed to be at higher risk of future mortality or permanent disability from sarcoidosis who have been treated with glucocorticoids or other IST and have continued disease, the guidelines suggest the addition of infliximab to improve and/or preserve lung function and HRQoL (5). This was a conditional recommendation with overall low quality of evidence (5). Infliximab is a tumor necrosis factor inhibitor (TNFi) that has been shown to be effective in severe and refractory forms of pulmonary (49–62) and extra-pulmonary sarcoidosis (49, 63). TNFi (Infliximab and Adalimumab) have historically been regarded as "third-line agents" to be added in patients whose disease is uncontrolled on (or who develop significant toxicity to) "second-line therapy" (5, 7, 64). Infliximab has the most data supporting its use in sarcoidosis and is the preferred and more commonly prescribed "third-line agent" (5, 7, 64). Other advanced immunomodulating steroid-sparing agents suggested for use in patients with advanced and refractory disease include rituximab and Repository Corticotropin (RCI) (5). Practical suggestions and experience based recommendations on the use and management of TNFi in sarcoidosis have been published (64). The current widely accepted stepwise medications used in sarcoidosis are listed in **Table 1** and the stepwise approach to treatment in pulmonary sarcoidosis is shown in **Figure 1**. Treatment algorithms specific to other organ manifestations have been published (5).

Non-pharmacological therapies and palliation of symptoms

The main focus of this paper is the implementation and investigation of pharmacologic systemic anti-inflammatory therapy, yet it must be noted that there are various non-systemic and non-pharmacologic therapies that have shown benefit in palliating symptoms. The intended use of systemic anti-inflammatory therapy is to halt or reverse active or partially active sarcoidosis along with remission of associated symptoms and physical impairment. Symptoms and physical impairment arising solely from irreversible tissue damage resulting from previously active disease is usually not amenable to anti-inflammatory treatment. Innumerable combinations of both pharmacological and non-pharmacological palliative measures exist to augment HRQoL by ameliorating organ-specific symptoms and physical impairment related to irreversible tissue damage (28). These include psychological, nutritional, strategic coping, mindfulness, physical/respiratory/occupational therapy, and possibly supportive pharmacology such as mucolytics, inhalers, anti-emetics etc. (28). Exercise and physical training are areas that are gaining momentum as systemic non-pharmacological treatment in inflammatory diseases and should be leveraged more frequently in sarcoidosis (65–69). Physical training/exercise is arguably both a systemic treatment that can modulate inflammation and a non-pharmacological therapy that cultivates physical capacity through amplification of neuromuscular and vascular networks and other bio-mechanical pathways that reduce symptom burden regardless of sarcoidosis disease activity status (67, 69–71).

Treatment failure in sarcoidosis

Systemic medications offer hope for reversing the progression of moderate to severe disease activity and if successful provide the opportunity to re-gain global function as close to a person's baseline as possible. As stated above, the systemic treatment armamentarium in sarcoidosis is limited, while the likelihood of treatment failure is reported to be fairly high (23). It is crucial to understand what constitutes treatment failure, the types of treatment failure, and whether the "failure" is salvageable. At the heart of treatment success and adherence, is SDM (72). SDM conveys knowledge that ties disease behavior to anticipated expectations of efficacy and considers side effects, potential toxicity and how toxicities are avoided (72, 73). SDM assesses and discusses a person's treatment priorities, expectations and desires and has been shown to potentially influence response to therapy (74). Being a powerful component of clinical management, national cost-free training protocols on comprehensive SDM skill development for clinicians are becoming increasingly accessible (75, 76).

Commonly, treatment failure is interpreted as a drug being unsuccessful in inducing disease resolution or eliciting disease control. While this is true, there are other causes and, additionally, varying shades of lack of therapeutic responsiveness which must be recognized in order to preserve the use of an effective or partially effective drug. Firstly, an absolute lack of treatment responsiveness must prompt consideration of either wrong diagnosis, inactive sarcoidosis with high damage burden or medication non-adherence (requiring exploration through SDM). If active sarcoidosis is confirmed, there may be genetic

TABLE 1 Current widely accepted stepwise medications for the treatment of Sarcoidosis (5, 7, 48, 64).

Historic designation	Drug name	Usual dosage	Major toxicity	Drug monitoring	Comments
"First-Line"	Prednisone/ Prednisolone	20 mg/day initial dose, tapered to 5–10 mg QD to QoD	Weight gain, Diabetes Mellitus, Hypertension, Osteoporosis, Cataracts, Glaucoma, Sleep disturbance, Depression	Blood pressure and serum glucose monitoring, Bone density, Eye exams Body mass index	Causes cumulative toxicity that is dose and duration dependent.
"Second-Line" (Anti-metabolites)	Methotrexate	10–15 mg once a week PO Maybe given SQ if severe GI intolerance	GI Intolerance, Hepatotoxicity, Leukopenia, Fatigue, Pneumonitis.	CBC, LFT, renal function Folate supplementation is recommended.	Preferred anti-metabolite Teratogenic; avoid in pregnancy in both males and females of child-bearing age. Cleared by kidney, avoid in significant renal failure. Doses < 15 mg/week associated with inefficacy.
	Azathioprine	50–250 mg QD	Nausea, Leukopenia, Hepatotoxicity, Risk of Infections, Cutaneous and Lymphoproliferative Cancers.	CBC, LFT	Consider check TPMT level at initiation
	Leflunomide	10–20 mg QD	Nausea, Leukopenia, Hepatotoxicity, Peripheral Neuropathy, Pneumonitis	CBC, LFT, renal function	Due to long half-life, cholestyramine may be necessary to clear drug and its metabolites in toxicity. Teratogenic, avoid in pregnancy and breastfeeding. Cleared by kidney, avoid in significant renal failure
	Mycophenolate Mofetil	500–1,500 mg BID	Diarrhea, Leukopenia, risk of infections, Lymphoproliferative, and Cutaneous cancers	CBC, LFT Negative hepatitis B/C screening and negative IGRA are required prior to initiation	Less experience in sarcoidosis than other agents. Non-nephrotoxic
"Third-Line" Reserved for patients who have failed prior treatment with steroids and/or anti-metabolites	Infliximab or Biosimilars	3–5 mg/Kg IV at weeks 0, 2 and every 4–6weeks	Infections, allergic reactions. Contraindicated in demyelinating neurologic disease, active tuberculosis, deep fungal infections, prior malignancy, and severe CHF	Monitor for allergic reactions Screen for prior tuberculosis (negative IGRA testing) prior to initiation. Negative hepatitis B/C screening also advised.	Allergic reactions can be life threatening. Consider co-administration with Methotrexate to minimize formation of anti-drug antibodies.
	Adalimumab	40 mg SQ every 1–2 weeks	Infections, Allergic reactions Contraindicated in demyelinating neurologic disease, active tuberculosis, deep fungal infections, prior malignancy, and severe CHF	Monitor for allergic reactions Screen for prior tuberculosis (negative IGRA testing) prior to initiation. Negative hepatitis B/C screening also advised.	Less toxic than infliximab. Has been successfully used in patient's intolerant to infliximab.
	Rituximab	500–1,000 mg IV every 1–6 months	Infections	Screen for viral hepatitis. Check IgG level with chronic therapy	High risk for viral reactivation. Can lead to IgG deficiency.

(*Continued*)

TABLE 1 (Continued)

Historic designation	Drug name	Usual dosage	Major toxicity	Drug monitoring	Comments
	Repository corticotropin Injection (RCI)	40–80 Units SQ twice a week	Diabetes Mellitus, Hypertension, Anxiety, Edema, Weight gain, Cataracts, Glaucoma, Sleep Disturbance.	Blood pressure and serum glucose monitoring, Bone density, Eye Exams Body Mass Index	Need to wean prednisone quickly to avoid cumulative toxicity.
Others	Hydroxychloroquine	200–400 mg QD	Loss of vision GI side effects,—abdominal pain, anorexia.	Regular eye exams depending on age and renal function	Beneficial for cutaneous disease. Minimal impact in cardiac and neurologic disease.

CBC, complete blood count; LFT, liver function test; IGRA, interferon gamma release assay for tuberculosis; PO, per oral; SQ, subcutaneously; IV, intravenously; QD, daily; QoD, every other day; TPMT, thiopurine S-methyltransferase (TPMT) genotype or enzyme activity; IgG, Immunoglobulin G; GI, Gastrointestinal (Intolerance, Nausea, vomiting, diarrhea); CHF, congestive heart failure.

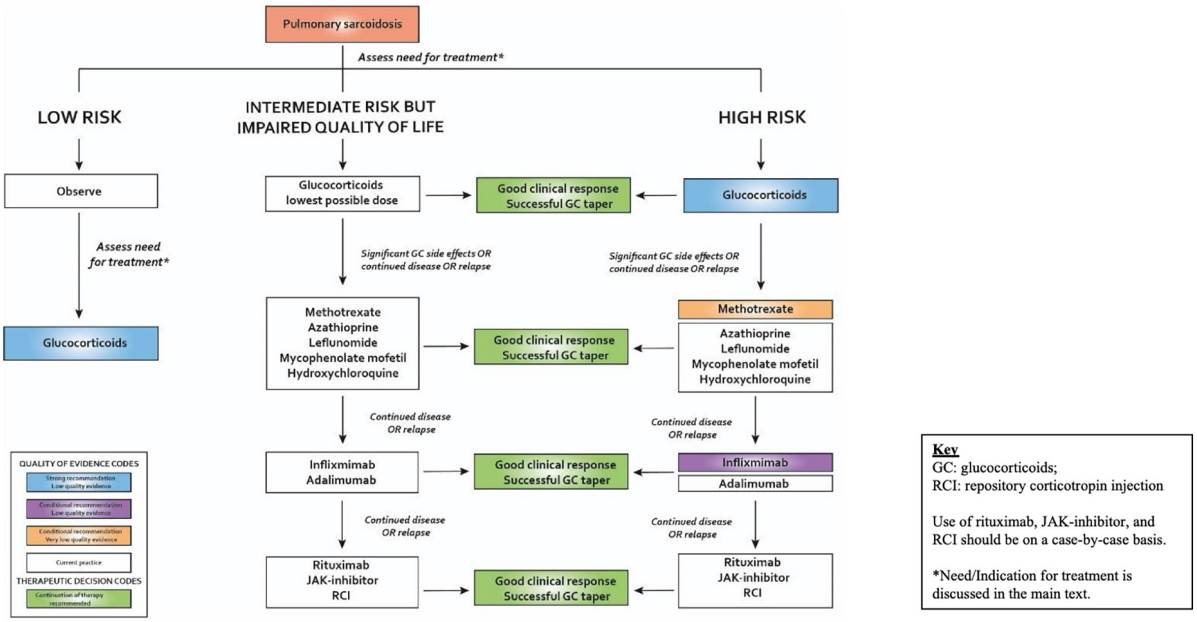

FIGURE 1
Approach to management of plumonary sarcoidosis. Reproduced with permission from the European Respiratory Society (ERS) clinical practice guidelines on the treatment of sarcoidosis (5). All rights reserved.

influences on bioavailability of a particular medication that require attention (77, 78). Partial responsiveness, wherein a monotherapy is insufficient to completely quell a disease activity level (that may be of high intensity, or that may outpace the efforts of a particular drug or drug dose) should still be considered valuable. A drug, eliciting partial responsiveness, used in combination with other agents, may provide value in keeping doses of more toxic agents minimal.

Tolerability is another cause of "treatment failure" and may have the greatest potential for salvaging efficacious systemic medication. Ongoing query into patient perceptions of side effects, as can be accomplished with patient self-reported measures, may support earlier interventions that effect tolerability. Being clinically inquisitive in gaining knowledge in the many strands of administration (e.g., route, frequency, dose division, rate of dose escalation, acclimation maneuvers, timing, nutrition, etc.) and palliation (e.g., anti-emetics, anti-diarrheal, etc.) to enhance tolerability, is pivotal (in the context of SDM) to preserving systemic medication use.

Toxicity accounts for another type of "treatment failure." This area requires dedicated knowledge to preventing and monitoring for toxic medication effects, as well as an opportunity to re-challenge. Often, once an

unanticipated toxicity occurs, a person's confidence in that medication is shaken.

Finally, it is important to note that none of the drugs used in sarcoidosis are "curative" and that relapses occur frequently with treatment interruption, medication holidays or with medication tapers. These relapses should not be interpreted as treatment failure but rather as disease recurrence following early discontinuation of treatment. In cases where a relapse occurs after at first a response was achieved, previously successful therapy should be re-instituted, and a more prolonged treatment course considered (79).

Ascertaining sarcoidosis vs. other etiology of worsening symptoms

For patients who develop new or worsening symptoms while on therapy, it is critical to consider and evaluate for other causes of these symptoms rather than routinely attributing worsening to failed therapy or to established progressive pulmonary or other organ sarcoidosis manifestation (80, 81). Common sarcoidosis-related complications exhibiting overlapping symptoms requiring consideration include cardiac involvement with either arrhythmia or heart failure, sarcoidosis-associated pulmonary hypertension (SAPH), small fiber neuropathy, and CNS symptoms (80, 82, 83). Fatigue, depression, sleeplessness, physical deconditioning, and obstructive sleep apnea are also very common medication-related, sarcoidosis-related, or non-sarcoidosis-related co-morbidities that may drive the appearance of worsening disease (80–82, 84). Entities contemporaneously seen in sarcoidosis that are crucial to consider are acute or sub-acute bacterial pneumonia, mycobacterial or mycotic infection, cardiovascular disease events common in the general population, and pulmonary embolism or lung and other cancers that have a higher temporal relationship to sarcoidosis than in the general population (85–95). **Figure 2** outlines a more global approach to treatment that emphasizes the need for comprehensive patient care (83, 84, 96).

Challenges affecting drug trials and drug development in sarcoidosis

Challenges with patient recruitment, patient selection, and cohort enrichment

Careful definition of the target cohort, as much as is possible, is critical in clinical trial design. Lack of attention to patient selection and cohort enrichment could generate data that significantly under-represent the efficacy of a good drug. A central premise of selection is establishing active sarcoidosis in the target organ(s) that will be sufficiently responsive to an efficacious treatment. This is accomplished in either or both of two major ways: (a) demonstration of inflammation consistent with sarcoidosis to the exclusion of other causes, which may be accomplished by positron emission tomography (PET)/computerized tomography (CT) scan in non-neurosarcoidosis, cardiac PET/cardiac magnetic resonance imaging (cMRI) in CS or brain MRI changes suggestive of edema/inflammation in neurosarcoidosis (97–102); (b) demonstration of clinically meaningful progressive disease in target organ(s) that is documented over a defined recent interval of time (for which other causes of worsening have been ruled out). Examples include worsening impairment or extent of disease on imaging, exercise tolerance or pulmonary function tests (PFTs) in lung involvement, worsening left ventricular ejection fraction (LVEF) on echocardiogram or cMRI in CS, or worsening limb strength or balance in CNS involvement. Effective selection also minimizes confounding factors that portend poor likelihood for significant improvement such as concomitant sarcoidosis co-manifestations that are minimally responsive to anti-inflammatory therapy examples of which include presence of severe SAPH in an interstitial lung disease (ILD) trial or extensive fibrosis in patients with pulmonary sarcoidosis. Both these co-manifestations are characterized on PFTs by very low forced vital capacity (FVC) and/or diffusing capacity for carbon monoxide (DLCO).

Additional challenges with patient recruitment include the need to promote diversity and ensure equitable enrollment of minorities and non-white participants. As much as is possible, trial populations should be reflective of the populations affected by the disease of interest. Sarcoidosis occurs three times more commonly in African Americans and presents with disproportionate severity in patients of a lower socioeconomic status (SES) (40, 42, 45, 103, 104), yet most trial populations thus far have not been reflective of this racial prevalence of disease (105, 106). Several studies show that African Americans are less likely to both qualify for and to participate in clinical trials due to several reasons such as mistrust in the system, lack of interest in clinical trials, fear and stigma associated with participation, and a perception that they may not be compliant with trial protocols (107–110). Several strategies that have improved clinical trial participation for minorities and the underserved in other diseases may also be deployed in sarcoidosis (108, 111, 112). These strategies center around the need to address mistrust and misconception, promote increased information and awareness of the benefits of trial participation, and ensure that investigators and study personnel are fully trained in cultural and racial competencies (108, 112). Ensuring feasibility studies are undertaken prior to commencement of large trials and involving patients in the design stages of trials will also help to address several of these challenges (113).

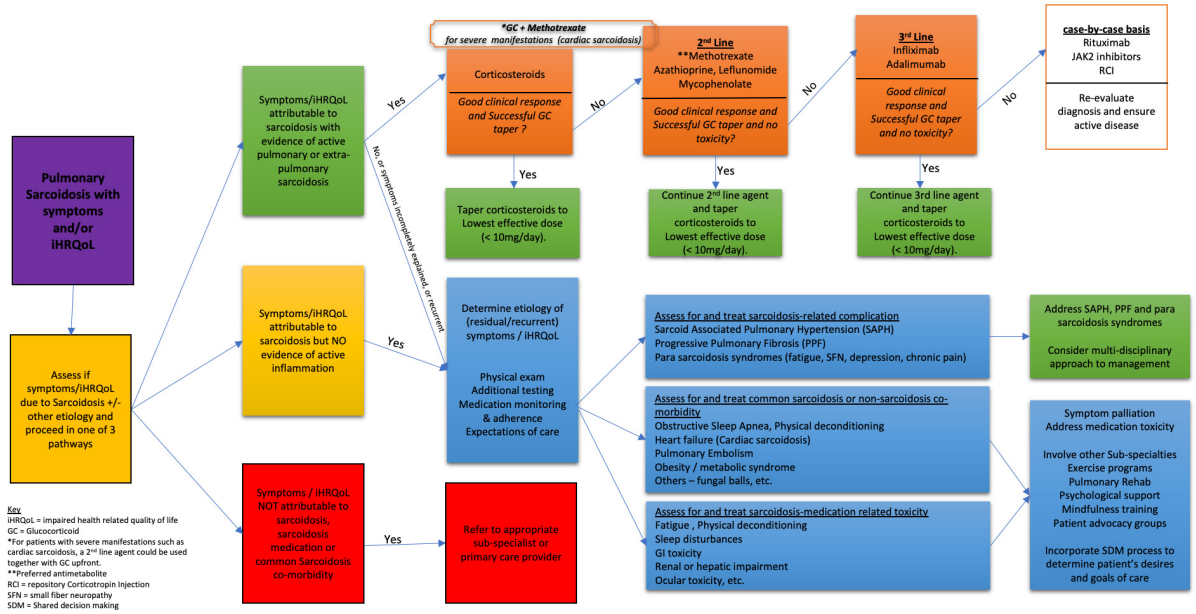

FIGURE 2
Global approach to treatment of sarcoidosis.

Studies focused on non-pulmonary manifestations of sarcoidosis face the unique challenge that very few patients (< 2% in one large cohort) (3) will have isolated non-pulmonary manifestations of sarcoidosis. Consequently, trial designs in pulmonary sarcoidosis may need to be adapted to enrich for other organ manifestations of disease without detracting from pulmonary endpoints, or conversely, to focus on a very small subset of patients with very clear-cut endpoints—such as focusing on chronic cutaneous sarcoidosis and using the sarcoidosis activity and severity index (SASI) (114, 115) as an endpoint or focusing on optic neuritis rather than all neurosarcoidosis. To this end, leveraging ongoing studies in biomarker, proteomics, and metabolomics research will be crucial to guide patient selections that enrich trial populations with patients that have active disease in multiple non-competing organs (116, 117). Routine use of PET scans to detect evidence of active disease in the lungs and extra-pulmonary organs, may also begin to address this issue (62, 118–122).

Besides RCTs, the use of large global registries with well phenotyped patients is also a critical step to systematically study various forms of high-risk or severe manifestations of sarcoidosis and their optimal treatment regimens. Sarcoidosis is a rare disease with heterogenous manifestations, and a potential marginal benefit of registries compared with RCTs is the ability to include a more heterogenous (and more representative) sarcoidosis population so as to gain large numbers and gain insight into the use of off-label therapeutics and novel therapeutic approaches for uncommon and severe disease manifestations. Several such registries are currently in existence and may begin to yield some much-needed information in this regard (14, 123–125).

Identifying appropriate endpoints

Another limitation of drug development in sarcoidosis has to do with identifying validated measures that reflect clinically meaningful response to therapy. Identifying and validating outcome measures is one of the greatest tasks at hand in organ-specific or multi-organ trial design in sarcoidosis. Even when considering a prevalent manifestation such as pulmonary sarcoidosis, the most used measures such as FVC can be flawed in capturing clinically meaningful change (126); and may miss a large subset of patients with ventilatory defects affecting other PFT parameters (127). Another example is though improvement in HRQoL along with the minimum clinically important difference (MCID) for various HRQoL instruments has been reported with some treatment regimens (128, 129), correlative changes in HRQoL to other endpoints such as physiologic function or steroid-tapering are still lacking.

The selection of outcome measures is predicated upon which outcomes or endpoints (whether primary, secondary, or exploratory) are deemed important in validating the hypothesis while remaining cognizant of the drug's mechanism of action and anticipated side effects. For example, in clinical trials targeting lung involvement and measuring changes in physiologic function as primary outcome, FVC, FEV1 or DLCO is often selected while changes in physical function

may warrant use of 6-min walk distance and other outcome measures assessing dyspnea, cough or HRQoL may be selected from a variety of PROMs intended to measure each of these as secondary or exploratory outcomes. As much as is possible, outcome measures should be authenticated to demonstrate content validity, reliability, discrimination between similar but different situations (e.g., SAPH vs. sarcoidosis-ILD, respiratory decline vs. anxiety), and responsiveness to changes over time that correlate with clinically meaningful change in disease state. Measures are more likely to be successful when they demonstrate high precision, easy interpretability, cost effectiveness, accessibility, and are without undue risk regarding patient safety, comfort, and fatigability. Measures that are easy and logical to complete and that provide immediate real time feedback to patients are also more likely to be accepted.

As noted, the ERS guidelines stress the two major reasons for treatment: avoid organ loss or death (danger) and/or improve HRQoL. Unfortunately, none of currently available literature has used these as a specific endpoint. Since mortality from sarcoidosis, death alone as an endpoint has not been a practical primary endpoint, the time to clinical worsening (TTCW) which includes a composite of predefined endpoints such as disease-related hospitalization, death, transplantation, or worsening of 6-min walk or FVC of 5–10% have been used as primary endpoints in trials for SAPH and pulmonary fibrosis (130, 131). However, most patients entering trials still have enough reversible disease making hospitalization, death, or lung transplantation far less likely. Thus, these endpoints may not be sensitive in a treatment trial for anti-inflammatory therapy.

Improving HRQoL should be a core outcome of all clinical trials in sarcoidosis (5). Unfortunately, the use of diverse PROMs makes comparison across studies difficult. There is a need to identify core sets of outcome measures for organ-specific and systemic sarcoidosis, respectively. The recently convened Sarcoidosis Clinical Outcomes Task Force (SCOUT) has identified several commonly reported outcome measures in pulmonary sarcoidosis with a view to develop a set of core outcome measures that can be uniformly applied across studies in pulmonary sarcoidosis (22).

Another endpoint accepted as reasonable is the ability to taper steroid dosage (steroid-sparing) (22). While reduction of steroids is clinically meaningful and important to patients, subjects enrolled in the placebo arm of placebo-controlled studies, are expected to be unable to achieve steroid reduction, thus remaining on moderate steroid doses for prolonged periods. This is problematic for a few reasons. The symptoms of prolonged steroid use can be intolerable to patients especially as patients are increasingly aware of steroid-sparing treatment alternatives, thus creating a vulnerability to patient retention. While drop-out in the placebo arm may appear to be data in favor of treatment, unless drop-out is a primary, or at least secondary, endpoint this information will not be captured as a meaningful outcome. Another is an ethical concern considering the availability of steroid-sparing agents and the well-known short and long-term toxicities of prolonged steroid use. Further, as sarcoidosis clinical trials become more plentiful, investigators are going to select among the studies they feel are optimal for their patients' health and safety and steroid withdrawal studies are unlikely to be preferred among other available studies. Other concerns for the use of steroid-sparing as an endpoint exist. Sarcoidosis is a multi-organ disease; therefore, the target organ may not be the organ that relapses when steroids are withdrawn thus creating conflicting trial results. For example, a patient in a pulmonary sarcoidosis trial may develop new or worsening uveitis as prednisone is withdrawn, while lung function remains stable. This information needs to be captured in trials. Using a customized approach to TTCW as an endpoint will capture these adverse events. Next, patients on medium to high dose steroids may develop steroid withdrawal symptoms unrelated to the efficacy of the trial drug and these may confound trial results. These concerns notwithstanding, several studies in pulmonary sarcoidosis have shown statistically significant steroid-sparing (132, 133) and it remains an important outcome to patients dealing with sarcoidosis and the toxic effects of steroids (5, 21, 22). Measures to limit the extent of steroid toxicities or to pro-actively manage their onset may be necessary for patients on placebo who require escalating steroid doses (28).

A possibly more efficient and patient-centered strategy in non-neurosarcoidosis might be the use of changing PET/CT values over time. The use of PET/CT is likely to shorten trial length, confer greater precision of change (126), allow for the flexibility of enrolling patients with clinically active disease whether treatment naïve, on corticosteroids or a steroid-sparing agent and confer the ability to use the addition or tapering of non-study drug as an outcome. Although very attractive as an end point, apart from CS, changes in PET/CT scans have not yet been validated as outcome measures in sarcoidosis and controversies exist as to whether SUVmean or SUVmax should be used (98, 122, 134). Further, changes in PET/CT imaging have not always correlated to changes in clinical parameters (56, 98, 122, 134–138). Nonetheless, use of change in PET/CT values over time remains a very promising endpoint and work on establishing its role in this regard remains ongoing.

Hierarchical composite endpoints (HCE) whereby multiple relevant outcomes or components ranked in order of clinical importance/relevance and combined into a single ordinal outcome may also be considered. These components would have to be adapted to both the organ of interest and the study drug under investigation and should capture both the most favorable and least desirable aspects of a drug or intervention (139, 140). For example, a trial evaluating the role of a new molecule in pulmonary sarcoidosis, may wish to evaluate its role as a steroid-sparing agent while concomitantly assessing its toxicity profile, and effect on HRQoL and FVC. Such a study may wish to

prioritize steroid-sparing and toxicity profile or HRQoL over FVC and may consequently design a HCE wherein the highest (best possible) rank is given to patients who are able to taper steroids to < 10 mg/day of prednisone or prednisone equivalent, have no reported toxicity, experience a clinically significant improvement in HRQoL, and have a prespecified improvement in FVC. Patients who are unable to taper steroids, but who otherwise meet all the other criteria may be given a second rank and so on and so forth—with the worst rank given to patients who do not meet any of the prescribed criteria. The study would then identify and report what proportion of patients are able to achieve a certain rank or higher as an outcome. HCE have not been used in sarcoidosis trials, however, several non-sarcoidosis trials suggest that they provide a sensitive endpoint to detect treatment effect with smaller sample sizes and in shorter time periods (139).

Table 2 lists the various potential endpoints of clinical trials in sarcoidosis.

Subject retention

Subject retention relies on principles of patient safety, comfort, anticipating medication tolerability and cost of participation. This necessitates incorporation of preventive measures and management of toxicities related to steroid-tapering studies. Such measures include protocols to ensure gastric, bone, endocrine and cardiovascular protection as well as measures to address psychiatric and sleep disturbance that follows any available published guidelines for prolonged steroid use. On the other hand, investigator protocols to help manage side effects of study drug that are anticipated to be frequent and decrease study drug tolerability, should be carefully developed with supportive communication aids and patient brochures in the event side effects arise.

Fair reimbursement for participation

Patients are the most valuable element in clinical trials, and it is their participation that enables science and treatment development to advance. Most patients are motivated to participate in trials. However, "motivation" to benefit self or others is not enough for a patient to be able to participate in a clinical trial, as participation in clinical trials often requires a level of financial stability and job security that allows compliance with complex trial schedules and prolonged time off work for multiple study visits—sometimes at centers far away from home. Consequently, only patients that can afford the financial and other losses associated with clinical trials are able to participate without the support of financial coverage for expenses and collateral costs of participation (141, 142). For patients diagnosed with sarcoidosis—who have been shown to experience significantly less earnings, higher work-lost days, greater risk of job loss, and greater difficulty qualifying for income support for lost wages due to disease (42, 143, 144)—participation in clinical trials may present a significant socio-economic burden that few are equipped to bear.

Patient costs of research trial participation can be divided into two broad categories. D*irect expenses* paid by patients to attend visits (travel, meals for long visits) and *collateral costs* to the patient (time off work, the stress of negotiating time off from work, time recording in and dealing with technical issues related to e-diaries, potential electricity for charging, and data use for e-diaries). A potential third category might be *procedural burden* which might relate to the complexity and potential discomfort of study procedures. While a fourth category might be *probability of benefit* which relates to foregoing other available treatment to participate in a placebo trial or a trial of a medication without clinical precedent, or as is common in sarcoidosis to remain on prolonged toxic medications such as prednisone. Participation in clinical trials occurs on top of the usual anticipated annual financial losses for patients and family members related to the disease itself (28, 42, 143, 145). Subjects will continue to need to take sick days and vacation days or lose wages for comprehensive care of their health status that is not supplied within the context of the trial. Supporting fair reimbursement for clinical trial participation protects diversity and inclusion of non-white and lower SES participants who could not afford the financial loss associated with RCT participation (141, 142, 146). The validity of coercional incentive related to reimbursement is rapidly losing ground and recent studies supports the position that payment to economically vulnerable populations is ethically justified and indeed desirable when certain conditions are met (141, 142, 147–149).

Patient research partners

Some of the most valuable guidance on feasibility, subject retention, and natural history of disease come from patients themselves. Patients are experts in their disease and have an idea of what other patients are willing to tolerate and make trade-offs for. For e.g., the likelihood that patients will remain on stable dose of steroids if study drug shows no efficacy, or the degree of imposition of a daily e-diary. Patient research partners (PRPs) are now accepted as an important element of successful clinical trial design (150). Inclusion of PRPs from the inception of a study imparts expertise on foundational aspects of trial success such as feasibility and subject retention (150, 151). PRPS provide their general expertise which leverage advantages in brainstorming solutions and offering insight from their unique lens on disease behavior (152). There is work to be done on remuneration for PRP effort and their involvement through to publication of results with appropriate acknowledgments, but that is beyond the scope of this manuscript (153, 154).

TABLE 2 Proposed endpoints for clinical trials in sarcoidosis (5, 21, 22).

Organ involvement	Domain	Measure	Comments
Pulmonary sarcoidosis	**Symptoms	Dyspnea—mMRC, BDI/TDI Cough—Leicester scale Fatigue—FAS	This should be customized to capture multi-organ and/or extra pulmonary involvement.
	*Physician judgment	Clinical judgment of improvement, worsening or progression.	This is applicable to systemic and all organ-specific forms of sarcoidosis.
	*Steroid sparing	% Reduction in steroid dose, Cumulative steroid dose, Duration of time at minimal steroid doses, % Of participants able to achieve steroid taper to < 10 mg/day.	Consider analyzing drop-out from placebo arm as a secondary outcome. Confounding results may occur from withdrawal from steroid or flare-ups in non-target organs. Measures of steroid toxicity and ways of addressing them need to be put in place.
	Radiology/evidence of activity	Changes in PET/CT chest imaging	Changes in PET scans will need to be defined in terms of SUVmean or SUVmax. There is a need to determine what constitutes a meaningful difference in SUV levels.
	*Medication toxicity/tolerance	Serious AEs, Life threatening AEs, AEs leading to discontinuation of therapy Other AEs	This should be captured in all clinical trials and tailored to investigational drugs and organ system targeted.
	Pulmonary function	FEV1, FVC, DLCo, CPI	There is a need to determine what is clinically meaningful disease specific change in FVC, FEV1 and DLCo for patients with pulmonary sarcoidosis. The CPI has also been validated as a prognostic severity marker in pulmonary sarcoidosis.
	Exercise capacity	6MWD	There is a need to determine what constitutes meaningful change in 6MWD for patients with pulmonary sarcoidosis.
	*HRQoL	SGRQ, SF-36, SAT-Lung FAS KSQ General Health; KSQ Lung	Various PROMs have been used to capture HRQoL. There is a need to create core sets of outcome measures for organ specific and systemic sarcoidosis.
	Mortality	Mortality often not feasible Consider composite outcome—TTCW	TTCW is a predefined composite endpoint that can be customized to capture such events as disease-related hospitalization, all-cause hospitalization, death, transplantation, worsening of 6MWD, PFT or symptom burden.
Cutaneous Sarcoidosis	Cutaneous sarcoidosis disease activity HRQoL	PGA, SASI, CSAMI, Photographs SAT skin, KSQ Dermatology Questionnaire, SAT Fatigue	
Cardiac Sarcoidosis	Symptoms Radiology/Evidence of Disease Activity Exercise Capacity Mortality	Arrythmias/arrythmia burden cPET Scan, cMRI, Echocardiogram (LVEF) 6MWD Mortality is often not feasible. Consider composite outcomes assessing all-cause hospitalization, cardiac hospitalization,	Note that mortality will likely never be feasible in view of rarity of disease and much improved prognosis. Though composite outcomes are more achievable, sample size is likely to be prohibitive in view of rarity of disease and much improved prognosis.
Neurosarcoidosis	Imaging/evidence of disease activity HRQoL	MRI Measures assessing cognitive functioning, Functional independence, strength measures of limbs, General Health status questionnaires.	
Others Ocular Renal Hypercalcemia	HRQoL measures	General and organ specific HRQoL measures	This can be customized for each organ involved.

**Should be customized to reflect the specific organ(s) of interest.

*Applicable to all organ manifestations of disease.

HRQoL, health related quality of life; TTCW, time to clinical worsening; PFT, pulmonary function tests, FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; DLCo, diffusion capacity for carbon monoxide; SGRQ, Sant Georges Respiratory Questionnaire; SF-36, Short form-36; SAT-Lung, sarcoidosis assessment test lung component; FAS, fatigue assessment score; KSQ, Kings sarcoidosis questionnaire; mMRC, modified Medical Research Council; BDI/TDI, baseline dyspnea index/Transitional dyspnea index; PET/CT, positron emission tomography/computed tomography scan; cPET, cardiac PET scan; SUV, standardized uptake Value; MRI, magnetic resonance imaging; cMRI, cardiac MRI; AE, Adverse event; 6MWD, 6-min walk distance; PGA, Physician Global Assessment; SASI, sarcoidosis activity and severity instrument; CSAMI, cutaneous sarcoidosis activity and morphology instrument; LVEF, left ventricular Ejection Fraction; CPI, composite physiological index; a weighted index of pulmonary function variables.

The immunopathogenesis of sarcoidosis as it influences therapeutic drug trials

The sarcoid granuloma is the immunohistopathologic hallmark of sarcoidosis (1, 96, 155). It has been shown to result from an aberrant CD4 + Th1/Th17 cell mediated immune response to a yet unknown (presumptive) environmental or occupational exposure/stimulus in a genetically predisposed individual (96, 156–160). The immunologic cascade resulting in sarcoid granuloma formation has been described and is outlined in **Figure 3**. It may broadly be categorized into three main phases: granuloma formation, granuloma propagation/expansion, and persistence of granuloma associated with chronic disease and progression to fibrosis (96). Not every patient progresses through all the three stages to fibrosis. Most patients will have resolution or stabilization of their disease, and this can occur at any stage (96, 157). Only 10–30% of patients will develop a chronic non-remitting disease with progression to fibrosis (8, 161, 162). Certain HLA subtypes and African American ancestry have been associated with chronic progressive fibrotic disease (156, 163).

As noted above, the inciting "antigenic" stimulus in sarcoidosis is unknown, however, several studies support that an inhaled infectious, organic, or inorganic antigen acquired from various occupational and environmental exposures such as moldy environments, metalworking, firefighting, agricultural employment, and occupational exposures to insecticides and building supplies is implicated in disease etiology (96, 156, 164). A double hit theory either from the same "antigen" with a prolonged intervening latent period, or multiple insults from various synergistic "antigens" has been proposed (96, 165). The inciting antigen may arise from one or more sources, such as Propionibacteria, mycobacteria, or inorganic fibers. A dysregulated immune response to antigenic stimulus (or stimuli) from mycobacterial catalase-peroxidase G (mKatG), the 6-KDa early secreted mycobacterial antigenic protein (ESAT-6), and Propionibacterium nucleic acids has been associated with a granulomatous condition (166–175). A possible role for serum amyloid A (SAA) inappropriately accumulated in response to mycobacterial infection/exposure, or another unknown stimulus has also been suggested (176, 177). Although possible exposure to mycobacterial proteins remains one of the most plausible etiologic risk factors for sarcoidosis, a recently published large RCT did not show any clinical or physiologic response to anti-mycobacterial therapy in patients with pulmonary sarcoidosis despite a significant reduction in ESAT-6 levels (106); and several studies have failed to culture mycobacteria from sarcoidosis tissues (178, 179).

The inflammatory response in sarcoidosis is initiated by the innate immune system through activation of membrane-bound pattern recognition receptors (mbPRRs) on the surface of antigen presenting cells (macrophages and dendritic cells) at the alveolar epithelial surface (168). These mbPRR include toll-like receptors (TLR), RIG-I-like receptors, and nucleotide-binding oligomerization domain and leucine-rich repeat-containing receptors (NLRs) which constitute one component of the NLRP inflammasome (168, 180). Activation of alveolar macrophages (*via* TLR) results in activation of effector proteins (such as caspase-1) and cleavage of inactive forms of interleukin (IL)-1β and IL-18 into their active forms (168, 180). Activated alveolar macrophages produce large amounts of several proinflammatory and Th1-skewing cytokines such as IL-1, IL-6, IL-8, IL-12, IL-18, interferon (IFN)-γ, and TNF-α (96, 157, 168). These cytokines in conjunction with several chemokines and chemokine receptors (CXCR3, CCR5, CCR6) also released by activated macrophages activate the adaptive immune system and upregulate the process of granuloma formation and expansion through activated CD4 + and CD8 + T-cells (96, 157, 168).

The core of the sarcoid granuloma consists predominantly of activated CD4 + T helper (CD4 + Th) lymphocytes with rare scattered CD8 + T cells and B cells in the periphery (157). The adaptive immune response in sarcoidosis is a predominant CD4 + type 1 helper (CD4 + Th1) response (181). Activated alveolar CD4 + T-lymphocytes produce high levels of IFN-γ, IL-2, and TNF-α as well as high levels of IL-12 and 1L-18 which skew the immune response toward a Th1 pathway and cause increased expression of Th1-associated chemokines CXCR3 and CCR5 which amplify the Th1-oriented response (96, 157, 182, 183). The activated CD4 + Th1 cells also upregulate the Th1-specific transcription factor (T-box transcription factor 21 [TBX21]) which promotes further differentiation of CD4 + Th cells down the Th1 pathway (96, 184). TBX21 activates/regulates the Janus kinase and signal transducer and activator of transcription (JAK-STAT) pathway and controls the Th1 hallmark cytokine IFN-γ (96). The JAK-STAT pathway has been proposed as a potential drug target in sarcoidosis (18, 185, 186).

Alveolar CD4 + Th lymphocytes may also differentiate down a Th17/Th17.1 effector pathway under the influence of IL-1, IL-6, and TGF-β (157, 187–189). Th17 cells produce IL-17 and INF-γ, and their survival and proliferation is dependent on IL-23 (187, 190, 191) which regulates the process of Th17 cell differentiation (157, 160, 191–193) and has also been shown in some cases to initiate a more proinflammatory process (resulting in persistence of the sarcoid granuloma) through the production of granulocyte-macrophage colony stimulating factor (GM-CSF) (96, 194–196). The Th17/17.1 pathway is less frequently employed in sarcoidosis but has been implicated in the development of chronic progressive disease (156, 188, 197). A large phase II multi-center randomized trial that evaluated the role of Ustekinumab (a fully human IgG1 monoclonal antibody directed against IL-12/IL-23) in patients with chronic pulmonary and cutaneous sarcoidosis refractory to corticosteroids found that there was no significant

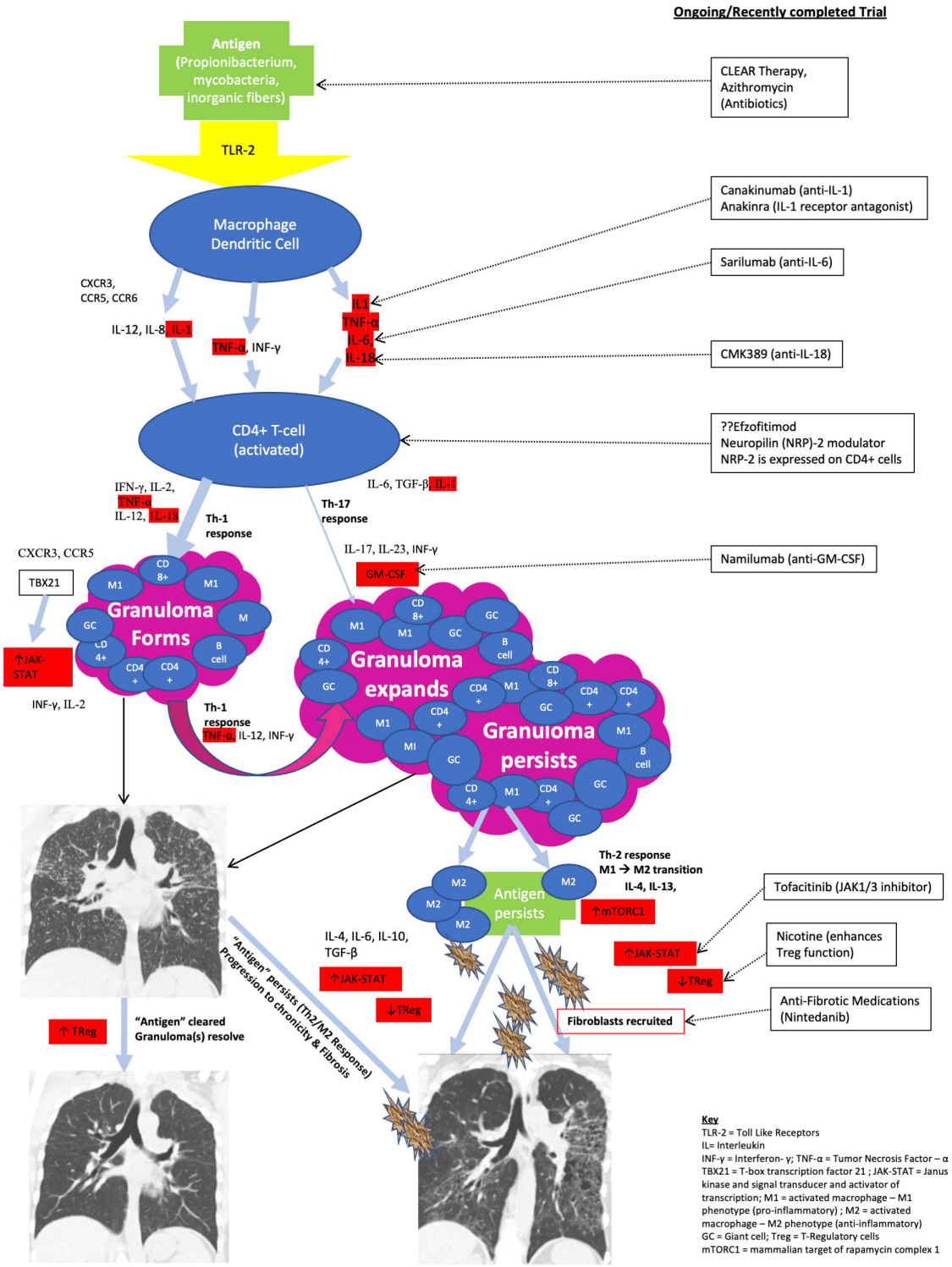

FIGURE 3
Immunologic cascade resulting in sarcoid granuloma formation.

difference in pulmonary function, health related quality of life (HRQoL) or skin assessment score in patients on Ustekinumab vs. placebo after 6-months of therapy (105). A drug trial directed against GM-CSF in patients with chronic refractory pulmonary and CS is ongoing and will be discussed further below (198, 199).

Failure to clear the inciting antigen and persistence of a dysregulated immune response has been associated with the development of chronic disease and progression to fibrosis in sarcoidosis (200). Activated macrophages drive the inflammatory process associated with granuloma formation. In the classic antigenic model, phagocytic clearance of the offending pathogen results in resolution of the inflammation and the granuloma, however, persistence of the antigenic stimulus results in ongoing inflammation and propagation of the sarcoid granuloma (201). Alveolar macrophages may be classified as M1 or M2 depending on the cytokine microenvironment (168). MI macrophages are activated by IFN-γ and produce proinflammatory cytokines (TNF-α and IL-12) whereas M2 macrophages are generated in the presence of Th2 cytokines (IL-4 and IL-13) and produce immunosuppressive, immunoregulatory, anti-inflammatory and profibrotic cytokines (168). IL-4, IL-6, IL-10, and TGF-β are anti-inflammatory cytokines that inhibit IL-2 and INF-γ and facilitate fibroblastic recruitment leading to extracellular matrix deposition and fibrosis (202, 203). It is thought that transition from a Th1/M1 predominant pro-inflammatory cytokine response to a Th2/M2 anti-inflammatory cytokine response promotes persistence of the sarcoid granuloma and development of chronic disease (96, 202, 203). IL-13 promotes the differentiation of M1 to M2 macrophages and has also been shown to activate the metabolic check point kinase mammalian target of rapamycin complex 1 (mTORC1) (96, 204). mTORC1 has been implicated in granuloma formation through its role in activating macrophages and promoting their differentiation into epithelioid cells and multinucleated giant cells (96, 185, 204). Impaired autophagy resulting from excessive stimulation of mTORC1 pathway has been implicated in the failure to eliminate antigens and shown to contribute to granuloma persistence and chronicity (96, 204, 205).

Other immune mechanisms associated with persistence of the sarcoid granuloma and thus of the development of chronic progressive fibrotic disease include activation of the JAK-STAT pathway (185, 186) and impaired immunosuppressive function of T-regulatory cells (Tregs) (206–208). Studies show that sarcoid derived Tregs fail to inhibit production of TNF-α, INF-γ, and IL-2 all of which contribute to granuloma growth and expansion (206, 207, 209).

Ongoing, future, and recently concluded clinical drug trials in sarcoidosis

ClinicalTrials.gov is a publicly available database of all privately and publicly funded clinical trials conducted around the world. It includes all studies conducted within the 50 states and in over 200 countries including Japan and Europe (210).

A search on ClinicalTrials.gov of all ongoing, future, and completed interventional clinical trials in sarcoidosis yielded 173 results. Limiting the search to adults (age 18 and over), drug intervention, and early Phase 1 through Phase 4 trials, as well as excluding trials of devices or behavioral interventions, and studies that have been withdrawn, suspended, or terminated resulted in 69 studies. During manual review of these studies, duplicate entries as well as trials evaluating nutritional supplements, non-granulomatous manifestations of sarcoidosis, and diagnostic, radiologic, and non-drug interventions were excluded. We also excluded trials completed before 2018. Trials evaluating the same molecule for different disease manifestations (such as for pulmonary and CS) or where an early phase trial has been completed and late phase trial initiated were acknowledged as independent studies. Twenty-eight trials were identified. Fourteen of these trials are in pulmonary sarcoidosis, four in SAPH and three in CS. There are only two trials each in cutaneous and multi-organ sarcoidosis and one each in hepatic, CNS sarcoidosis and sarcoidosis affecting the calcium and Vitamin D homeostatic balance (**Table 3** and **Figure 4**). As at the time of publication, nine of these trials are actively recruiting, three are anticipated to start recruiting and another three are reported as active but not recruiting. Three of the studies are reported to have an unknown status, however, on further literature review, one of these studies has been completed with results published (131). An additional ten studies are reported as completed; six of these have preliminary results yet only three of these study results have been published in peer-reviewed journals (106, 128, 211) and one in abstract form (132). Of the 15 active and/or anticipated studies, 11 are evaluating drug molecules and four are evaluating alternative treatment approaches using already established drugs as listed in **Table 1**. Three of the active drug intervention trials are each in pulmonary sarcoidosis and SAPH (27% each), two are in CS (18%) and there is only one ongoing study in each of hepatic, calcium/Vit D homeostatic imbalance and multi-organ disease. **Figure 4** outlines the search process used to identify trials included in this manuscript and **Table 3** provides a summary of the active, future and recently completed trials in sarcoidosis.

Select studies evaluating novel therapeutic agents and alternative therapeutic regimens are further discussed below. Studies in SAPH are not discussed further as they are considered outside the scope of this manuscript.

Studies in pulmonary sarcoidosis

Most of the studies in pulmonary sarcoidosis are evaluating new molecules and two are evaluating alternative treatment approaches aimed at minimizing steroid exposure. Two recently completed studies with divergent results provide excellent learning opportunities and are reviewed.

TABLE 3 Summary of ongoing, anticipated and recently completed studies in sarcoidosis from 2018 to 2022.

Organ system	Study title	NCT number	Study status	Sample size/ completion date	Primary outcome (s)	Secondary outcome measure(s)
Pulmonary sarcoidosis	Efficacy and safety of IV Efzofitimod in Patients with Pulmonary Sarcoidosis	NCT05415137 (Phase 3) NCT03824392 Phase 2/completed	Planned but not started	#264/January 2025 #37/July 2021	Steroid tapering at 48 weeks Safety and Tolerability	Change in FVC Change in KSQ-Lung score Steroid tapering, cumulative steroid dose, Immunogenicity.
	RCT of Hydroxychloroquine Combined with Low-dose Corticosteroid in Pulmonary Sarcoidosis. (QUIDOSE)	NCT05247554 (Phase 3)	Planned but not started	#200/March 2024	Change in FVC at 26 weeks	Not stated
	Efficacy and Safety of SQ Namilumab in Participants with Chronic Pulmonary Sarcoidosis (RESOLVE-Lung)	NCT05314517 (Phase 2)	Actively recruiting	#100/January 2025	Change in FVC at 26 weeks	Steroid sparing, Safety and Tolerability, Change in PROs (not specified) Cumulative steroid dose and toxicity Change in SASI and ePOST, Change in HRCT and PET imaging, Change in 6MWD
	Efficacy, Safety and Tolerability of IV CMK389 in Patients with Chronic Pulmonary Sarcoidosis	NCT04064242 (Phase 2)	Actively recruiting	#66/July 2023	Change in FVC at 16 weeks	Steroid tapering, Composite index of change in FVC & 6MWD, Change in FEV1, 6MWD Change in PET imaging
	Effectiveness of Methotrexate vs. Prednisolone as First-line Therapy for Pulmonary Sarcoidosis (PREDMETH)	NCT04314193 (Phase 4)	Actively recruiting	#138/January 2025	Change in FVC at 24 weeks	Time to pulmonary (FCV) improvement Change in DLCO Change in Biomarkers (sACE, sIL-2R, T-cell biomarkers). Change in KSQ (all domains), CRQ, GRC, EuroQol, FAS Change in mMRC Medication tolerance and change in PESaM
	Efficacy and Safety of Two Glucocorticoid Regimens in the Treatment of Sarcoidosis (SARCORT)	NCT03265405 (Phase 4)	Actively recruiting	#86/June 2022	Relapse or Treatment failure at 18-months	Time to relapse or treatment failure, Proportion of patients with response to therapy, change in FVC, cumulative prednisone dose, prednisone toxicity, HRQoL (SHQ and FAS)
	Phase II Investigation of Antimycobacterial Therapy on Progressive, Pulmonary Sarcoidosis	NCT02024555 (Phase 2)	Completed	#97/April 2019	Change in FVC at week 16	Radiographic improvement (CXR) Change in 6MWD, Dyspnea, Change in FAS, SGRQ, KSQ, Adverse events Change in FEV1
	Nicotine Treatment for Pulmonary Sarcoidosis: A Clinical Trial Pilot Study	NCT02265874 (Phase II)	Completed	#57/Nov 2021	Change in FVC	Change in CT imaging
	Azithromycin a Treatment for Pulmonary Sarcoidosis CAPS	NCT04020380 (Phase 2)	Completed	#21/June 2020	Change in cough count at 12 weeks	Change in severity of and urge to cough Change in Leicester cough questionnaire Change in KSQ total score

(*Continued*)

TABLE 3 (Continued)

Organ system	Study title	NCT number	Study status	Sample size/ completion date	Primary outcome (s)	Secondary outcome measure(s)
	Tofacitinib Hypothesis-generating, Pilot Study for Corticosteroid-Dependent Sarcoidosis	NCT03793439 (Phase 1)	Completed	#5/June 2021	Steroid Sparing (50% reduction in CS requirement) at week 16	Change in STAT1 mediated Genes by peripheral blood RNA sequencing
	ActharGel in Participants with Pulmonary Sarcoidosis (PULSAR)	NCT03320070 (Phase 4)	Completed	#55/November 2021	Change in FVC and DLCO at week 24; change in HRCT	Change in FAS, steroid taper
	Study of efficacy, safety, and tolerability of ACZ885 (Canakinumab) in Patients with Pulmonary Sarcoidosis	NCT02888080 (Phase 2)	Completed	#40/March 2019	Change in FVC at week 24	Change in PET/CT, HRCT Change in 6MWD Change in FEV1, DLCO
Fibrotic pulmonary sarcoidosis (FPS)	Pirfenidone for Progressive Fibrotic Sarcoidosis. (PirFS)	NCT03260556 (Phase 4)	Completed	#60/March 2020	Time To Clinical Worsening (TTCW)	Change in FVC and Composite physiologic Index
SAPH	Safety and efficacy of oral selexipag in participants with SAPH (SPHINX)	NCT03942211 (Phase 2)	Actively recruiting	#74/September 2024	Pulmonary Vascular Resistance (PVR) week 26	Not stated
	Inhaled Treprostinil in patients with SAPH (SAPPHIRE)	NCT03814317 (Phase 2)	Actively recruiting	#10/October 2022	PVR at week 16 Mean Pulmonary artery pressure (mPAP) at week 16	Change in 6MWD Change in FEV1 and FVC Change in cMRI Change in BNP and WHO functional class
	A dose escalation study to assess the safety and efficacy of pulsed inhaled nitric oxide in subjects with pulmonary hypertension associated with pulmonary fibrosis or sarcoidosis on long term oxygen therapy.	NCT03727451 (Phase 2)	Active, not recruiting	#17/March 2022	mPAP PVR, Pulmonary capillary wedge pressure (PCWP), cardiac output (CO) and change in 6MWD at week 16	Safety and tolerability Distance saturation product, Dyspnea HRQoL using St. Georges Questionnaire
	Riociguat for Sarcoidosis Associated Pulmonary Hypertension (RioSAPH)	NCT02625558 (Phase 4)	Status unknown	#60/Oct 2018	TTCW	Adverse Events, Change in FVC, HRQoL (instrument not specified), 6MWD
Cardiac sarcoidosis	A study to assess the safety, tolerability, and efficacy of SQ Namilumab in Participants with Active Cardiac Sarcoidosis. (RESOLVE-Heart)	NCT05351554	Planned but not started	#30/January 2024	Safety and tolerability (Incidence of adverse events)	Change in cPET imaging, arrhythmia burden and echocardiogram findings. Hospitalization for cardiac events. Cumulative steroid dose and toxicity. Change in FAS and subject Global assessment
	Interleukin-1 Blockade (daily SQ Anakinra for 4 weeks) for Treatment of Cardiac Sarcoidosis (MaGiC-ART)	NCT04017936 (Phase 2)	Actively recruiting	#28/December 2023	Change in C-reactive protein at 28-days	Change in cPET and cMRI. Serious cardiac events (summation of hospitalizations and death due to cardiac causes)

(*Continued*)

TABLE 3 (Continued)

Organ system	Study title	NCT number	Study status	Sample size/ completion date	Primary outcome (s)	Secondary outcome measure(s)
	Cardiac Sarcoidosis Randomized Trial (CHASM-CS-RCT)	NCT03593759 (Phase 3)	Actively recruiting	#194/December 2024	Change in perfusion and rest scores on cPET scan	Mortality, Cardiovascular hospitalizations, medication related adverse events, GC toxicity, medication compliance, BMI and HRQoL (KSQ, SF 36, SAT), ventricular arrhythmia burden, complete heart block, echocardiography
Cutaneous Sarcoidosis	Open-label Trial of Tofacitinib in Cutaneous Sarcoidosis and Granuloma Annulare	NCT03910543 (Phase 1)	Completed	#15/June 2021	Change in CSASI at 26 weeks	Change in Skindex, Change in PET-CT
	A Clinical Study of Tranilast in the Treatment of Sarcoidosis	NCT03528070 (Early Phase 1)	Status unknown	#56/December 2020	Change in size of skin lesion Change in FVC at 12-months	Not stated
Multi-Organ Sarcoidosis	Sarilumab in Patients with Glucocorticoid-Dependent Sarcoidosis	NCT04008069 (Phase 2)	Active, not recruiting	#15/July 2027	Flare-free survival at 2-weeks	Change in ePOST score, FACIT-F, SASI, 68/66 Joint evaluation, Steroid sparing. change in FVC, FEV1, change in liver and renal function
	Efficacy of Remission-induction Regimen with Infliximab for Severe Extrathoracic Sarcoidosis (EFIRTES)	NCT03704610 (Phase 3)	Completed	#31/September 2021	Change in ePOST score at week 6	Change in ePOST score at week 22
Others	Vitamin D Homeostasis in Sarcoidosis Ursodeoxycholic Acid (UDCA) for Hepatic Sarcoidosis CNS Sarcoidosis and Acthar Gel	NCT03621553 (Phase 4) NCT03602976 (Phase 2) NCT02298491 (Phase 4)	Active, not recruiting Completed Completed	#90/December 2023 #10/July 2023 #4/Nov 2020) (completed)	Change in Lung Function at week 24 Reduction in ALP and GGT Total number of lesions assessed at 1 year	Change in KSQ, 6MWD Change in serum CBC, Vit D, sACE, CRP and several other biomarkers Change in PET/CT and bone density scores Not stated. HRQoL (Treatment satisfaction QoL) measures, change in PDDS, MoCA, SF-36 and Beck depression Inventory-11

CRQ, Chronic Respiratory Questionnaire; KSQ, Kings Sarcoidosis Questionnaire; GRC, Global rating of change Scale; PESaM, Patient Experience and Satisfaction with Medication Questionnaire; FAS, Fatigue Assessment Scale; PET, positron tomography emission scanning; Cpet, cardiac PET scan; cMRI, cardiac magnetic resonance imaging scan; LGE, late gadolinium enhancement; ePOST, extrapulmonary physician organ severity tool; SASI, Sarcoidosis Activity and Severity Index; CSASI, Cutaneous Sarcoidosis Activity Index; FVC, Forced Vital Capacity; FEV1, Forced Expiratory Volume in 1 s; SKindex, skin-related quality of life metric; BNP, Brain Natriuretic Peptide; WHO, World Health Organization; CS, Corticosteroid; ALP, Alkaline phosphate; GGT, gamma glutamyl transferase; SAT, Sarcoidosis Assessment Tool; PDDS, Patient Determined Disease Steps; MoCA, Montreal Cognitive Assessment.

Studies of novel therapeutic agents in pulmonary sarcoidosis

Study of the efficacy, safety and tolerability of CMK389 in patients with chronic pulmonary sarcoidosis (NCT04064242)

This is a multi-national randomized double-blind placebo-controlled phase 2 study evaluating the safety, efficacy and tolerability of CMK389 in patients with chronic pulmonary sarcoidosis (212). Patients will receive a single intravenous (IV) infusion of CMK389 (vs. placebo) every 4 weeks for 16-weeks. Eligible subjects must be symptomatic, and on concomitant therapy with prednisone and methotrexate (or azathioprine) (212). Patients are excluded if they have significant pulmonary hypertension or extensive pulmonary fibrosis (> 20%) as determined by the grading system by Walsh et al. (213). The primary study outcome measure is change in FVC (212). Secondary outcome measures include exercise capacity [6MWD (214)], a composite index of pulmonary physiology (relative reduction in FVC > 10% or FEV1 = 10% or DLCo = 15%) and exercise capacity (relative reduction of 6MWD = 50 m), steroid sparing, and change in maximum and mean standardized uptake value (SUVmax and SUVmean) on PET/CT scan (116, 215). The projected study completion date is July 2023 with an expected enrollment number of sixty-six patients (212).

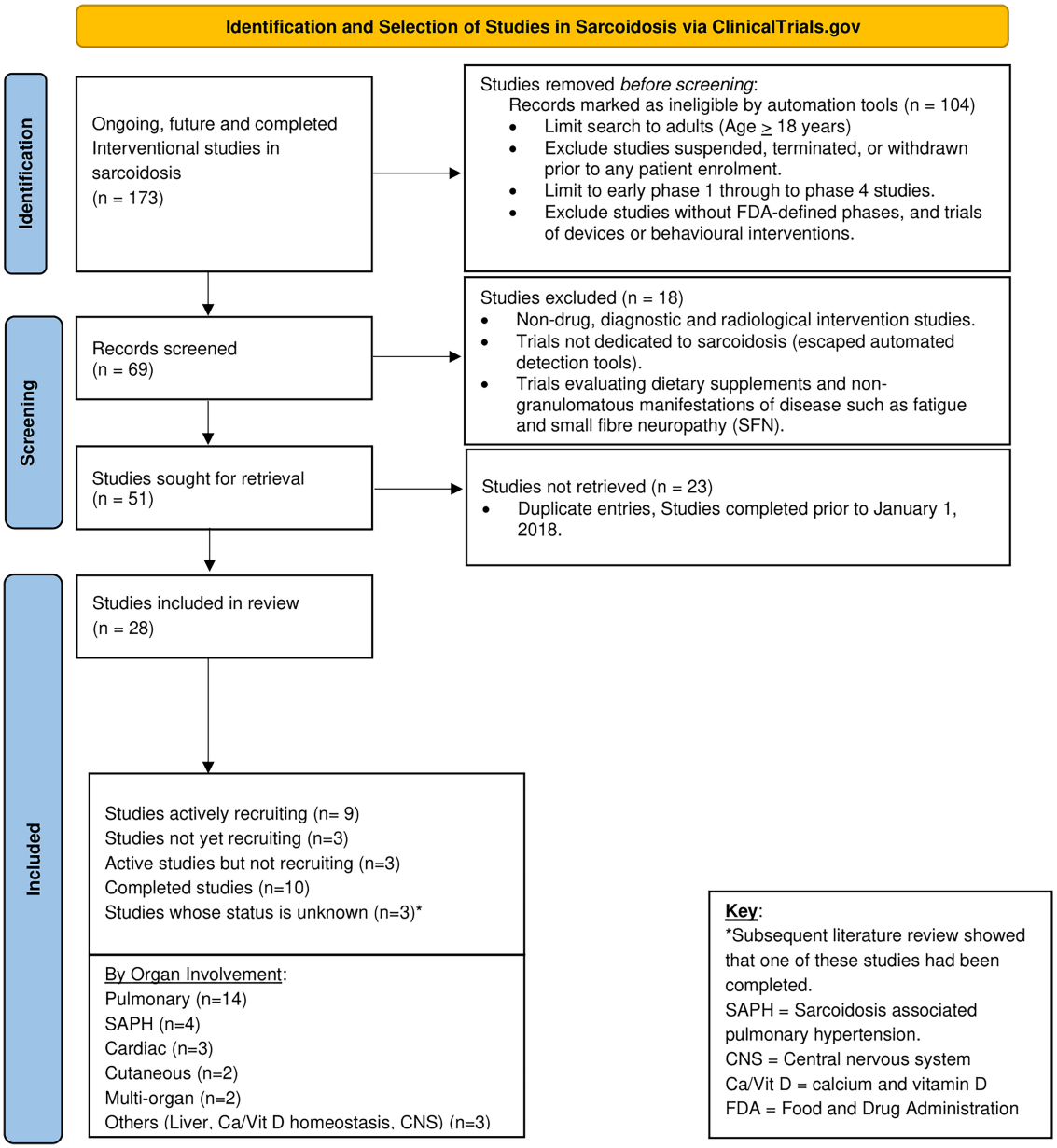

FIGURE 4
Active, future, and recently concluded clinical trials in sarcoidosis in the past 5-years (2018–2022).

CMK389 is a fully human IgG1 monoclonal antibody directed against IL-18. Pre-clinical studies suggest that it selectively binds to and inhibits IL-18 activity. Significantly elevated levels of IL-18 have been found in serum and BAL fluids (BALF) of patients with pulmonary sarcoidosis and have been shown to play a significant role in the immunopathogenesis of the sarcoid granuloma formation (**Figure 3**) (216–219). IL-18 is a monocyte/macrophage derived pro-inflammatory cytokine which works synergistically with IL-12 to enhance IFN-γ production from Th1 cells (220, 221). On its own, IL-18 is weak at stimulating IFN-γ production, however, in conjunction with IL-12 it leads to enhanced IFN-γ production (221, 222). Studies in patients with pulmonary sarcoidosis showed that IL-18 also stimulates increased levels of IL-18 receptor (IL-18R) expression which activates AP1 and the transcription factor NF-κB leading to enhanced IL-2 gene expression and concomitant T-cell activation with an enhanced expression of Th1 cytokines (218, 219). IL-18 is produced as a procytokine which is cleaved intracellularly by caspase-1 to a mature biologically active form (219). Studies in Japanese patients with sarcoidosis suggest that

IL-18 gene polymorphisms may be associated with an increased genetic risk of developing sarcoidosis (223).

RESOLVE-lung: A study to assess the efficacy and safety of Namilumab in patients with chronic pulmonary sarcoidosis (NCT05314517)

This is a randomized double-blind placebo-controlled phase 2 trial with open label extension (OLE) evaluating the safety and efficacy of anti-GM-CSF antibody (Namilumab) in patients with chronic pulmonary sarcoidosis (224). Participants will be randomized to receive a subcutaneous (SQ) injection of Namilumab (or placebo) every 4-weeks for a total of 26-weeks followed by an optional 28-week OLE of active study drug for patients who complete the 26-week double blind treatment period (224). As with CMK389, this trial targets patients with symptomatic chronic pulmonary sarcoidosis refractory to steroids. Patients with significant pulmonary fibrosis (=20%) or SAPH are excluded. The primary study outcome is a change in pulmonary function (assessed by the FVC). Other outcome measures include safety and tolerability of Namilumab, corticosteroid sparing effect, improvement in exercise tolerance (6MWD), overall improvement in HRQoL, and improvement in extrapulmonary organ manifestations of disease (ePOST score) (63). Similar to CMK389, improvement in lung parenchymal disease burden and extent of lung inflammation determined by changes in High-Resolution Computed Tomography Scans (HRCT) and SUVmean changes on PET scan will also be assessed (224). This study aims to enroll 100 participants worldwide and will run through January 2025.

Namilumab is a fully human IgG1 monoclonal antibody that binds with high affinity to GM-CSF and neutralizes its function (225, 226). It has been evaluated in patients with rheumatoid arthritis (227, 228), plaque psoriasis (229) and in hospitalized patients with severe COVID-19 pneumonia (230). While it was found to be effective in controlling symptoms and improving inflammation in patients with rheumatoid arthritis (227, 228) and severe COVID-19 pneumonia (230), it did not have any effect on patients with psoriasis (229). It is currently not FDA approval for any of these indications. GM-CSF is a hematopoietic growth factor produced by T-cells, alveolar macrophages, and fibroblasts (194). It has several proinflammatory effects and increased levels have been found in BALF of patients with active pulmonary sarcoidosis where it has been shown to correlate with disease activity (194, 195). The exact role of GM-CSF in the immunopathogenesis of the sarcoid granuloma is unclear, however, it has been shown to be involved in the alveolar cytokine network that promotes the formation and maintenance of granulomatous inflammation in patients with chronic sarcoidosis (**Figure 3**) (231).

Autoantibodies to GM-CSF (GM-CSFab) have been identified in patients with autoimmune pulmonary alveolar proteinosis (PAP) where they are known to be highly pathogenic (232–234). PAP is a rare life-threatening autoimmune disease characterized by accumulation of excess surfactant in the alveoli causing respiratory failure and predisposing to severe infections (232–234). There are several case reports of detection of GM-CSFab in sarcoidosis patients who subsequently developed PAP (235, 236) and there is concern that neutralizing GM-CSF activity in sarcoidosis patients may precipitate or unmask a co-existent PAP. Studies of Namilumab in patients with Rheumatoid arthritis did not reveal any evidence of lung damage, new-onset PAP, or evidence of opportunistic infections consistent with neutrophil dysfunction known to occur in patients with PAP, however, ongoing monitoring is warranted (228).

Efficacy and safety of intravenous Efzofitimod (ATYR1923) in patients with pulmonary sarcoidosis (NCT05415137)

This is the most advanced ongoing drug trial in pulmonary sarcoidosis. It is a multicenter randomized double-blind placebo-controlled phase 3 study evaluating the safety and efficacy of two IV doses of Efzofitimod (3 and 5 mg/kg) given every 4 weeks to patients with pulmonary sarcoidosis receiving stable doses of oral corticosteroids taken with or without additional immunosuppressant therapy (237). As with the other two trials above, a forced steroid taper is planned. The study plans to enroll 264 patients worldwide for completion in January 2025. Patients to be enrolled must be symptomatic from their disease (mMRC at least 1) and have evidence of disease associated impaired HRQoL (assessed by KSQ-Lung score < 70) (238). As with the other trials above, patients with advanced pulmonary fibrosis, clinically significant SAPH or other advanced and severe forms of extra-pulmonary sarcoidosis are excluded (237). The primary study outcome is steroid-sparing, and the secondary outcome measures are change in FVC and HRQoL as assessed by the Kings Sarcoidosis Questionnaire (KSQ)-Lung Score (239). This study is unique in incorporating HRQoL as a criterion for study enrollment and as a high-priority secondary outcome.

The exact mechanism of action of Efzofitimod in sarcoidosis is unclear. It is a novel immunomodulator that selectively binds the immunoregulatory receptor Neuropilin-2 (NRP2). Neuropilins (NRP) are multifunctional, single pass transmembrane, non-tyrosine kinase surface receptors expressed on all vertebrates (240, 241). Two isoforms (NRP1 and NRP2) have been identified and have been shown to be expressed in various subsets of innate and adaptive immune cells (macrophages, dendritic cells, T cells, B cells, and mast cells) where they regulate cell development, migration, recruitment, and modulate the overall immune response (240, 241). NRP2 is expressed on CD4 + effector T-cells, Treg cells, and in alveolar, bronchial, and intravascular macrophages (240). Immormino et al. found that NRP2 levels in alveolar macrophages were upregulated in a neutrophilic asthma model following challenge with an inhaled antigen/irritant suggesting that NRP2 regulates

airway inflammation and plays a role in that disease (242). Two exploratory/preliminary studies in sarcoidosis showed that NRP2 is expressed in sarcoid granulomas (243, 244), however, its exact role in granuloma formation is unknown (241).

Efzofitimod is a fusion protein comprised of an immunomodulatory histidyl-tRNA synthetase (HisRS) domain fused to a human IgG1 Fc fragment. HisRS is an important pathogenic antigen (Jo-1 antigen) in autoimmune myositis (245–247) and patients with a history of Jo-1 antibody positivity or who screen positive for Jo-1 antibody are excluded from the study (237). The study will also monitor for the development of anti-Jo1 antibodies in study participants. A recently completed phase 2 study of Efzofitimod (ATYR1923) in patients with pulmonary sarcoidosis showed that this drug was safe and well tolerated and there was no signal of increased immunogenicity or formation of anti-Jo1 antibodies (study results published in abstract form) (132).

Studies evaluating alternative treatment approaches in pulmonary sarcoidosis

PREDMETH: Effectiveness of methotrexate vs. prednisolone as first-line therapy for pulmonary sarcoidosis (NCT04314193)

This is a unique first of its kind randomized prospective trial designed to compare the efficacy and side-effects of monotherapy with methotrexate vs. prednisone as first-line therapy for patients with pulmonary sarcoidosis (248). Enrolled patients (treatment naïve pulmonary sarcoidosis patients) will be randomized to receive oral methotrexate (15 mg weekly to be increased to 25 mg weekly) vs. oral prednisone (starting at 40 mg daily to be tapered to 10 mg daily) for 24 weeks followed in both groups by another 18-month period of regular care (25, 248). Patients will be required to perform hospital visits and in addition, will perform weekly home spirometry and record symptoms and medication side-effects *via* a home monitoring application. The primary study objective is to investigate the efficacy and tolerability of methotrexate as first-line therapy in patients with pulmonary sarcoidosis compared to prednisone (25). Secondary objectives are to gain more insights in response to therapy in individual patients by home spirometry and PROMs including the Fatigue Assessment Scale (FAS), the King's Sarcoidosis Questionnaire (KSQ), Global Rating of Change Scale (GRoC), Chronic Respiratory Questionnaire (CRQ), Patient Experience and Satisfaction with Medication Questionnaire (PESaM) and Euroqol-5D-5L questionnaire (EQ-5D-5L) (25). Several biomarkers including (sACE, sIL-2R, Monocyte and T-lymphocyte numbers) will also be examined to find predictors of response to therapy, disease progression and chronicity, and to further improve understanding of the underlying disease mechanism.

The primary study endpoint is change in hospital measured FVC between baseline and 24 weeks (25). The secondary study endpoints include time to pulmonary improvement measured by home-spirometry (home-FVC), percentage of patients with =5 and =10% improvement or decline in FVC and DLCO at 4, 16, and 24 weeks, improvement or decline in HRQoL, experiences and satisfaction with medications, severity and impact of side-effects compared between prednisone and methotrexate, adherence to treatment schedule, and number of patients who discontinue or switch medication (25). Changes in biomarkers over time, and correlation between biomarkers and clinical parameters will also be evaluated as exploratory endpoints (25).

This study is ongoing in The Netherlands and results are expected in January 2025. If this study confirms the hypothesis that methotrexate is as effective as prednisone as first-line therapy for pulmonary sarcoidosis, but with fewer side effects, it will provide an important "steroid alternative" regimen for a small but not insignificant population of patients for whom a "steroid-alternative" or "steroid-avoidance" regimen is essential.

QUIDOSE: A randomized controlled trial of hydroxychloroquine combined with low-dose corticosteroid in pulmonary sarcoidosis (NCT05247554)

This is another large phase 3 RCT that is designed to evaluate the hypothesis that a regimen with lower cumulative prednisone doses will be as effective as one with higher cumulative prednisone doses with less toxicity and better HRQoL measures (34, 35). Patients will be randomized to receive Hydroxychloroquine 400 mg daily for 6-months combined with low-dose prednisone 20 mg daily for 1 month followed by 10 mg daily for 5 months (cumulative prednisone dose of 1,820 mg) vs. initial monotherapy therapy with prednisone 40 mg daily for 4 weeks followed by 30 mg daily for 2 weeks, then 20 mg daily for 2 weeks, then 15 mg daily for 2 weeks then 10 mg daily for 14 weeks for a total 6-month cumulative dose of 2,870 mg of prednisone (249). The primary study outcome is difference in percent predicted FVC between inclusion and 6-months in the two groups.

Hydroxychloroquine is an antimalaria drug that has been shown in case reports and small studies to be effective in patients with cutaneous and pulmonary sarcoidosis, sarcoidosis associated hypercalcemia, and in select patients with neurosarcoidosis (250–254). The mechanism of action of hydroxychloroquine in sarcoidosis is varied. It has anti-inflammatory properties and has been shown to interfere with antigen presentation, prevent T-cell activation, inhibit Toll like receptor signaling, and reduce production of inflammatory cytokines by T and B cells (255, 256). It is very well tolerated with only minimal gastrointestinal side effects reported (257).

Hydroxychloroquine has been associated with retinal toxicity and patients with known retinopathy or maculopathy

are excluded from this study (258, 259). This study is based in France and the planned completion date is March 2024.

SARCORT: Efficacy and Safety of Two Glucocorticoid Regimens in the Treatment of Sarcoidosis (NCT03265405)

is an ongoing randomized parallel assignment open label study in India evaluating the safety and efficacy of a treatment regimen of low dose prednisone (given as 20 mg/day for 8 weeks, followed by 15 mg/day for 8 weeks, 10 mg/day for 4 weeks and 5 mg/day for 4 weeks) vs. medium dose prednisone (given as 40 mg/day for 4 weeks, followed by 30 mg/day for 4 weeks, 20 mg/day for 4 weeks, 15 mg/day for 4 weeks, 10 mg/day for 4 weeks and 5 mg/day for 4 weeks). Participants in both groups will be followed for evidence of disease relapse at the end of 18-months (260). The study authors hypothesize that a higher initial dose of prednisone will be more effective in preventing post treatment relapse than a lower prednisone dose. Enrolled patients will have symptomatic pulmonary sarcoidosis with evidence of impaired pulmonary function (FEV1 $<$ 80%) and/or active extra-pulmonary sarcoidosis requiring treatment. The primary study outcome is the proportion of subjects with a relapse or treatment failure at the end of 18-months. Secondary outcome measures include difference in mean time to relapse, proportion of patients with disease stabilization, improvement, or resolution of disease at 18-months, and the difference in mean FVC at the end of the 6-months. Other secondary outcomes of interest include cumulative prednisone dose, steroid toxicity/adverse effects and HRQoL measured using the Sarcoidosis Health Questionnaire (SHQ) (261) and the FAS.

It is to be noted that patients enrolled in both the SARCORT (260) as well as QUIDOSE (249) trials could potentially be exposed to cumulative steroid doses shown in prior trials to be associated with reduced HRQoL and potentially increased toxicity (34, 35, 262). Patients randomized to the low-dose prednisone arm in SARCORT will receive a 6-month cumulative prednisone dose of 2,380 mg vs. 3,360 mg for patients randomized to the medium dose prednisone arm (260). Participants in the QUIDOSE trial will likewise be exposed to cumulative prednisone doses of 1,820 mg (low dose arm) vs. 2,870 mg in the steroid mono-therapy arm (249). While there is no definition for what quantity of cumulative prednisone doses qualify as "high," "medium," or "low" dose, Judson et al. (35) found that patients who received $>$ 500 mg of prednisone in the preceding year ("high dose prednisone group") had worse HRQoL (35). Cox et al. also reported similarly reduced HRQoL in patients prescribed prednisone, however, the cumulative prednisone doses were not reported (262). More recently, Broos et al. (34) observed that there was no significant difference in the number of patients who experienced an exacerbation or relapse during tapering in patients who receive a 12-month cumulative prednisone dose of 4,000 mg or more ("high dose prednisone group") vs. those who received a lower 12-month cumulative dose ("low dose prednisone group") (34). Additionally, patients who received higher prednisone doses had more toxicity and increased weight gain, and there was no correlation between prednisone dose and pulmonary function as assessed by FVC (34). These studies serve to emphasize the need to report cumulative prednisone doses in all sarcoidosis trials; and importantly also, to clearly define what constitutes a low vs. high dose steroid regimen in cumulative prednisone doses and not just as a final prednisone dose of $<$ 10 mg/day.

Recently completed drug trials in pulmonary sarcoidosis

There have been several recently completed early phase trials of novel therapeutic agents in sarcoidosis.

Nicotine treatment for pulmonary sarcoidosis: A clinical trial pilot study (NCT02265874)

This randomized double-blind placebo-controlled phase 1b/2a pilot study evaluated the safety, efficacy, and tolerability of transdermal nicotine (vs. placebo) given as a daily 21 mg patch for 24 weeks in patients with active symptomatic pulmonary sarcoidosis receiving a maximum daily dose of 10 mg prednisone (or prednisone equivalent) without any concomitant 2nd or 3rd line therapy (211, 263).

Several epidemiologic studies show that cigarette smokers and smokeless tobacco users have a twofold reduced risk of developing sarcoidosis (264–267). Nicotine has a potent immunomodulatory activity on T-cell-mediated inflammation *via* α7 nicotinic cholinergic receptors (α7nAChR) which signal through the JAK-STAT pathway (268–272). At high concentrations, nicotine suppresses antigen-mediated TNF-α production (273), increases the suppressive action of Treg cells (274), and suppress Th1- and Th17-type immune responses (269, 270). These responses appear to be more pronounced in the lungs and are thought to create a microenvironment that results in inhibition of granuloma formation (211, 263, 275).

This study was conducted between October 2015 and January 2019 in two centers in the U.S. and enrolled 49 patients with pulmonary sarcoidosis randomized to receive transdermal nicotine vs. placebo for 24 weeks (211). Study participants were never smokers or former smokers and were required to be non-smoking for at least 6-months prior to study enrollment (211). Patients were followed with serial PFTs, quantitative lung texture score (based on computed tomography (CT) texture analysis), Fatigue Assessment Score (FAS), and HRQoL measures (263). Overall, Nicotine treatment was well tolerated and safe. There was a clinically significant (2%, 70 cc) improvement in FVC and a trend to improvement in FAS. There was no change in HRQoL scores or change in radiographic burden of disease as assessed by serial CT texture analysis (211). Similar to findings

from an earlier study (276), Nicotine was found to be non-addictive (211).

While the results of this study appear positive, it is important to note that it was a small study and larger Phase 3 trials to further evaluate the role of transdermal nicotine as a therapeutic option for sarcoidosis are needed. To ensure a secure place for Nicotine as a treatment option in sarcoidosis, future studies will need to demonstrate improvements in HRQoL and in pulmonary parenchymal disease burden in addition to improvements in pulmonary function. It will also be important to continue to document that prolonged use of Nicotine remains non-addictive. The study was performed in relatively mild patients, with only a quarter on low dose prednisone at time of study entry. Several study participants were newly diagnosed treatment naïve patients (211). A place for nicotine as a first-, second- or third line treatment option in sarcoidosis will need to be determined.

Phase II investigation of antimycobacterial (CLEAR) therapy on progressive, pulmonary sarcoidosis (NCT02024555)

This recently completed phase 2b multi-center randomized double blind placebo controlled trial evaluated the role of antimicrobial therapy given as a concomitant regimen of Levofloxacin 500 mg daily, Ethambutol 1,200 mg daily, Azithromycin 250 mg daily and Rifampin 600 mg daily (or Rifabutin 300 mg daily) for 16 weeks (CLEAR regimen)—to patients with chronic progressive pulmonary sarcoidosis (CPPS) (106). Participants were required to have evidence of parenchymal or nodal disease on chest radiograph (CXR) and met criteria for CPPS if they had any of the following: (1) 5% decline in absolute percentage predicted of FVC or DLCO on serial measurements over 24-months; (2) radiographic disease progression on CXR observed on a side-by-side comparison; or (3) decline in dyspnea score, as measured using the transition dyspnea index (TDI) (106, 124). Patients with end stage fibrotic pulmonary disease [Scadding stage IV on CXR (277)] were excluded. Patients were allowed to continue their baseline immunosuppressive regimen, however, patients on prednisone (or equivalent) doses $>$ 40 mg/day or receiving biologic medications within 6-months of the study were excluded (106).

The study enrolled 97 patients (52% female and 29% African American) across four sites in the U.S. 49 patients were randomized to receive CLEAR regimen and 48 patients to Placebo. Each patient received 8 weeks of four drugs (or matching placebo) followed by 8 weeks of two drugs (or matching placebo) based on individual tolerance and toxicity profile during the first 8 weeks (106). The primary endpoint was change in FVC, however, change in 6MWD, HRQoL, adverse events grades and ESAT-6-specific immune responses were also reported. Overall, this study did not find any benefit of CLEAR therapy over placebo in patients with CPPS (106). Patients on CLEAR therapy had a significant decline in ESAT-6 immune responses, however, there was no corresponding change in FVC or 6MWD at the end of 16-weeks (106). Furthermore, patients randomized to active intervention reported worse HRQoL scores than patients on placebo (106).

The results of this study are in complete contradiction with results of prior studies that provided evidence suggestive of a potential benefit of CLEAR therapy in patients with pulmonary (278) and cutaneous (279) sarcoidosis. The reasons for this discordance are unclear and several plausible theories have been advanced (106). While it is entirely possible that the negative trial results may be accounted for by the presumption that mycobacteria has no role in the etiopathogenesis of sarcoidosis (and thus use of antimycobacterial agents will have no effect), the authors acknowledge that methodological flaws in the study design provide more likely explanations (106). These design flaws will hopefully serve to guide future trials (19). One of the major concerns surrounding the negative study results center on patient selection. It is speculated that by selecting patients with evidence of disease progression over 24 months (in lieu of a shorter duration), the authors may have inadvertently selected for patients with chronic stable disease for whom additional therapy provided no added benefit while incurring additional burden (and negative HRQoL impact) of taking additional pills with increased toxicities. In further support of this point is the observation that patients were selected based on CXR findings without other biomarker evidence of disease activity. Several studies have shown that CXRs are insensitive at detecting active disease in patients with sarcoidosis, and high-resolution CT (HRCT) and 18F-FDG PET scans have been advocated for this purpose (62, 118–122). The authors note that use of 18F-FDG PET scans to detect active disease was not common practice at the time of study design (106). Another concern raised is the absence of a forced steroid or other immunosuppressant (IST) taper (106). Study participants were continued on stable doses of steroids and IST, and this may have blunted the ability to determine the additive contribution of CLEAR therapy while negatively impacting HRQoL due to an overall increased pill burden.

Regardless of the reason for the results obtained, it is important to note that they do not reflect on all antibiotic trials in sarcoidosis. A recently concluded non-controlled open label phase 1b study of Azithromycin 250 mg taken once daily for 3-months in 21 patients with pulmonary sarcoidosis presenting with chronic cough found that Azithromycin led to improved cough metrics and HRQoL measures (280). Patients in this latter study were maintained on corticosteroid monotherapy. Larger phase 2b RCTs of Azithromycin are planned. Perhaps, trials with a reduced pill burden and forced corticosteroid or concomitant IST taper may yield different results.

Other recently completed studies in pulmonary sarcoidosis

Several other studies have been recently completed in patients with pulmonary sarcoidosis. These studies evaluated the role of Canakinumab (a fully human anti-IL1β monoclonal antibody) (NCT02888080) (281); and RCI (NCT03320070) (282) in patients with pulmonary sarcoidosis. Canakinumab is a novel therapeutic agent and the role of IL-1 in sarcoid granuloma formation has been addressed above.

RCI is a complex mixture of prolonged-release adrenocorticotropic hormone (ACTH) and other pituitary peptides (283). It has a complex multi-mechanistic action in sarcoidosis that is distinct from that of corticosteroids (283) and several studies have found RCI to be corticosteroid sparing in sarcoidosis and other autoimmune diseases (126, 133, 283, 284). One of the key outcomes of the RCI phase 4 study (PulSAR trial) is the effect of RCI on the Sarcoidosis Treatment Score (STS) which is a composite measure that captures multiple facets of pulmonary sarcoidosis including pulmonary function (FVC and DLCO), fatigue, HRQoL, fatigue, corticosteroid taper and lung parenchymal changes on HRCT scan (285). Although RCI has a mechanism of action that is distinct from corticosteroids, its side effect profile is identical to that of corticosteroids, and it is important that patients on RCI are rapidly tapered off corticosteroids to minimize toxicity (286).

Friedman et al. also recently published results from a recently concluded small phase 1 proof of concept study evaluating the role of Tofacitinib given as a 5 mg oral pill twice daily for 16-weeks in 5 patients with pulmonary sarcoidosis (128). They found that addition of Tofacitinib allowed 3 out of 5 (3/5) patients with steroid refractory pulmonary sarcoidosis to taper their prednisone to <5 mg daily (128). Additionally, patients reported improved symptom burden and HRQoL scores (128). One patient was withdrawn from the study due to worsening neurosarcoidosis despite stable pulmonary disease. This is a very small study and larger studies are needed.

Studies with a focus on extra-pulmonary and multi-organ sarcoidosis

Sarilumab in patients with glucocorticoid-dependent sarcoidosis (NCT04008069)

This is a small single center phase II study that is planned to enroll 15 patients with steroid refractory pulmonary and extra-pulmonary sarcoidosis randomized to Sarilumab vs. placebo (287). Sarilumab is a recombinant humanized IgG1monoclonal antibody directed against IL-6 receptor (288–290). It is FDA approved for the treatment of moderate to severe rheumatoid arthritis refractory to TNFi therapy and other disease modifying anti-rheumatic drugs (DMARDs) (288–290). The well-established role of IL-6 in the formation of the sarcoid granuloma (**Figure 3**) (157, 291, 292) and evidence that levels of IL-6 correlate with sarcoidosis disease activity and severity (293–295) make it a unique target for sarcoidosis.

All enrolled patients will receive Sarilumab given as a subcutaneous injection every 2 weeks for 16 weeks and will undergo a forced steroid taper. Patients who successfully taper off steroids by week 16, will be randomized to receive continued SQ injections of Sarilumab vs. placebo for an additional 12-weeks. The primary outcome of interest is flare-free survival of Sarilumab treated patients defined as ability to remain off prednisone and other therapies while on Sarilumab. This study targets patients with steroid refractory (prednisone dose 10–60 mg/day) and multi-organ disease. It has a unique study design that incorporates patients with pulmonary sarcoidosis enriched for presence of non-life-threatening multi-organ disease. In addition to patients with pulmonary sarcoidosis, the target population includes patients with active glucocorticoid-dependent sarcoidosis affecting the lymph nodes, liver, kidneys, spleen, bone, soft tissues, skin, and/or eyes while excluding patients with fibrotic pulmonary sarcoidosis (FPS), CNS sarcoidosis, and CS (287). Furthermore, the primary study outcome of flare-free survival could also be considered a correlate of TTCW, and the investigators have incorporated drop-out from placebo arm as a component of this primary outcome measure (287). As expected, the secondary outcome measures assess effect of therapy on multiple organs and include change in pulmonary function (FVC, FEV1), extrapulmonary physician organ severity tool (ePOST) score (63), SASI and size of skin lesions (for cutaneous sarcoidosis), 68/66 Joint evaluation (296), and kidney and renal function. Change in fatigue scores using the Functional Assessment of Chronic Illness Therapy-Fatigue subscale (FACIT-F) (297, 298) are also monitored, however, no other specific HRQoL PROMs are mentioned. This study is projected to be completed in July 2025 and will address a much-needed niche in sarcoidosis (287).

Phase I hypothesis generating study evaluating the role of Tofacitinib in cutaneous sarcoidosis (NCT03793439)

Tofacitinib is a JAK1/JAK3 inhibitor that is currently approved for the treatment of Rheumatoid arthritis refractory to conventional DMARD therapy (299, 300). Anecdotal case reports of successful treatment of patients with refractory cutaneous sarcoidosis using JAK inhibitors (301–303), and immunological evidence of JAK-STAT pathway activation in patients with sarcoidosis (186, 304) has raised interest

in the possible use of JAK inhibitors in patients with sarcoidosis (18).

Damsky et al. (305) recently published an open-label study of ten patients with cutaneous sarcoidosis. The authors found that treatment led to significant improvement of skin lesions using the previously validated cutaneous sarcoidosis activity and morphology instrument (CSAMI) scoring system. In detailed studies, the skin changes were associated with significant changes in the inflammatory profiles. There was some evidence of response for extra-cutaneous manifestations. However, the number of patients with extra-cutaneous disease were small and the end points were poorly described. Nevertheless, this was an important validation of the original case reports (305).

EFIRTES: Efficacy of remission-induction regimen with infliximab for severe extrathoracic sarcoidosis (NCT03704610)

This study conducted in France is focused on patients with extra-pulmonary multi-organ sarcoidosis (306). It is a randomized placebo-controlled phase 3 multi-center study designed to assess the efficacy of a loading dose of infliximab given as a 5 mg/Kg infusion (vs. Placebo) every 2-weeks for two doses (day 0 and 15) in patients with active or recurrent multi-organ sarcoidosis despite ongoing treatment with a first-line immunosuppressive drug. Patients are randomized to receive a loading dose of infliximab (vs. placebo) in the first 2 weeks, followed in both arms by open label injection of infliximab for a total of 3 additional doses in the intervention arm and 5-additoinal doses in the placebo arm. The primary outcome measure is the percentage of patients who have an ePOST score < 1 in all organs (including absence of hypercalcemia) at week 6 regardless of the corticosteroid dosage received, and the secondary outcome measures include the percentage of patients who completed a forced steroid taper while on active intervention and who have an ePOST score < 1 without any evidence of relapse or treatment failure (306). As with the two other studies referenced above, there is no specific mention of HRQoL assessments.

It is to be noted that whilst there is broad consensus that HRQoL is an important and relevant outcome in sarcoidosis research (5, 22, 113), in the current landscape of pharmaceutical trials, HRQoL does not appear to have been accorded a place as a top-tier endpoint and is often only given cursory attention as a secondary endpoint. Additionally, many studies refer to the measurement of HRQoL but do not define if they are measuring all dimensions of HRQoL using condition specific measures or generic measures or in fact health status and symptom burden rather than HRQoL *per se* (26). This lack of attention and transparency around the choice of PROMs will need to be addressed in future studies. Having a dedicated set of core outcome measures that are both organ- and systemic-disease specific will also help to address this.

Studies in severe forms of disease: Fibrotic pulmonary sarcoidosis/cardiac sarcoidosis

Studies in fibrotic pulmonary sarcoidosis

About 10–30% of sarcoidosis patients develop progressive fibrotic disease and it is associated with a significantly increased morbidity and mortality (307, 308). The immuno-etiologic factors driving the transition from chronic sarcoidosis to fibrotic disease are poorly understood (200). While most experts believe that ongoing unbridled granulomatous inflammation is the key driver of fibrosis (200, 309, 310), recently published work showed that fibrotic foci (FF) known to be the hallmark drivers of fibrosis in idiopathic pulmonary fibrosis (IFP) were also present in patients with FPS, and that the gene and protein expressions were similar despite differing initiation pathways (311). This finding suggests that management of patients with FPS will need to address loss of lung function due to both the ongoing inflammation as well as to progressive fibrosis. Thus, studies pointing toward the use of a combined anti-fibrotic and immunosuppressive agent such as mycophenolate mofetil (312, 313) which has demonstrated global significant improvement in lung function in patients with systemic sclerosis, may be worth investigating (314, 315). This is in contradistinction to (or perhaps in addition to) anti-fibrotic agents which demonstrated only deceleration of progressive fibrosis without improvement in other important disease parameters such as HRQoL, cough, dyspnea, or systemic manifestations (316). Patients with FPS express various clinical phenotypes typified in some patients by alternating periods of rapid disease progression followed by periods of stability and in others by a slowly progressive indolent disease course (317, 318).

The INBUILD trial (319) revealed that Nintedanib (an oral intracellular tyrosine kinase inhibitor) was associated with a statistically significant deceleration in disease progression and loss of lung function in patients with progressive fibrosing interstitial lung disease (PF-ILD) regardless of the radiographic pattern of disease or the underlying etiology (319). Since that study was published, the term PF-ILD has now been replaced with progressive pulmonary fibrosis (PPF) (320) and a recently published systematic review and GRADE based meta-analysis of available data regarding the use of Nintedanib in patients with PPF found that a generalized conclusion about the effects of Nintedanib in all the various subtypes of PPF could not be made (321). A *post hoc* analysis of the

INBUILD study found that of the 663 patients enrolled, only 12 patients (<2% of the study population) had sarcoidosis with evidence of PPF (322). 4 of these patients received Nintedanib and 8 received placebo (321, 322). Further evaluation of these 12 patients showed that there was no consistent effect of Nintedanib on lung function (321). Furthermore, none of these patients contributed data to all-cause mortality, adverse treatment effects, or to time to first exacerbation or death (321). When grouped into radiographic patterns, only sarcoidosis patients with a radiographic UIP pattern [as described by Raghu et al. (320)] derived benefit from Nintedanib; however, there were only three such patients and two of them were randomized to placebo (321). Consequently, the role of Nintedanib or other anti-fibrotic therapy in FPS remains unknown.

There is currently no ongoing trial evaluating the role of anti-fibrotic therapy in patients with FPS. A small recently completed phase 2 study that evaluated the role of pirfenidone (vs. placebo) in patients with FPS enrolled 16 patients and was underpowered to determine pirfenidone efficacy (131). In that study, only patients with > 20% fibrosis on HRCT or DLCO < 40% met the clinical end point of time to clinical worsening (TTCW), which was defined as death, lung transplant or > 10% absolute drop in percent predicted FVC at the end of the study period (131).

Patients with FPS present varied radiographic profiles which may correlate with lung function (317, 318, 323). In evaluating the role of anti-fibrotic therapy in sarcoidosis, it will be important to determine how the radiographic pattern of disease [UIP vs. non-UIP pattern (320)] interacts with DLCO in determining which patients respond to therapy. It will also be important to clearly define what disease progression means in patients with FPS as not all the FPS patients with clinical or radiographic worsening will warrant anti-fibrotic therapy (320). Finally, the role of anti-fibrotic therapy in preventing or prolonging time to "acute exacerbation" of disease in FPS will need to be determined. Baughman and Lower (324) showed that patients with FPS experience a high frequency of acute worsening events or "acute exacerbations" (about three per year), however, this was more common in patients who had evidence of bronchiectasis on HRCT (324). A recently reported study of Roflumilast in patients with FPS, notes that these exacerbations were characterized by increased cough and sputum production; and use of the phosphodiesterase 4 (PDE-4) inhibitor (Roflumilast) was associated with a lower rate of acute events than placebo (129). A prior report also found that "acute exacerbations" in patients with FPS responded to short courses of prednisone therapy (325). While Nintedanib increased the time to first exacerbation in patients with both IPF and PPF (326, 327), the mechanism of exacerbation in patients with IPF and other forms of PPF has not been associated with underlying bronchiectasis and particularly in IPF, has not shown consistent response to corticosteroids (328–330).

Studies in cardiac sarcoidosis

There are several ongoing interventional drug trials evaluating new molecules and therapeutic treatment approaches in patients with CS.

RESOLVE-Heart (NCT05351554) is a randomized double-blind placebo-controlled phase 2a study that is planned to evaluate the safety, efficacy, and tolerability of Namilumab in patients with active CS (331). It is a hybrid study with two planned cohorts. Patients enrolled in Cohort A will be randomized to receive SQ Namilumab vs. matching placebo, while all patients in cohort B (open Cohort) will receive active intervention. All study participants will continue their stable doses of prednisone and other background IST for the study duration (30 weeks). The primary study outcome is the safety and tolerability of Namilumab (measured by the incidence and severity of treatment emergent adverse events, serious adverse events, and adverse events leading to discontinuation), however, the effect of intervention on cumulative arrythmia burden, HRQoL, ability to achieve steroid taper, changes in left ventricular ejection fraction (LVEF) and other echocardiographic variables will also be assessed. Patients will have a cardiac PET scan prior to and after enrollment and mean changes in SUVmax will be assessed. The study is expected to begin enrollment soon and expected completion date is January 2024. The mechanism of action and rationale for use of Namilumab in sarcoidosis has been discussed above.

MAGiC-ART (NCT04017936) is an open label Phase 2 pilot study evaluating the role of IL-1 blockade in patients with CS (332, 333). Study participants will be randomized to receive daily subcutaneous injections of Anakinra plus standard of care (vs. standard of care only) for 4 weeks (332, 333). The primary study outcome is change in plasma C-reactive protein (CRP) at 28-days, and secondary outcome measures include change in cardiac inflammation and fibrosis measured by cardiac PET scan and cardiac MRI, and number of serious cardiac events measured by the sum of hospitalizations and deaths from cardiac causes from baseline to 28-days (333). Patients to be enrolled must have evidence of abnormal myocardial uptake and an elevated CRP at baseline (332). In addition to its role in sarcoid granuloma formation (**Figure 3**), IL-1 has been shown to play a significant role in the pathophysiology of heart disease including ischemic heart disease and heart failure (334, 335). Patients with clinically active CS, and evidence of active inflammation on FDG-PET scans have demonstrated evidence of active inflammasomes with IL-1β activity in biopsies of granulomatous lesions obtained prior to cardiac surgery (336). Anakinra is a recombinant human IL-1 receptor antagonist that is currently FDA approved for the treatment of Rheumatoid arthritis (337). Several studies suggest that treatment with Anakinra has been associated with a reduced incidence of heart failure in patients with myocardial infarction, and an improved cardiorespiratory fitness and HRQoL in patients with systolic

heart failure (338–340). There are no prior trials of Anakinra in sarcoidosis. This study is projected to enroll 28 participants and is planned for completion in December 2023 (333).

CHASM CS-RCT (NCT03593759) is a large phase 3 non-inferiority multi-center multi-national RCT designed to evaluate the optimal initial treatment strategy for patients with active CS (24, 341). Newly diagnosed treatment naïve CS patients will be randomized to initiate treatment with prednisone monotherapy or combination therapy with prednisone and methotrexate given for the first 6-months after diagnosis (24). The primary study endpoint is the degree of myocardial fibrosis and scarring measured by the summed perfusion rest score (SPRS) obtained on the 6-month follow-up PET scan (24, 341). Presence and extent of fibrosis (SPRS) was chosen over suppression of inflammation (SUV mean/max) as a primary endpoint consistent with the primary aim of therapy in sarcoidosis being to prevent end organ damage due to fibrosis and not merely to suppress inflammation (24). Secondary study endpoints include mortality, cardiovascular hospitalizations, medication adverse events, glucocorticoid toxicity, generic and disease specific HRQoL, extra-cardiac disease activity, ventricular arrhythmia burden, left, and right ventricular ejection fractions and FDG uptake, several biomarkers, and the burden of late gadolinium enhancement on cardiac MRI (24, 341). This is the largest study in CS and will provide very valuable information on the optimal initial therapeutic option in CS. The rationale for this study is based on findings from several small studies suggesting that CS patients treated with an initial regimen of corticosteroids and IST at diagnosis had better outcomes (reduced rate of relapse and improved or stable LVEF) than those treated with steroid monotherapy (342, 343). Furthermore, these differences persisted even if other IST were subsequently added (342).

J-ACNES (UMIN 000025936) is the Japanese Antibacterial Drug management for CS, an ongoing multicenter open-label RCT designed to investigate the effect of antibiotic treatment in addition to standard corticosteroid therapy in patients with CS (344). The primary objective of this trial is to investigate the clinical benefit and safety of antibiotic therapy (clarithromycin plus doxycycline) in addition to corticosteroids in patients with CS and is based on findings from several studies identifying *Propionibacterium acnes* in sarcoid granulomas of myocardial tissue (344). Newly diagnosed treatment naïve CS patients with evidence of abnormal myocardial uptake will be randomized to receive either corticosteroid therapy plus antibiotic (ABD group) or corticosteroids alone (standard group) for 6-months followed by an observation period of 4.5 years during which all study participants will receive standard corticosteroid therapy (344). The primary study endpoint is change in total SUV at 6 months vs. baseline. Secondary study endpoints include frequency of corticosteroid dose escalation, change in the maximum and mean SUV and change in LVEF at 6- and 12-months, a composite of major adverse cardiovascular events (cardiovascular death, lethal arrythmias and hospitalization for heart failure) at 6, 12, 36, and 60 months, frequency of adverse events and treatment discontinuation, and change in various biomarkers including sACE, lysozyme level, and sIL-2R levels at 6 and 12-months (344). The rate of reduction in plasma *P. acnes* lipoteichoic acid concentration (ACNEX) at 6 and 12 months will also assessed (344). A minimum of 80 patients will be enrolled, however, the final sample size will be dependent on findings from a planned interim analysis (344).

Looking ahead to improve future clinical drug trials

This is an exciting time for therapeutic trials and trials evaluating new treatment approaches in sarcoidosis. For the first time in over two decades, preliminary guidance for the diagnosis and treatment of sarcoidosis have been published and work has begun on defining a set of core outcome measures that can be uniformly applied in clinical trials (1, 5, 22). Additionally, for the first time since 2014 (105, 106), there is a renewed focus on evaluating new molecules and new therapeutic targets for sarcoidosis. While some much-anticipated trials and therapeutic regimens have returned negative results (106), it is important to understand that these trials teach as much or more about the disease process and sarcoidosis clinical trial conduct than those with positive results. Ongoing challenges to clinical trials abound, yet these are not insurmountable.

The outlook to improve future drug trials in sarcoidosis should look to:

- Enhance and diversify subject recruitment and retention by eliminating all real and perceived barriers to trial participation. This includes an active outreach to underserved and minority communities.
- Clearly define target cohorts and optimize mechanisms of ascertaining who has active disease prior to trial enrollment. Concomitant with this is the need to clearly define physiologic and clinical endpoints of disease that are consistent with the pathobiology of sarcoidosis and at the same time meaningful to patients.
- Determine a core set of organ-specific HRQoL and patient-centered outcome measures that will enable comparison of trial results across studies and minimize redundancy and waste of resources.
- Optimize clinical trial designs to enrich study populations of pulmonary sarcoidosis for other organs of interest without detracting from pulmonary specific endpoints.
- Build in protocols that address and manage steroid toxicities for studies whose primary outcome is "steroid-sparing"; while at the same time seeking new and safer endpoints that capture clinical deterioration and lack

of response to therapy without incurring significant steroid toxicities.
- Prioritize studies evaluating the appropriate management of FPS. There is a high morbidity and mortality associated with FPS, yet this group of patients are routinely excluded from clinical trials. Recent findings of the similarities between fibrosis in sarcoidosis and IPF and increasing availability and efficacy of anti-fibrotic therapy should serve as a catalyst for further attention to this severe disease manifestation. The various phenotypes of FPS need to be elucidated and optimal treatment approach for these patients determined.
- Finally, while this manuscript was limited to only pharmacologic intervention, it is to be noted that there have been advancements in non-pharmacological management of sarcoidosis, and these will need to be studied alongside the pharmacologic interventions for a more holistic approach to patient care.

Having come this far, the sarcoidosis community needs to maintain an unflinching resolve to ensure that large scale well designed RCTs be performed for all future therapeutic interventions and that each subsequent/updated guideline reflect an improved grade of evidence for guideline recommendations.

Conclusion

Over 50% of patients with sarcoidosis require treatment for their disease yet none of the medications currently used for sarcoidosis treatment has been rigorously studied in large RCTs (5, 17) and evidence supporting use of these medications is weak in the best of cases.

There are several ongoing RCTs evaluating new therapeutic molecules, novel therapeutic targets and steroid alternative treatment regimens in sarcoidosis with several yielding positive early phase results (211).

While these ongoing trials and future potential trials face significant challenges, it is important to note that these challenges are not insurmountable, and several potential solutions have been proffered in this manuscript.

Author contributions

All authors wrote, edited, reviewed, and contributed key concepts to the manuscript. All authors approved the submitted version.

Conflict of interest

OO had served as principal investigator (PI) for clinical trials conducted by aTyr, Novartis, and Kinevant and provides consultation for Xentria. LS had received research assistance, conducts research, provides consultation or has been a speaker for Argenx, aTyr, Boehringer Ingelheim, Corbus, Eicos, Horizon, Janssen, Kadmon, Kinevant, Mallinckrodt, and United Therapeutics. AR had received speaker fees from Boehringer Ingelheim and Hoffman La Roche. RB had research grants from Janssen, Mallinckrodt, aTyr, Novartis, and Gilead. RB has been a consultant and speaker for Mallinckrodt. All authors attest that there were no potential conflicts of interests with respect to the research, authorship, and/or publication of this manuscript.

Publisher's note

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Integrative bioinformatics analysis to explore a robust diagnostic signature and landscape of immune cell infiltration in sarcoidosis

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Background: The unknown etiology of sarcoidosis with variable clinical features leads to delayed diagnosis and limited therapeutic strategies. Hence, exploring the latent mechanisms and constructing an accessible and reliable diagnostic model of sarcoidosis is vital for innovative therapeutic approaches to improve prognosis.

Methods: This retrospective study analyzed transcriptomes from 11 independent sarcoidosis cohorts, comprising 313 patients and 400 healthy controls. The weighted gene co-expression network analysis (WGCNA) and differentially expressed gene (DEG) analysis were performed to identify molecular biomarkers. Machine learning was employed to fit a diagnostic model. The potential pathogenesis and immune landscape were detected by bioinformatics tools.

Results: A 10-gene signature SARDS consisting of *GBP1*, *LEF1*, *IFIT3*, *LRRN3*, *IFI44*, *LHFPL2*, *RTP4*, *CD27*, *EPHX2*, and *CXCL10* was further constructed in the training cohorts by the LASSO algorithm, which performed well in the four independent cohorts with the splendid AUCs ranging from 0.938 to 1.000. The findings were validated in seven independent publicly available gene expression datasets retrieved from whole blood, PBMC, alveolar lavage fluid cells, and lung tissue samples from patients with outstanding AUCs ranging from 0.728 to 0.972. Transcriptional signatures associated with sarcoidosis revealed a potential role of immune response in the development of the disease through bioinformatics analysis.

Conclusions: Our study identified and validated molecular biomarkers for the diagnosis of sarcoidosis and constructed the diagnostic model SARDS to improve the accuracy of early diagnosis of the disease.

KEYWORDS

sarcoidosis, diagnostic model, WGCNA, machine learning, immune infiltration, functional analysis, biomarker

Introduction

Sarcoidosis is a systemic autoimmune disease characterized by non-caseous necrotizing epithelioid granulomas that can affect various organs and tissues such as the lung, eye, skin, heart, and nervous system, with a predominance in young and middle-aged people (1, 2). About 25% of these patients present a chronic, progressive process. Eventually, it can lead to irreversible pathologies, including pulmonary fibrosis, cirrhosis, fatal arrhythmias, and blindness, seriously affecting patients' life quality and longevity (3, 4). Sarcoidosis has a mortality rate of ∼7% over a 5-year follow-up period (5). However, patients have significant heterogeneity in the tissues and organs involved, clinical manifestations, responses to treatment, and prognosis, leading to the diagnosis of sarcoidosis relying on a comprehensive assessment of clinical presentation, imaging, and pathology characteristics. Invasive methods such as pathological biopsies are still constrained by samples' accessibility, resulting in delayed diagnosis. Therefore, easily accessible diagnostic approaches are necessary to help patients be diagnosed as early as possible before irreversible pathology to avoid delaying the optimal time for treatment. Given that the etiology of sarcoidosis has not been elucidated, the first line of therapy for sarcoidosis patients is oral glucocorticoids (5), despite their severe side effects (6).

With the rapid advances in bioinformatics, the assessment of blood transcriptional signature may provide a fast, easily accessible, and convenient screening approach to identify potential molecular biomarkers for diagnosing disease and explore the latent pathogenesis and immunological characteristics to provide additional therapeutic perspectives for better individual treatment. Molecular biomarkers from the blood transcriptome are widely used for disease diagnosis and pathogenesis exploration. Several studies have documented that gene expression profiling of peripheral blood could be used as biomarkers in multisystem diseases and immune-related disorders (7–11), like monitoring multiple sclerosis progression (12) and response to treatment and distinguishing active tuberculosis from other infectious and inflammatory diseases (13).

Our study collected 11 microarray cohorts of sarcoidosis patients from the Gene Expression Omnibus (GEO). Our study is committed to identifying specific gene profiles correlated with sarcoidosis through bioinformatics analysis and constructing a robust sarcoidosis diagnostic signature (SARDS). Additionally, the results might provide new insights into the pathogenesis, immune characteristics, and potential treatment options for sarcoidosis.

Methods

Data collection and processing

We downloaded the gene expression profile from GEO (http://www.ncbi.nlm.nih.gov/geo/) by searching "sarcoidosis." The inclusion criteria were as follows: (i) The datasets contained total RNA gene expression microarray data; (ii) the datasets included sarcoidosis and normal samples: the samples can be one of the five forms, including whole blood, peripheral blood mononuclear cells (PBMC), bronchoalveolar lavage (BAL) cells, and lung tissue; and (iii) the datasets had five samples of both sarcoidosis and normal patients at least. The data processing procedure of the research was illustrated in the workflow (Figure 1).

Weighted gene co-expression network analysis

The consensus WGCNA approach was employed to cluster genes with similar expression patterns and filter out clusters of co-expressed genes called "modules" which are highly associated with sarcoidosis via the "WGCNA" R package (14). First, the expression of genes was ranked by standard deviation, and the top 5,000 genes were selected for the subsequent analysis. Next, hierarchical cluster analysis was performed to determine whether there were outlier samples. Soft-thresholding power was based on scale-free analysis and set as the lowest power with a scale-free topology model fit >0.9 by the "pickSoftThreshold" function in the "WGCNA" R package. The soft threshold and the gene similarity matrix calculated by Pearson correlation values between each gene pair were used to construct the adjacency matrix. Subsequently, the adjacency matrix was

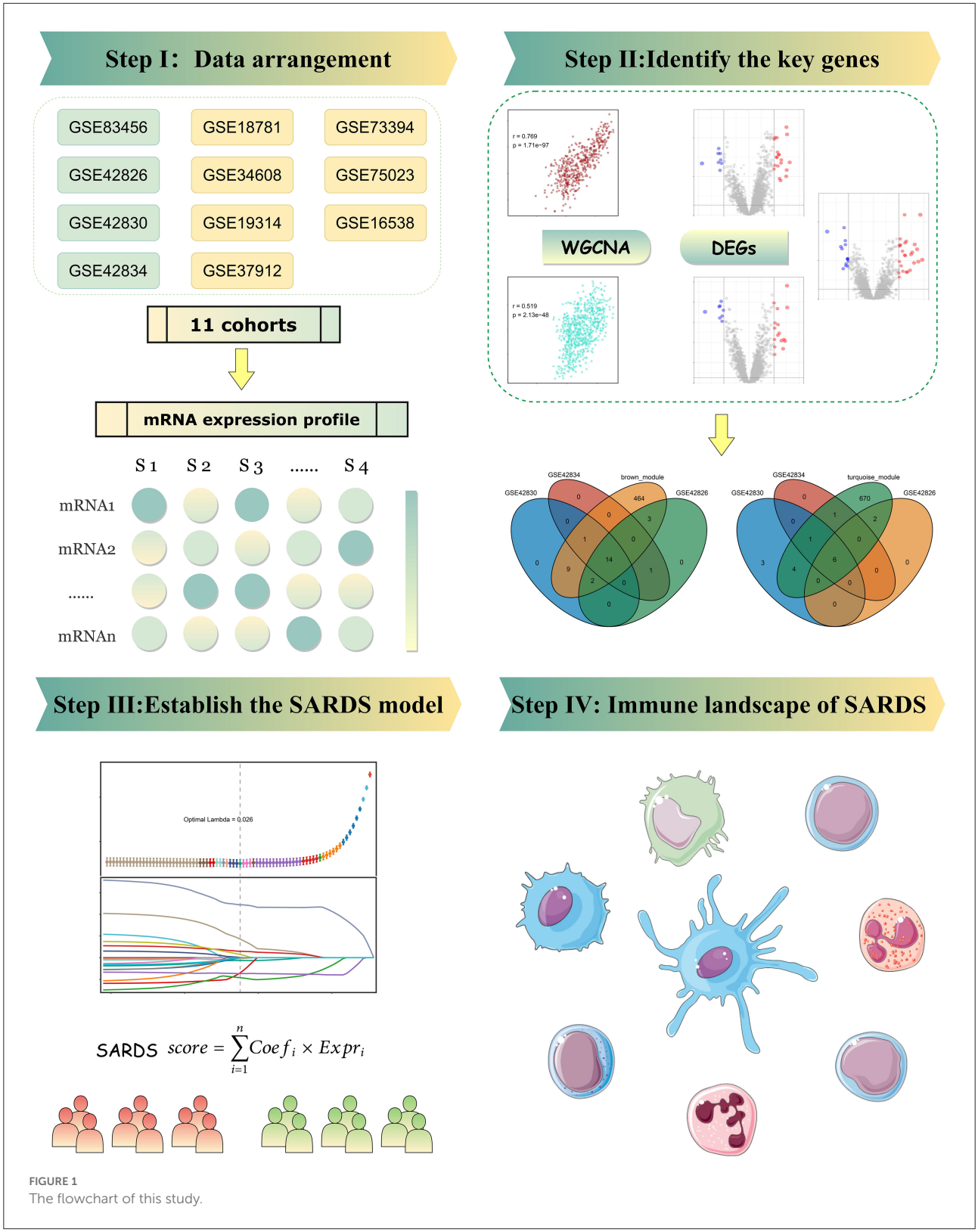

FIGURE 1
The flowchart of this study.

transformed into a topological overlap matrix (TOM) and a 1-TOM, reflecting the similarity and dissimilarity between genes separately. MinModuleSize was set to 50 to ensure each module had a minimum of 50 genes. Deep Split was set to two to identify modules using dynamic tree cut. MEDissThres was set to 0.2 to cluster module eigen genes (MEs). These genes were

classified into different MEs calculated to represent the gene expression. We calculated the correlation between diagnosis and MEs to screen key modules for further analysis. The genes with high gene significance and module membership were considered essential genes.

Functional enrichment analysis

The functional enrichment analysis contained Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis. The biological processes, cellular components, molecular functions, and relevant pathways of the genes in the key MEs were implemented via the "clusterProfiler" R package (15). According to the Benjamini–Hochberg procedure, the *P*-adjusted value was further computed. Set *P*-adjusted value <0.05 as statistically significant.

Identification of key regulative genes

The differentially expressed genes (DEGs) between sarcoidosis and normal blood samples were screened via the "limma" R package. We set *P*-adjusted value <0.05 and $|\log2 \text{ fold change (logFC)}| > 1$ as the threshold of DEGs. The intersection of the GSE83456 positively correlated module genes with the upregulated differentially expressed genes of GSE42826, GSE42830, and GSE42834 was believed to be the key upregulated genes of sarcoidosis. Similarly, the intersection of the GSE83456 negatively related module genes with the downregulated differentially expressed genes of GSE42826, GSE42830, and GSE42834 was thought to be the key downregulated genes of sarcoidosis.

LASSO machine learning algorithm

The least absolute shrinkage and selection operator (LASSO) was performed to obtain a robust diagnostic performance model. LASSO is a popular algorithm that is broadly utilized in medical studies (16–18). 10-fold cross-verification was performed to determine the Lambda minimum. This machine learning algorithmic procedure was implemented with the "glmnet" R package. In addition, LASSO can obtain relevant genes for the diagnosis of sarcoidosis for further mechanistic studies. The receiver operator characteristic (ROC) curves were generated, and the area under the ROC curve (AUC) assessed the performance of the disease diagnostic model.

Gene set enrichment analysis

The normalized enrichment scores (NES) were calculated for sarcoidosis based on the diagnostic model scores on GO terms and KEGG pathway in the Molecular Signature Database (MSigDB) via all GO gene sets (c5.go.v7.4.symbols.gmt) and KEGG gene sets as Gene Symbols (c2.cp.kegg.v7.4.symbols.gmt), respectively. We set $|\text{NES}| > 1.50$, and *P*-adjusted value <0.01 as cutoff criteria.

Evaluation of immune landscape

Single sample GSEA (ssGSEA) (19) that generates enrichment scores for a single sample was used to explore differences in immune cell infiltration between sarcoidosis and normal samples. The abundance of the 24 immune infiltrating cells was calculated and visualized by the "GSVA" R package (v1.42.0). In addition, correlation coefficients between the diagnostic model scores and the immune cell abundance of the samples were calculated to investigate the significant immune cells involved in sarcoidosis and the immune mechanisms.

Statistical analysis

Data processing, statistical analysis, and plotting were carried out in the R 4.1.2 software. The correlation between two continuous variables was assessed using Pearson's correlation coefficient. Comparisons of categorical variables were done using the Chi-square test, while comparisons of continuous variables were done using the Wilcoxon rank-sum test or *t*-test. *P*-value <0.05 was determined to be statistically significant.

Results

Data acquisition from GEO

A total of 313 sarcoidosis patients from 11 public datasets were collected for further analysis (Supplementary Table 1). A total of 11 datasets were selected: six came from whole blood samples (GSE42834, GSE83456, GSE42826, GSE42830, GSE18781, and GSE34608), two came from PBMC samples (GSE19314 and GSE37912), two came from BAL cells samples (GSE73394 and GSE75023), and one came from lung tissue samples (GSE16538). The baseline characteristics can be found in Supplementary Table 2. The gene expression data of four datasets (GSE83456, GSE42834, GSE42826, and GSE42830) were used to screen the essential

genes. The seven remaining datasets were used as the validation sets.

Identification of key modules through WGCNA

The GSE83456 dataset was used to identify the key MEs related to sarcoidosis. First, no outlier sample was removed based on the sample tree, and then totaling 110 samples, the top 5,000 genes were used for WGCNA. The soft-thresholding power was set to nine to fit the scale-free network (Figures 2A,B). Second, the gene similarity matrix was constructed as an adjacency matrix according to the Pearson correlation values. The adjacency matrix was converted to the TOM and 1-TOM, reflecting the similarity and dissimilarity between genes separately. Third, the co-expression modules in the network were identified using the "cutreeDynamic" function, and all genes were clustered into 10 modules. These modules were further merged to nine MEs using the "mergeCloseModules" function plotted by clustering dendrogram (Figure 2C) and heatmap of the eigengene adjacency (Figure 2D). Figure 2E shows the heatmap of the topological overlap matrix of genes selected by WGCNA. The relationships between the MEs and sarcoidosis were visualized in the module-trait relationship diagram (Figure 2F).

Enrichment analyses of genes in key modules

Among the nine modules, the brown module was the most positively correlated module with sarcoidosis, including 493 genes, and the correlation between module membership and gene significance was 0.769 ($P < 0.0001$) (Figure 3A). The turquoise module was the most negatively correlated module, including 684 genes and the correlation was 0.519 ($P < 0.0001$) (Figure 3B). Figure 3C displayed that the genes of the brown module were significantly enriched in "defense response to virus," "defense response to symbiont," "response to virus," "type I interferon signaling pathway," and "regulation of innate immune response" in GO terms. Figure 3D illustrated that the genes of the turquoise module were significantly enriched in "ncRNA processing," "ncRNA metabolic process," and "ribonucleoprotein complex biogenesis" in GO terms. The enriched KEGG pathways of the brown module genes, including "Epstein-Barr virus infection," "Influenza A," "Antigen processing and presentation," and "Allograft rejection" were shown in Figure 3E. The enriched KEGG pathways of the turquoise module included the "RNA degradation," "Th17 cell differentiation," and "T cell receptor signaling pathway" (Figure 3F). The enrichment analysis results indicated that inflammatory and immune cells played an essential role in the process of sarcoidosis.

Identification of DEGs

The DEGs between sarcoidosis and normal patients were explored by the "limma" R package. In the GSE42826 dataset, 20 significantly upregulated genes and eight significantly downregulated genes were defined, shown as a volcano plot and heatmap in Figures 4A,B. The GSE42830 dataset identified 26 considerably upregulated genes and 14 significantly down-regulated genes, shown in Figures 4C,D. Similarly, in the GSE42834 dataset, 16 upregulated genes and 8 significantly downregulated genes were defined, demonstrated as a volcano plot and heatmap in Figures 4E,F.

Construction of a sarcoidosis diagnosis signature via machine learning

First, the key regulated genes were screened to further model construction by the intersection of the genes in the key module of WGCNA and the significantly regulated genes in the three datasets. Fourteen key upregulated genes were screened through the intersection of the brown module genes of WGCNA and the significantly upregulated genes of the three datasets (Figure 5A). Likewise, six key downregulated genes were screened through the intersection of the turquoise module genes and the significantly downregulated genes of three datasets (Figure 5B). A total of 20 key genes have been exploited as stable and reliable sarcoidosis diagnostic signatures (SARDS) to diagnose sarcoidosis at the gene level by applying the LASSO algorithm. The optimal lambda was 0.026 when the LASSO regression partial likelihood deviation was minimized (Figure 5C). Therefore, 10 key genes with non-zero LASSO coefficients were considered the main variables in the diagnostic model (Figure 5D). The 10 genes were *GBP1*, *LEF1*, *IFIT3*, *LRRN3*, *IFI44*, *LHFPL2*, *RTP4*, *CD27*, *EPHX2*, and *CXCL10* with the coefficients 0.244, −0.0925, 0.0855, −0.0732, −0.0703, 0.0292, 0.0149, −0.0131, −0.00522, and 0.000941, respectively. The SARDS was established with the following formula: SARDS score = 0.445 + 0.244 × Exp *GBP1* - 0.0925 × Exp *LEF1* - 0.0855 × Exp *IFIT3* - 0.0732 × Exp *LRRN3* - 0.0703 × Exp *IFI44* + 0.0292 × Exp *LHFPL2* + 0.0149 × Exp *RTP4* - 0.0131 × Exp *CD27* - 0.00522 × Exp *EPHX2* - 0.000941 × Exp *CXCL10*.

SARDS validation in different cohorts

ROC curves were used to assess the diagnostic efficacy of SARDS in 11 cohorts. The GSE83456, as the training cohort, performed an excellent AUC of 1.00 (Figure 6A). The

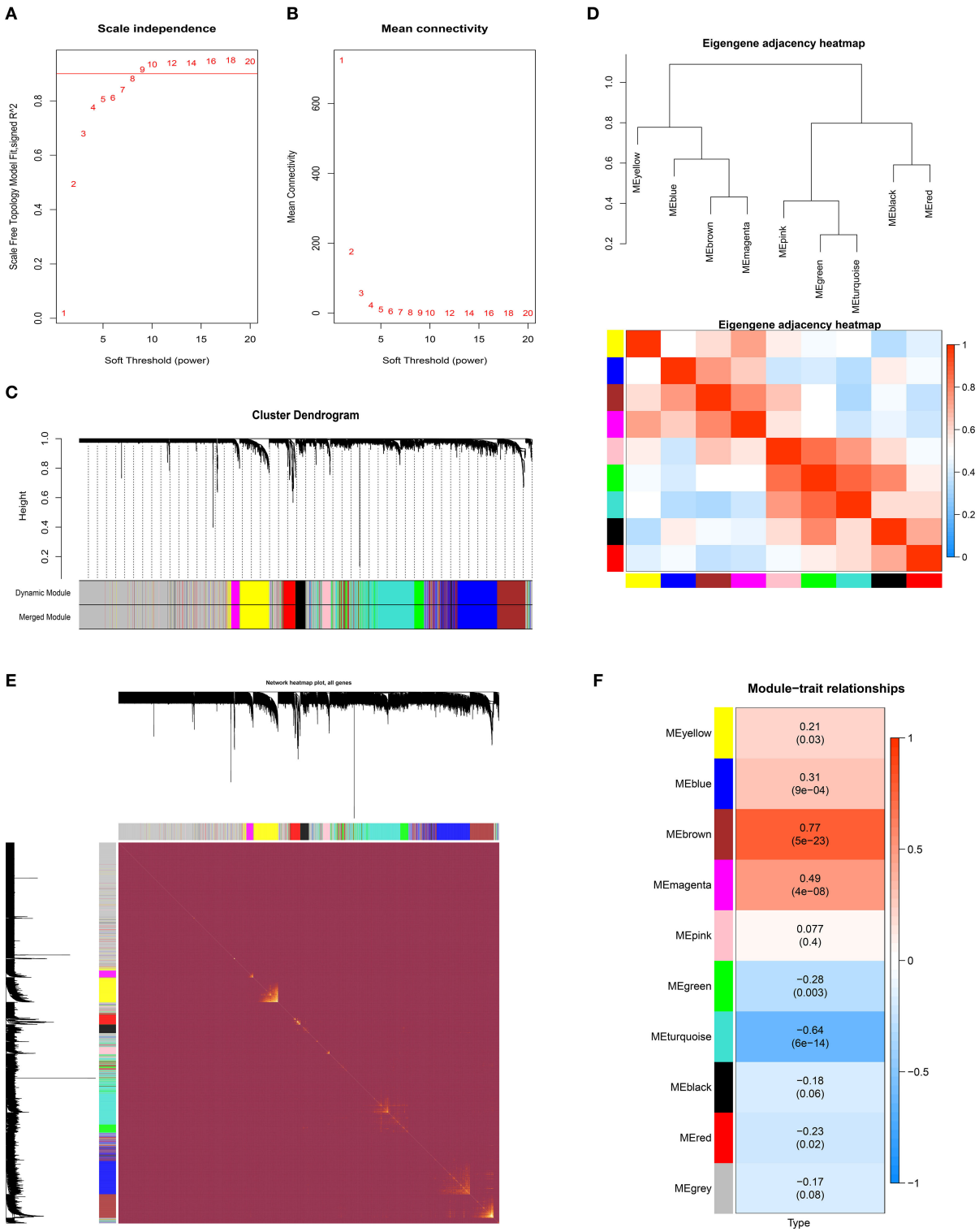

FIGURE 2
The weighted gene co-expression network analysis of sarcoidosis. **(A)** Scale-free topological model fit at various soft-thresholding powers. **(B)** Mean connectivity for different soft-thresholding powers of the network. **(C)** Gene clustering dendrograms based on hierarchical clustering under optimal soft-thresholding power. **(D)** The heatmap of the eigengene adjacency. **(E)** The heatmap of the topological overlap matrix of genes selected by WGCNA. **(F)** The relationships between MEs and sarcoidosis.

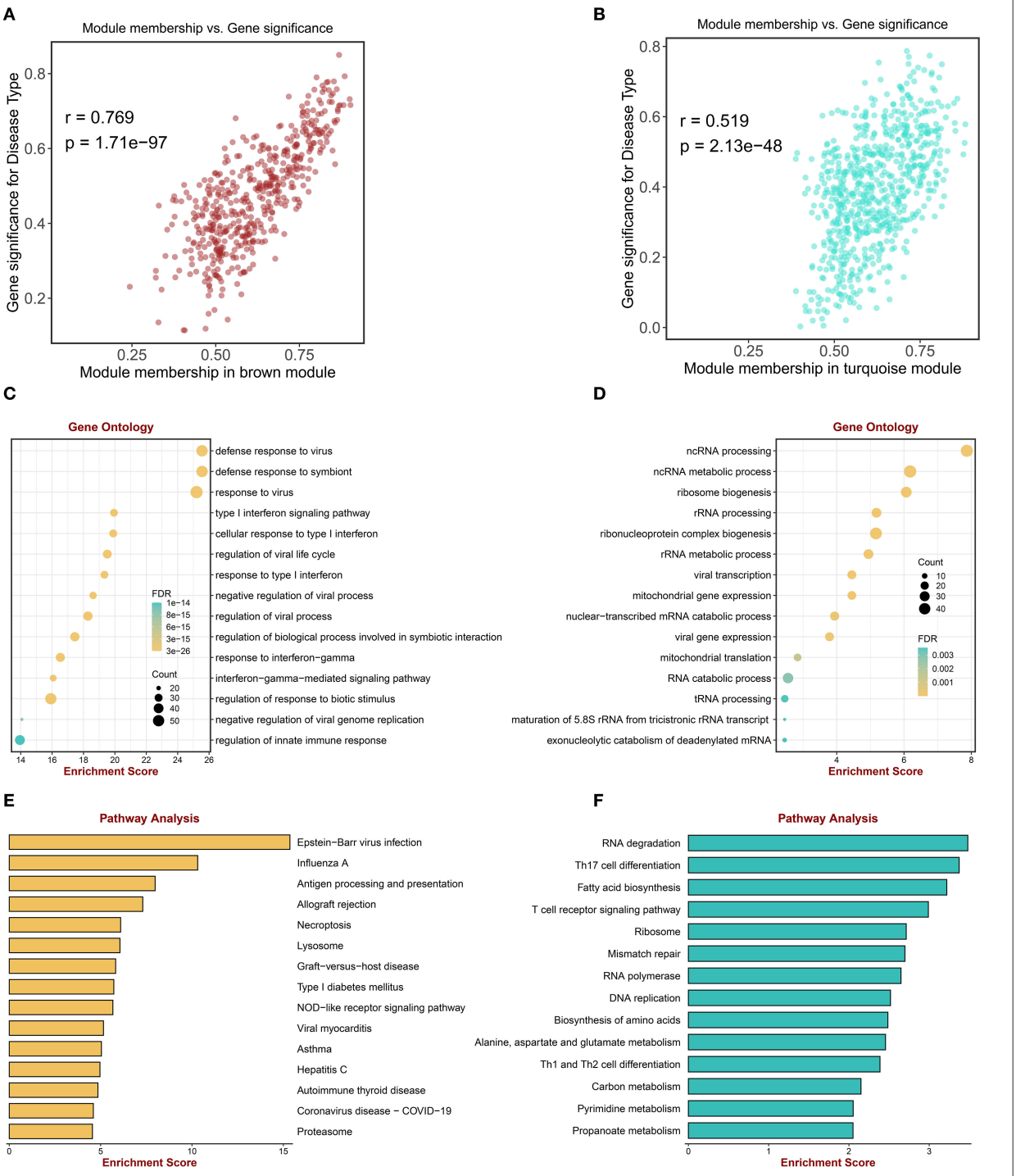

FIGURE 3
Enrichment analysis of genes in key MEs. **(A)** The scatter plot correlation between the brown module membership and gene significance. **(B)** The scatter plot of correlation between the turquoise module membership and the gene significance. **(C)** Go enrichment analysis of genes in the brown module. **(D)** GO enrichment analysis of genes in the turquoise module. **(E)** KEGG pathway analysis of genes in the brown module. **(F)** KEGG pathway analysis of genes in the turquoise module.

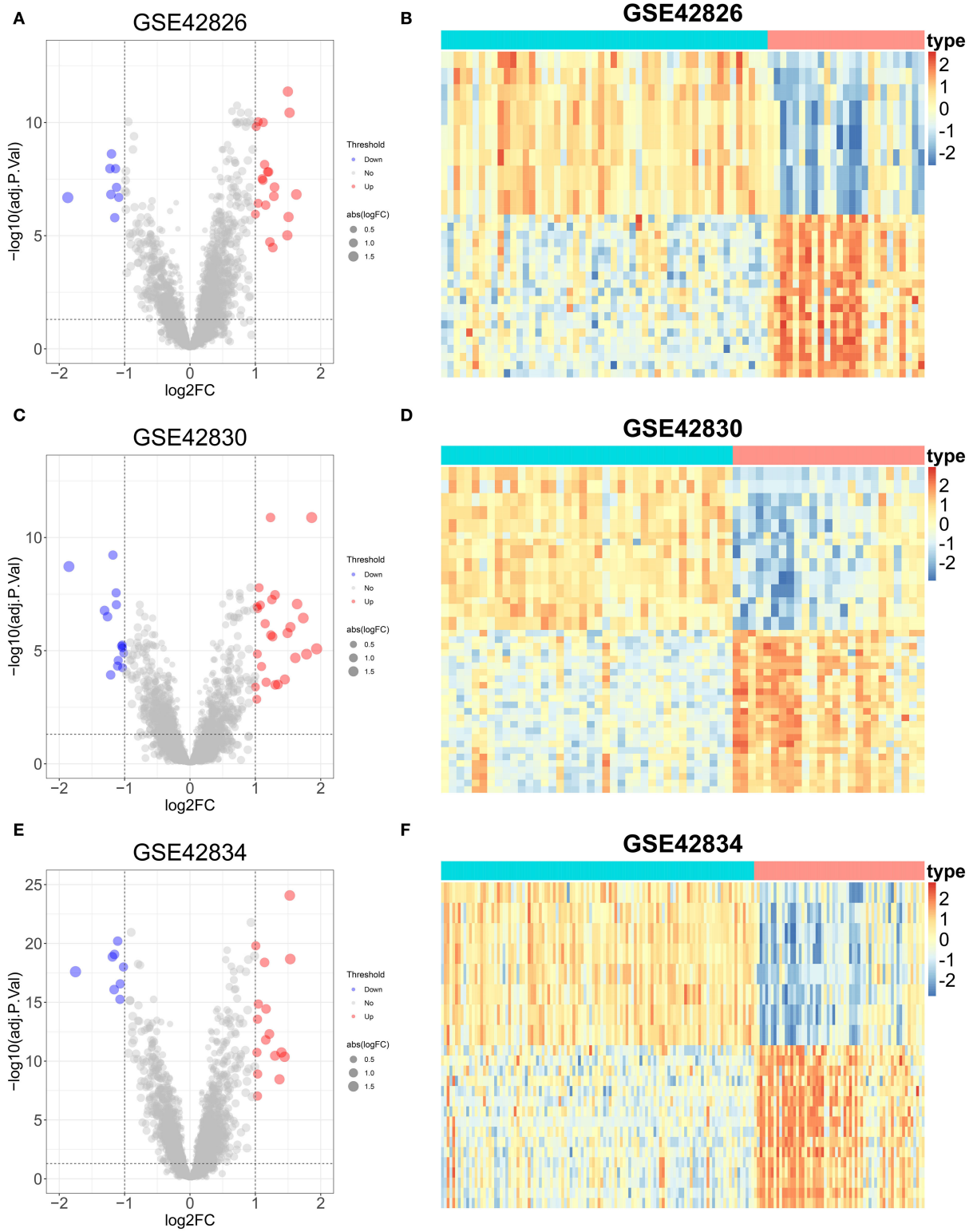

FIGURE 4
Differential expression genes analysis of three sarcoidosis datasets. **(A)** The volcano plot of DEGs in GSE42826. **(B)** The heatmap of the top 50 DEGs in GSE42826. **(C)** The volcano plot of DEGs in GSE42830. **(D)** The heatmap of the top 50 DEGs in GSE42830. **(E)** The volcano plot of DEGs in GSE42834. **(F)** The heatmap of the top 50 DEGs in GSE42834.

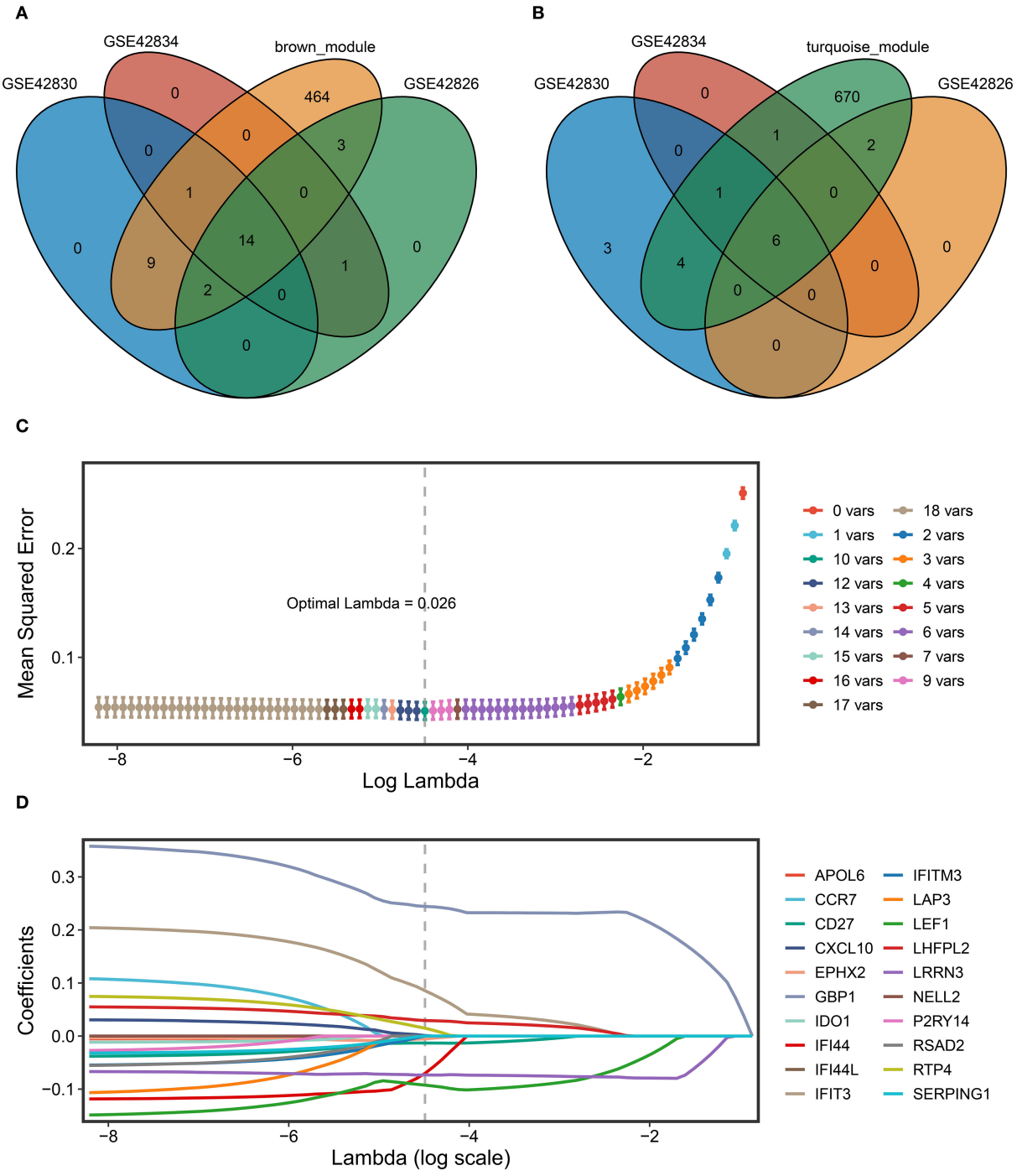

FIGURE 5
The construction of the SARDS based on the LASSO algorithm. **(A)** The Venn diagram of the intersection between the upregulated DEGs in three datasets and genes in the brown module. **(B)** The Venn diagram of the intersection between the downregulated DEGs in three datasets and genes in the turquoise module. **(C)** Determination of the optimal lambda was obtained when the partial likelihood deviance reached the minimum value, and further generated the key gene with nonzero coefficients. **(D)** LASSO coefficient profiles of the candidate gene for SARDS construction.

GSE42830, GSE42834, and GSE42826, which were involved in screening the key genes, performed outstanding AUCs of 0.987, 0.951, and 0.938 (Figures 6B–D). The GSE34608 and GSE18781, as the validation dataset of whole blood samples, had superior diagnostic efficacy in that AUCs were 0.972 and 0.960 (Figures 6E,F). Meanwhile, the cohorts with samples of

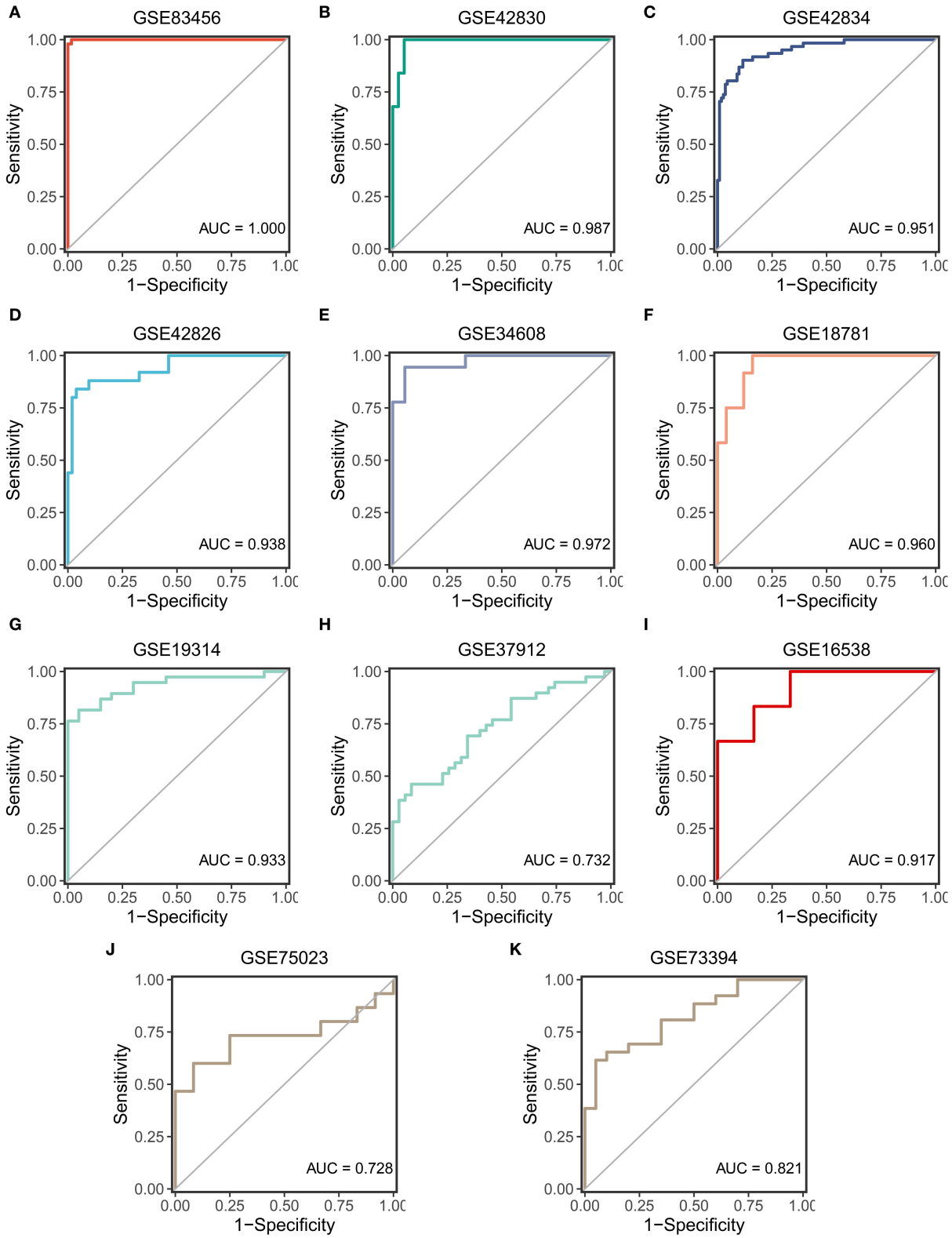

FIGURE 6
The validation of the SARDS in 11 cohorts. **(A)** The ROC curve of the modeling dataset (GSE83456). **(B–K)** The ROC curves of validation datasets (GSE42830, GSE42834, GSE42826, GSE34608, GSE18781, GSE19314, GSE37912, GSE16538, GSE75023, and GSE73394).

PBMC, BAL cells, and lung tissue were enrolled in the validation cohort of the SARDS. The GSE19314 and GSE37912 of PBMC samples performed AUCs of 0.933 and 0.732 (Figures 6G,H). The GSE16538 of lung tissue had an AUC of 0.917, shown in Figure 6I. The GSE75023 and GSE73394 displayed the great AUCs of 0.728 and 0.821 (Figures 6J,K). The SARDS has been proven to be a robust and reliable diagnostic model of sarcoidosis.

Exploration of mechanisms based on the SARDS score

The correlation between SARDS scores and gene expression was calculated for gene sequencing to detect latent mechanisms of sarcoidosis by applying GSEA. The most important GO terms and the KEGG pathways were displayed in the ridge plot (Figures 7A,B). Among these, Figure 7C depicted the top five positively relevant GO terms, including "Response to interferon gamma," "Response to type I interferon," "Antigen processing and presentation of exogenous peptide antigen via MHC class I," "Defense response to virus," and "Myeloid leukocyte mediated immunity." Figure 7D depicted the top five negatively relevant GO terms, comprising "Nuclear transcribed mRNA catabolic process nonsense mediated decay," "ncRNA processing," "Ribosome biogenesis," "Ribonucleoprotein complex biogenesis," and "ncRNA metabolic process." On the other hand, Figure 7E described the top five positively correlated KEGG pathways, consisting of "Leishmania infection," "Lysosome," "Toll like receptor signaling pathway," "Proteasome," and "Graft vs. host disease." Likewise, Figure 7F described the top five negatively correlated KEGG pathways, consisting of "Ribosome," "RNA degradation," "Alanine aspartate and glutamate metabolism," "Nucleotide excision repair," and "Spliceosome."

The immune landscape of sarcoidosis

Given that sarcoidosis is a systemic inflammatory disease of unknown mechanisms, it is essential to exploit the immune microenvironment of sarcoidosis patients. The ssGSEA algorithm was performed to estimate the infiltration abundance of 24 types of immune cells between sarcoidosis and normal patients. The heatmap and boxplot demonstrated the fraction and expression differences of 24 types of immune cells in the GSE83456 cohort (Figures 8A,B). It was evident that the superior abundance of the anchorage-dependent cell (aDC), macrophages, immature dendritic cells (iDC), neutrophils, plasmacytoid dendritic cells (pDC), eosinophils, Th1 cells, and mast cells and the inferior infiltration of T cells, Central Memory T cell (Tcm), T follicular helper cell (TFH), CD8 T cells, B cells, Th2 cells, and T helper cells were the immune signatures of the sarcoidosis patients. The correlations between different immune cells were shown in the heatmap (Figure 8C). The T helper cells and CD8 T cells showed the strongest positive correlation, and DC and T helper cells showed the strongest negative correlation. The correlation between the SARDS score and immune infiltration was shown in Figure 8D. We can see that the infiltration level of aDC cells ($r = 0.680$, $P < 0.0001$), macrophages ($r = 0.591$, $P < 0.0001$), iDC ($r = 0.423$, $P < 0.0001$), neutrophils ($r = 0.355$, $P = 0.0001$), pDC ($r = 0.309$, $P = 0.0011$), and eosinophils (r = 0.213, $P = 0.0260$) were positively correlated with the SARDS score; the infiltration level of T cells ($r = -0.643$, $P < 0.0001$), Tcm ($r = -0.618$, $P < 0.0001$), TFH ($r = -0.531$, $P < 0.0001$), CD8 T cells ($r = -0.463$, $P < 0.0001$), B cells ($r = -0.420$, $P < 0.0001$), and Th2 cells ($r = -0.405$, $P < 0.0001$) were negatively associated with the SARDS score.

Discussion

The diversity of clinical symptoms in sarcoidosis and the lack of a single reliable diagnostic criterion make prompt and accurate diagnosis challenging. In addition, the etiology and pathogenesis of sarcoidosis remain unknown, making treatment available considerably limited. Therefore, exploring the latent mechanisms and constructing an accessible and reliable diagnostic model of sarcoidosis is vital for innovative therapeutic approaches to improve prognosis.

Our study was based on gene transcriptome analysis of 303 sarcoidosis samples and 400 normal controls. Two modules with the highest correlation to sarcoidosis were identified through WGCNA, namely brown and turquoise modules. The brown module, containing 493 genes, demonstrated that the defense response to the virus and innate immune response might play an essential role in the pathogenesis of sarcoidosis, which was consistent with the results of previous studies. Numerous immunological arguments had indicated that inadequate clearance of viral particles, accompanied by various immunodeficiencies, might be relevant to sarcoidosis disease (20). In patients with sarcoidosis, viruses have evolved strategies to evade or suppress host cell defenses utilizing the process of autophagy (20). The primary regulator of the innate immune response in sarcoidosis was the alveolar macrophage, which both produced pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), contributing to the production of granulomatous lesions, and acted as an antigen-presenting cell (APC) interacting with T cells via human leucocyte antigen (HLA) molecules and T cell receptors (21, 22). The turquoise module, containing 684 genes, revealed that non-coding RNA (ncRNA), including processing, metabolic processes and degradation, and immune cells, was strongly associated with sarcoidosis through enrichment analysis. Regulative ncRNAs can be classified into microRNAs (miRNAs), long ncRNAs

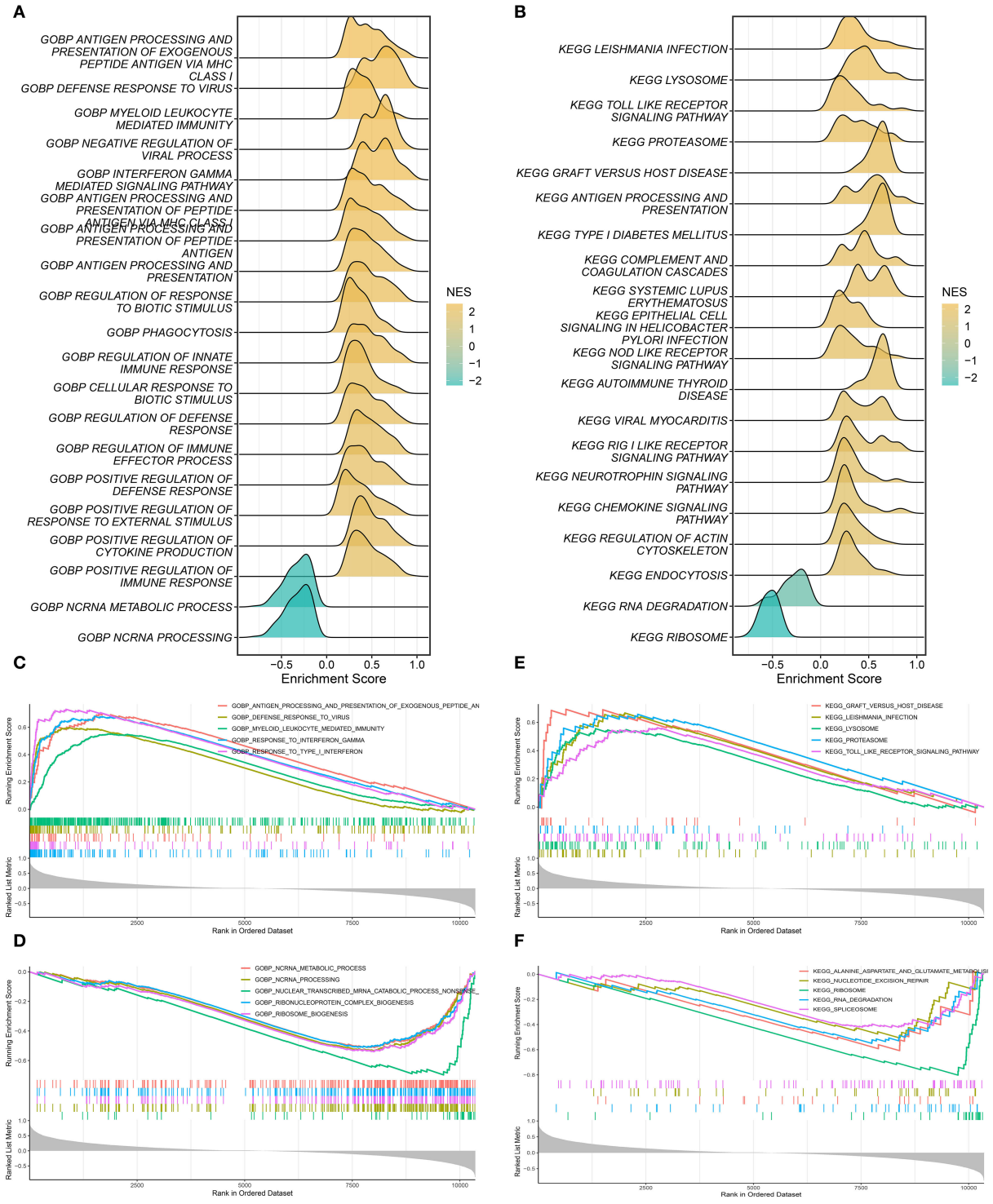

FIGURE 7
Gene set enrichment analysis. **(A)** The ridge plot of the top 20 GO terms with ranked genes of the GSE83456. **(B)** The ridge plot of the top 20 KEGG pathways with ranked genes of the GSE83456. **(C,D)** The positive and negative top five GO terms with ranked genes of the GSE83456. **(E,F)** The positive and negative top five KEGG pathways with ranked genes of the GSE83456.

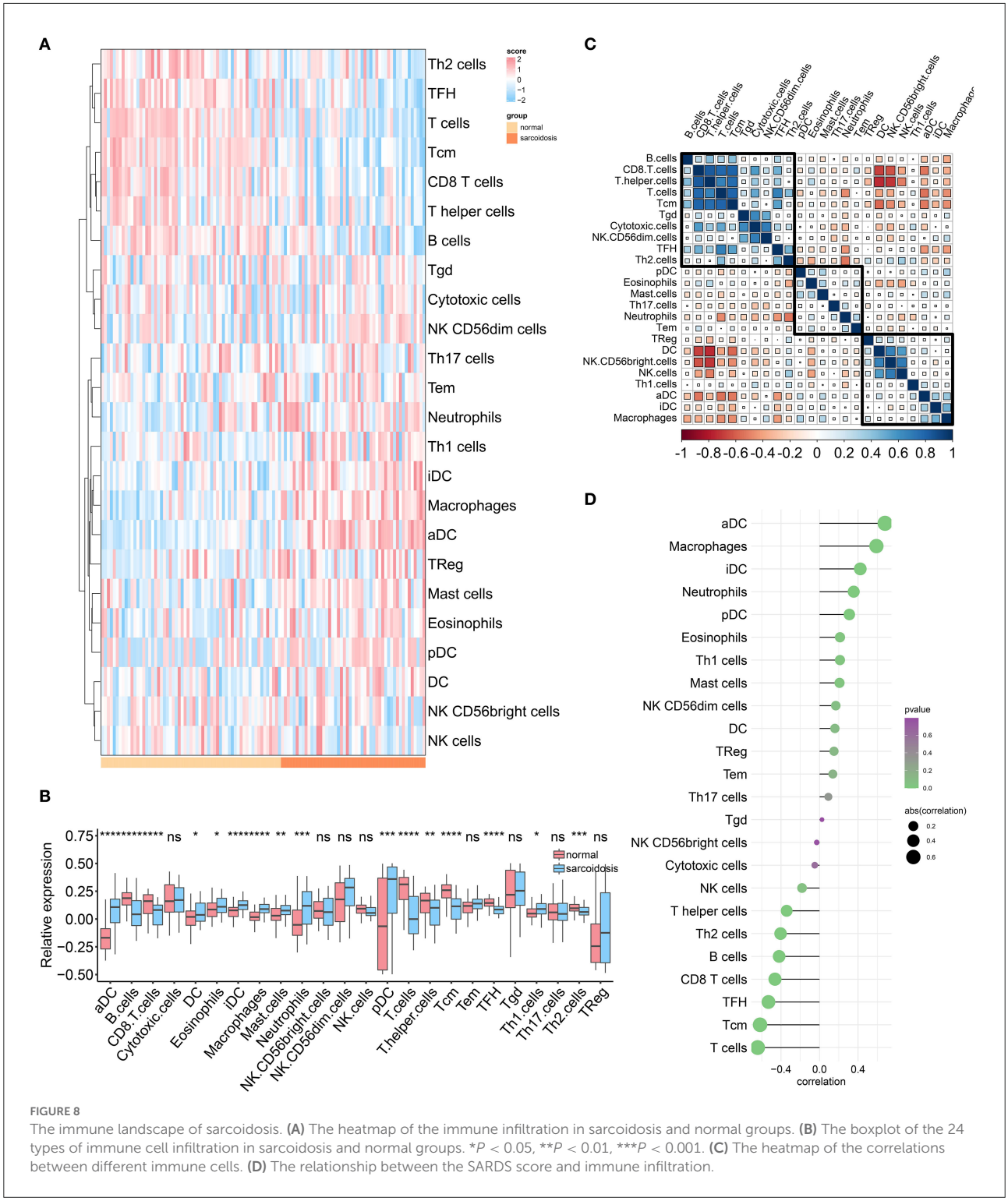

FIGURE 8
The immune landscape of sarcoidosis. **(A)** The heatmap of the immune infiltration in sarcoidosis and normal groups. **(B)** The boxplot of the 24 types of immune cell infiltration in sarcoidosis and normal groups. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. **(C)** The heatmap of the correlations between different immune cells. **(D)** The relationship between the SARDS score and immune infiltration.

(lncRNAs), and small interfering RNAs. Previous research has indicated potential associations between the dysregulation of some miRNAs and the diagnosis and prognosis of sarcoidosis (23, 24). In PBMCs from patients with sarcoidosis, levels of miRNA-34a were increased, which downregulated sirtuin (SIRT) 1 and stimulated the secretion of INF-γ (25). SIRT1 is an essential mediator of energy metabolism and tissue survival, and INF-γ is necessary to develop and maintain the

sarcoidosis status. These all contributed to the NF-κB-mediated inflammatory response in patients with sarcoidosis (26). In addition, miR-let7f, miR-15b, miR-16, miR-20a, miR-27b, miR-128a, miR-130a, miR-192, miR-221, and miR-222, miRNAs that target genes involved in angiogenesis and extracellular matrix remodeling, were found differentially expressed in a study between patients with sarcoidosis and controls (27). These genes were essential in the pathogenesis of sarcoidosis, including granuloma formation and fibrosis. Further research into the function of ncRNAs and immune cells in disease enhanced the understanding of the pathogenesis of sarcoidosis and provided new perspectives for translation into innovative therapeutic strategies (28).

Multiple reliable bioinformatics approaches were performed in our study to screen for essential molecular biomarkers associated with sarcoidosis. The intersection of WGCNA-associated module genes and differentially expressed genes was considered the critical gene, most strongly correlated with sarcoidosis and significantly differentially expressed in other sarcoidosis cohorts. A total of 20 genes were screened for dimensional reduction to construct the diagnostic model, and 10 genes were ultimately identified to the SARDS using the LASSO algorithm, including *GBP1*, *LEF1*, *IFIT3*, *LRRN3*, *IFI44*, *LHFPL2*, *RTP4*, *CD27*, *EPHX2*, and *CXCL10*. *GBP1*, as an IFN-γ-related gene in whole blood gene expression, was independently and positively correlated with T-bet+ frequency in Th17 cells, as the expression of T-bet in Th17.0 cells might indicate the degree of granulomatous inflammation in sarcoidosis patients (29). This result was consistent with previous enrichment analyses, demonstrating that IFN-γ and Th17 cells had an essential effect on the development and progression of granulomatous tissues in sarcoidosis (30, 31). Lymphoid enhancer-binding factor 1 (*LEF1*) is one of the Hippo signaling pathway hub genes, and it has been suggested that macrophage proliferation is related to the downregulation of the Hippo signaling pathway (32). The pathology of sarcoidosis is characterized by chronic granulomas with a core infiltration of macrophages and a peripheral infiltration of lymphocytes visually (33). Therefore, the downregulation of *LEF1* contributed to the diagnosis of sarcoidosis in our study. The single-cell analysis identified a new sub-group of macrophages called IFN-responsive macrophages (IFNRM) that expressed IFN-responsive genes (such as *IFIT3*) and secreted the cytokine CXC motif chemokine 10 (*CXCL10*), which regulated the proliferation and differentiation of satellite cells (34). On the question of *CD27*, the research found that the abundant B-cell infiltration in granuloma tissue indicated that B cells were directly involved in the inflammatory process in patients with sarcoidosis. And *CD27*(-) B cells may be a biomarker for treatment with TNF-α blocking agents. In addition, we found that *LRRN3*, *IFI44*, *LHFPL2*, *RTP4*, and *EPHX2* were all involved in diagnosing sarcoidosis, which might shed light on the mechanisms of sarcoidosis and provide potential biomarkers for diagnosis. Overall, results from 11 different cohorts of whole blood, PBMC, BAL cells, and lung tissue supported the diagnostic efficacy of the essential genes, with the splendid AUCs ranging from 0.938 to 1.000 in training datasets and ranging from 0.728 to 0.972 in validation datasets. He J et al. found that *BATF2* and *PDK4* could be used as diagnostic molecular markers for sarcoidosis through bioinformatics approaches in two cohorts (35). Our SARDS model, which combined the construction and validation of 11 cohorts, had higher diagnostic efficacy than other diagnostic models, further validating that SARDS was feasible and reliable in diagnosing patients with sarcoidosis.

Considering that both functional enrichment analysis and GSEA results based on SARDS scores indicated the involvement of immune cells and their processes in sarcoidosis, it was essential to explore the immune landscape in patients with sarcoidosis. This study found that the high infiltration of iDCs, macrophages, pDCs, neutrophils, and eosinophils and the low infiltration of T cells, Tcm, TFH, CD8 T cells, B cells, and Th2 cells constituted the immune microenvironment of sarcoidosis. As we know, dendritic cells comprise three lineages including myeloid DC (mDC), pDC, and Langerhans cells (LC). In the immune process of sarcoidosis, dendritic cells migrate to lymph nodes and participate in t-cell proliferation through t-cell receptors and costimulatory molecules (36). Subsequently, alveolar macrophages are activated to secrete TNF chemotactic leukocytes, which promote granuloma formation (21). The iDCs were enriched in BALF and skin lesions of patients, while mature DCs were located in lymph nodes (37). The pDCs resemble lymphocytes that produce large amounts of interferon-alpha (IFN-α) upon viral invasion, which is consistent with the results of the enrichment analysis regarding the viral response. Taken together, these results suggest that TNF is an important mediator in the pathogenesis of sarcoidosis. More surprisingly, TNF receptors are also abundant in DCs, making DCs possible for therapeutic targets. Following the present results, previous studies have demonstrated that patients with sarcoidosis had strong immuno-stimulability of DCs and macrophages in both the lung and blood (38, 39).

Overall, our research had limitations and strengths. The limitations of the study were that the cohorts in our study contained only diagnostic information lacking clinical aspects. Besides, the essential genes screened were not validated experimentally. Further studies need to be carried out to validate the value of the clinical application. However, it had the strength of a sufficiently large sample size of sarcoidosis, containing 313 patients and 400 healthy controls in 11 cohorts with four different sample sources. Validation in various sample sources and diverse cohorts compensated for experimental validation. In addition, advanced bioinformatics methods and machine learning algorithms reduce the impact of disease heterogeneity and confounding factors on diagnostic models. Collecting and analyzing circulating cells, indicative of pathogenic mechanisms

and immune characteristics, are less invasive and less costly for new diagnostic tools.

In summary, our study systematically identified a feasible and credible diagnostic signature (termed SARDS) comprising 10 essential molecular biomarkers for the diagnosis of sarcoidosis and validated its robustness and translation in multiple cohorts of different source types. The study also had significant implications in exploring the underlying pathogenesis and the immune landscape of sarcoidosis for innovative therapeutic strategies. Taken together, SARDS could be a promising tool to optimize the diagnosis and treatment of patients with sarcoidosis.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Author contributions

MD, ZL, and ZC designed the research. MD and ZL performed data acquisition and data analysis. PL, YW, and YuZ assisted with data analysis. MD wrote this manuscript. SW, YoZ, MF, RW, HX, and YR edited and revised this manuscript. All authors read and approved the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.942177/full#supplementary-material

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Pleural involvements in pulmonary sarcoidosis: A case report and review of the literature

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As a chronic and multisystemic granulomatosis of unknown origin, sarcoidosis can affect multiple organs throughout the body with variable progression and prognosis. Sarcoidosis may present with a battery of symptoms and signs, such as dyspnea, non-productive cough, uveitis, and erythema nodosum. Although the lungs and mediastinal lymph nodes are almost affected in sarcoidosis, involvements of the pleurae remain uncommon. Herein, we report a case of sarcoidosis with both pleural effusions and pleural nodules as confirmed by thoracoscopic pleural biopsy.

KEYWORDS

sarcoidosis, pleural effusion, pleural nodules, biopsy, thoracoscopy

Introduction

Sarcoidosis is a multisystem disease characterized by non-caseating granulomatous inflammation and its exact etiology remains unknown (1). It can affect different organs, especially the lungs, lymph nodes, skin, and eyes. Although lungs are involved in approximately 90% of patients with sarcoidosis, the pleurae are rarely affected in this perplexing disease (2). The frequency of pleural sarcoidosis was less than 3% and pleural effusion, pleural thickening, pleural nodules, and pneumothorax were major patterns of pleural involvements (3, 4). Sarcoidosis presenting as both pleural effusion and pleural nodules are even more unusual. The present report describes a case of sarcoidosis with pleural nodules and pleural effusion.

Case report

A 69-year-old female was admitted to our hospital in Oct 9, 2019 because of dry cough, dyspnea on exertion and fatigue for a month. She had no night sweats or weight loss. The patient was a nurse and she had neither previous disease nor history of smoking. She also had no history of exposure to foreign antigens inorganic particulates. In addition, she had no family history of rheumatologic or autoimmune diseases. On admission, temperature, pulse, respiratory rate, and blood pressure were 36.6°C, 79 beats/min, 21 breaths/min, and 126/88 mm Hg, respectively. Serum levels of electrolytes, brain natriuretic peptide, and liver & kidney function were normal. The levels of serum carcino-embryonic antigen (CEA), neuro-specific enolase (NSE), CYFRA 21-1, squamous cell carcinoma antigen (SCCA), and pro-gastrin-releasing peptide (Pro-GRP) were 1.24 ng/ml (0–5.0 ng/ml), 17.78 ng/ml (0–16.3 ng/ml), 1.54 ng/ml (0.1–3.3 ng/ml), 0.9 ng/ml (0–1.5 ng/ml), and 34.19 pg/ml (0–63 pg/ml), respectively. A computed tomographic (CT) scan of the thorax demonstrated left pleural effusions and linear opacity (**Figure 1**). The exudative, yellow fluid with the predominance of lymphocytes (85%) was detected by thoracentesis. The concentrations of CEA, NSE, CYFRA 21-1, SCCA, and Pro-GRP in pleural effusion were 0.62 ng/ml (0–5.0 ng/ml), 0.86 ng/ml (0–16.3 ng/ml), 11.95 ng/ml (0.1–3.3 ng/ml), 2.5 ng/ml (0–1.5 ng/ml), and 34.72 pg/ml (0–63 pg/ml), respectively. The levels of total protein, lactic dehydrogenase (LDH), glucose, and adenosine deaminase (ADA) in pleural effusion were 54.1 g/L (serum level in the same day was 66.80 g/L), 208 units/L (serum level in the same day was 200.0 units/L), 6.96 mmol/L (3.6–6.0 mmol/L), and value was 47.5 IU/L (<45 IU/L). PR3-anti-neutrophil cytoplasmic antibodies (ANCA) and MPO-ANCA were both negative in the serum. Cultures of the pleural effusion were negative for acid fast bacilli (AFB), fungi, and other organisms. A purified protein derivative (PPD) skin test was also negative. The cytological examination of exfoliated cells in pleural fluid revealed no malignant cells. To clarify the cause of pleural effusion, video-assisted thoracoscopy was performed and revealed a myriad of white nodules on both the visceral and parietal pleura, as well as on the diaphragm (**Figure 2**). Histological examination of the pleural biopsy sample demonstrated non-caseating granulomas and real-time quantitative polymerase chain reaction (PCR) for mycobacterium tuberculosis was negative (**Figure 3**). Stains for acid fast bacilli and fungi remained negative. These results, together with no evidence of other granulomatous disorders, were believed to be consistent with pleural sarcoidosis and this patient was started on prednisone 30 mg daily. All complaints regressed 2 weeks later and a follow-up CT 4 weeks later exhibited the left pleural effusion almost disappeared (**Figure 4**).

Discussion

The clinical features of pleural involvements in our patient seem to be interesting for several reasons. Firstly, this case of sarcoidosis is presented with both pleural effusion and pleural nodules. Pleural involvement in sarcoidosis was first recognized by Schaumann in 1933 and it can be manifested by pleural effusion, pleural nodules, pleural thickening, pneumothorax, hydropneumothorax, and chylothorax (4, 5). The true incidence of pleural involvements in sarcoidosis remains unclear because some cases of pleural sarcoidosis are asymptomatic (6). Chusid and Siltzbach reported that pleural involvements were histologically confirmed in 5 of 950 (0.5%) patients with sarcoidosis (7). Soskel et al. stated that pleural involvement occurred in about 3% of sarcoidosis). The first case of histologically proven pleural effusion caused by sarcoidosis was reported by Talbot et al. (8). Sarcoidosis-related pleural effusions were considered to be less than 3% of this entity and, when present, occur slightly more commonly in the right pleural cavity, although sometimes they can be bilateral (4). The typical finding in pleural effusions caused by sarcoidosis is a paucicellular exudate with the predominance of lymphocytes (9). The mechanism of pleural effusion formation may be analogous to that of other infiltrative diseases. Increased capillary permeability due to involvement of the pleura, obstruction of superior vena cava, lobar atelectasis, and trapped lung have been considered as a cause of pleural effusions secondary to sarcoidosis (10, 11). Pleural nodules, another manifestation of pleural sarcoidosis, were infrequent although the use of CT and thoracoscopy has increased awareness of this unusual site of involvement in sarcoidosis. These are often described as innumerable white nodules on both the parietal and visceral pleura (12, 13). Sarcoidosis-related pleural effusions and pleural nodules are unusual, and they occur concurrently in one patient, as described in our case, are even more uncommon.

Secondly, pleural involvement is the initial manifestation of sarcoidosis. Pleural manifestations caused by sarcoidosis may arise at the onset of this disease which is first diagnosed, as the case we have described, or at any time during the course of the known sarcoidosis (14). The development of pleural involvement in sarcoidosis seems to have no definite prognostic value (15). Thirdly, the pleural effusion and pleural nodules are associated with neither hilar adenopathy nor pulmonary infiltrate. The most common radiographic finding of sarcoidosis is bilateral hilar adenopathy. Other clinical features consist of interstitial lung disease, pulmonary nodules, skin lesions, and eye symptoms (12). Pleural sarcoidosis usually correlates with extrapulmonary involvement or extensively parenchymal lesions of the lung (7, 9, 16). In the present case, of great interest is pleural involvements are not associated with hilar adenopathy or pulmonary infiltrate.

Finally, the pleural involvement of sarcoidosis responds well to corticosteroids. Systemic corticosteroids are the mainstay of

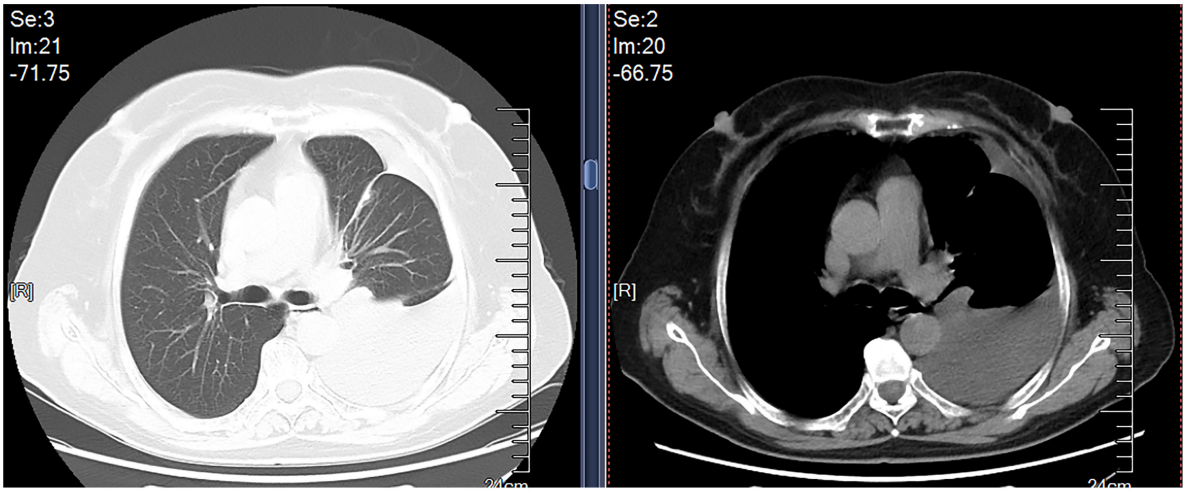

FIGURE 1
Chest CT scan showed left pleural effusions and linear opacity.

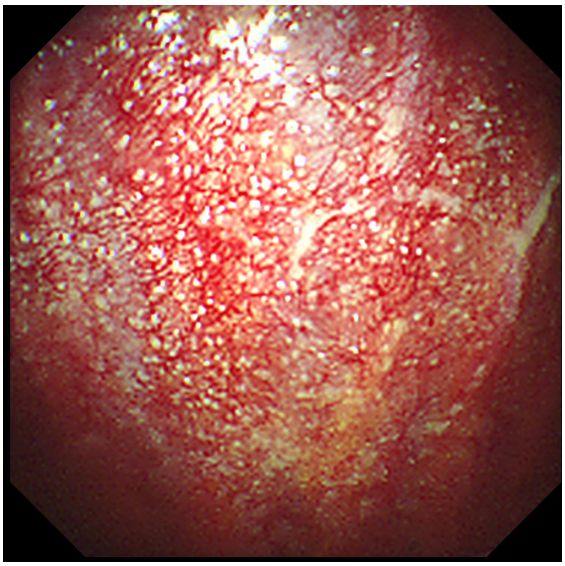

FIGURE 2
Thoracoscopy demonstrated amounts of white nodules on the pleurae.

treatment of sarcoidosis and also the most commonly used first-line therapy (17). Corticosteroids hamper the formation of granulomas and, as a result, are largely efficient against most active clinical manifestations (18). For recurrent or symptomatic patients of sarcoidosis with pleural involvement, corticosteroids are required (19). Asymptomatic pleural effusions are likely to resolve spontaneously. The time of spontaneous resolution ranges from 1 to 3 months (19). Our case responded well to oral corticosteroid therapy, resulting in marked improvement in both symptoms and chest radiological findings. Sarcoidosis related pleural effusions which resolve incompletely and develop to trapped lung may be relieved by decortication (11).

The diagnosis of sarcoidosis involving the pleura is based on the histologic evidence of non-caseating granuloma, the hallmark of sarcoidosis, and on excluding other granulomatous diseases, such as tuberculosis, fungal disease, and granulomatous polyvasculitis (2). In addition, some disorders including congestive heart failure and neoplasia, may be concomitant with sarcoidosis, must be ruled out. Our case showed no evidences of tuberculosis, fungal disease, vasculitis, and other granulomatous diseases. However, because the clinical

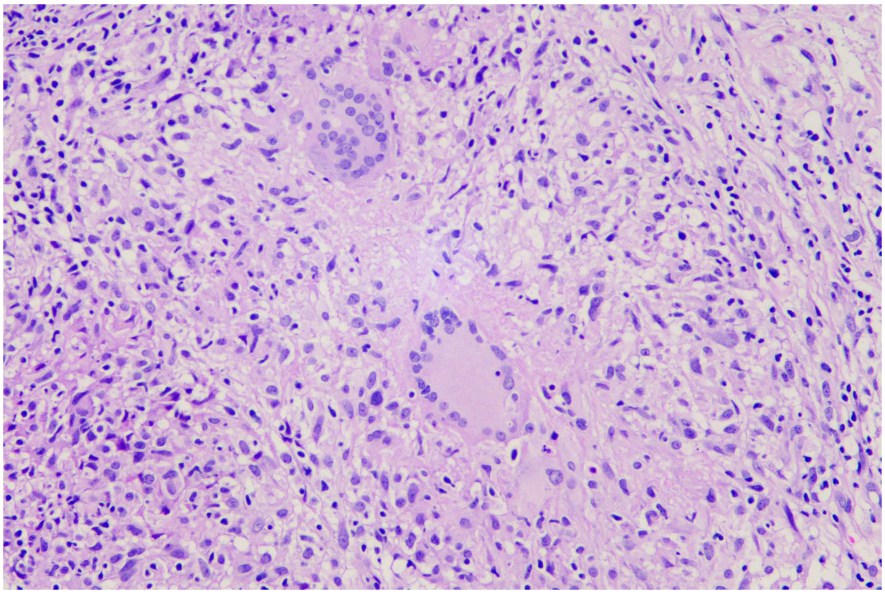

FIGURE 3
Histologic finding of non-caseating granulomas (hematoxylin-eosin, original magnification × 400).

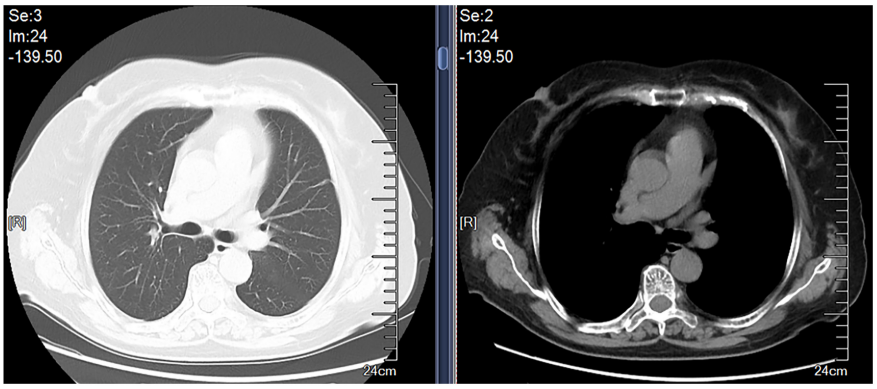

FIGURE 4
Follow-up chest CT showed a complete withdrawal of the left pleural effusion.

and pathological features of sarcoidosis and tuberculosis may mimic each other, the differentiation between the two entities remains a challenging problem. When the caseous necrosis is absent in biopsy samples, the real time PCR quantification for mycobacterium tuberculosis genome is a valuable test for differentiation between sarcoidosis and tuberculosis (20).

Medical thoracoscopy, a relatively less invasive and more efficient diagnostic method, plays a significant role in the diagnosis of pleural involvement in sarcoidosis, especially of pleural effusions and pneumothorax. By thoracoscopy, physicians can directly access and assess the pleural cavity, including the parietal, visceral and diaphragmatic pleura, and obtain adequate tissue sampling. Additionally, pleural fluid can be aspirated without complications during thoracoscopy. Therefore, thoracoscopy, an appropriate alternative technique, can provide doctors with important evidences to convince pleural sarcoidosis (21). Although thoracentesis or closed pleural biopsy can also help to diagnose, it is not easy for physicians to get the accurate pathologic evidence.

Conclusion

In summary, this case illustrates an unusual form of pleural involvement of sarcoidosis with pleural effusion plus pleural nodules. A definitive diagnosis of pleural sarcoidosis relies on the histological identification of non-caseating granulomas in the pleurae and on the exclusion of all other possible causes.

Although rare, pleural involvements in sarcoidosis should be considered in the differential diagnosis of pleural effusion and pleural nodules. As is the case with other forms of pulmonary involvement in sarcoidosis, these manifestations respond well to corticosteroids. It is believed that careful evaluation and vigorous treatment of pleural involvement in sarcoidosis is imperative.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the First Affiliated Hospital of Shandong First Medical University (Jinan, China). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

XJ, JL, and DL: conception and design. XJ, JL, HP, and FS: collection and assembly of data. XJ, JL, AZ, and DL: data analysis and interpretation. All authors: manuscript writing and final approval of manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Characteristics and survival of patients diagnosed with cardiac sarcoidosis: A case series

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Background: Sarcoidosis is a multiorgan system granulomatous disease of unknown etiology. It is hypothesized that a combination of environmental, occupational, and/or infectious factors provoke an immunological response in genetically susceptible individuals, resulting in a diversity of manifestations throughout the body. In the United States, cardiac sarcoidosis (CS) is diagnosed in 5% of patients with systemic sarcoidosis, however, autopsy results suggest that cardiac involvement may be present in > 50% of patients. CS is debilitating and significantly decreases quality of life and survival. Currently, there are no gold-standard clinical diagnostic or monitoring criteria for CS.

Methods: We identified patients with a diagnosis of sarcoidosis who were seen at the Simmons Center from 2007 to 2020 who had a positive finding of CS documented with cardiovascular magnetic resonance (CMR) and/or endomyocardial biopsy as found in the electronic health record. Medical records were independently reviewed for interpretation and diagnostic features of CS including late gadolinium enhancement (LGE) patterns, increased signal on T2-weighted imaging, and non-caseating granulomas, respectively. Extracardiac organ involvement, cardiac manifestations, comorbid conditions, treatment history, and vital status were also abstracted.

Results: We identified 44 unique patients with evidence of CS out of 246 CMR reports and 9 endomyocardial biopsy pathology reports. The first eligible case was diagnosed in 2007. The majority of patients (73%) had pulmonary manifestations, followed by hepatic manifestations (23%), cutaneous involvement (23%), and urolithiasis (20%). Heart failure was the most common cardiac manifestation affecting 59% of patients. Of these, 39% had a documented left ventricular ejection fraction of < 50% on CMR. Fifty eight percent of patients had a conduction disease and 44% of patients had documented ventricular arrhythmias. Pharmacotherapy was usually initiated for extracardiac manifestations and 93% of patients had been prescribed prednisone. ICD implantation

occurred in 43% of patients. Patients were followed up for a median of 5.4 (IQR: 2.4–8.5) years. The 10-year survival was 70%. In addition to age, cutaneous involvement was associated with an increased risk of death (age-adjusted OR 8.47, 95% CI = 1.11–64.73).

Conclusion: CMR is an important tool in the non-invasive diagnosis of CS. The presence of LGE on CMR in a pattern consistent with CS has been shown to be a predictor of mortality and likely contributed to a high proportion of patients undergoing ICD implantation to decrease risk of sudden cardiac death.

Clinical implications: Additional studies are necessary to develop robust criteria for the diagnosis of CS with CMR, assess the benefit of serial imaging for disease monitoring, and evaluate the effect of immunosuppression on disease progression.

KEYWORDS

cardiac sarcoidosis, cardiovascular magnetic resonance, inflammation, endomyocardial biopsy, cardiomyopathy

Introduction

Sarcoidosis is a multiorgan system granulomatous disease of unknown etiology. It is hypothesized that a combination of environmental, occupational, and/or infectious factors provoke an immunological response in genetically susceptible individuals, resulting in a diversity of manifestations throughout the body (1). Disease may be more prevalent in women and African Americans and its incidence varies by geographic location (2–4).

In the United States, cardiac sarcoidosis (CS) is diagnosed in 5% of patients with systemic sarcoidosis, however, autopsy results suggest that cardiac involvement may be present in over 50% of patients (5–7). These findings suggest that only a small proportion of patients with CS have clinically significant manifestations that prompted evaluation and led to diagnosis. Most patients diagnosed with CS have a history of sarcoidosis affecting other organ systems, most commonly pulmonary (8–10). Patients with predominant cardiac symptoms may have minimal extracardiac diseases, and therefore, sarcoidosis may not be considered in the differential diagnosis, further contributing to underdiagnosis. CS starts with the development of edema due to inflammation followed by granulomatous infiltration and eventual fibrosis and scarring (11). Cardiac manifestations may include conduction abnormalities, ventricular arrhythmias, heart failure, and sudden cardiac death (1, 8, 10, 12). CS can be debilitating and may significantly decrease quality of life as well as survival. Up to 25% of deaths from sarcoidosis in the United States have been attributed to cardiac involvement, whereas this figure is over 50% in Japan (11). Studies have demonstrated that a lower left ventricular ejection fraction (LVEF) is an important clinical predictor of mortality in patients with CS (13).

Currently, there are no gold standard clinical diagnostic criteria for CS. Multiple guidelines exist and are often discordant, although they involve similar elements, including the presence of clinical and radiological manifestations, non-caseating granulomas on biopsy from another organ system, and no evidence of an alternative etiology (9, 14–16). Endomyocardial biopsy in CS diagnosis is considered high risk and has low sensitivity (20–30%) due the patchy distribution of disease (8). These shortcomings have created a role for non-invasive imaging, such as cardiovascular magnetic resonance (CMR) and fluorodeoxyglucose-positron emission tomography (FDG-PET) to aid in diagnosing CS in conjunction with histopathology results from another organ system (17). The availability of advanced imaging techniques has resulted in increased recognition of underdiagnosed CS (17–19). The prevalence of CS has been reported as 3.7–54.9% with advanced imaging techniques, depending on the technique as well as the population under study (11).

As cardiac sarcoidosis is still considered quite rare, it can be challenging to conduct prospective studies to evaluate diagnostic methods and disease prognosis with various treatments, particularly given the heterogeneity of the disease. Case studies have been helpful in elucidating disease patterns, treatment exposure, and mortality that can impact future studies and management. We conducted a retrospective single center review of patients with biopsy-proven extracardiac sarcoidosis who were diagnosed with CS *via* CMR or endomyocardial biopsy to evaluate their cardiac manifestations, the prevalence of their extracardiac organ involvement, and mortality.

Methodology

Study population

Patients with an existing diagnosis of biopsy-proven extracardiac sarcoidosis seen at the Simmons Center for Interstitial Lung Disease at University of Pittsburgh Medical Center between 2007 and 2020 were selected for this review. Patients who underwent imaging with CMR and/or endomyocardial biopsy for the evaluation of CS were screened for eligibility. After exclusion of patients with negative CMR or pathology results, patients who were classified as having CS were selected. All patients were evaluated by a physician at the UPMC Dorothy P. and Richard P. Simmons Center for Interstitial Lung Disease.

Data collection

Results from CMR and endomyocardial biopsy pathology reports were obtained from the electronic health record. They were independently reviewed for CMR features of CS including late gadolinium enhancement (LGE) patterns and/or increased signal on T2-weighted imaging, and/or the presence of non-caseating granulomas on endomyocardial biopsy. Documentation on lung Scadding stage, extracardiac organ involvement, cardiac manifestations, comorbid conditions, treatment history, and vital status were extracted from the electronic health record.

Data analysis and definitions

Cases included in our analysis had either a CMR consistent with CS and/or a histopathological diagnosis of CS. CMR findings consistent with cardiac sarcoidosis including the presence of multifocal areas of LGE, subepicardial and midmyocardial LGE suggesting a non-infarct pattern, and extension of LGE across the interventricular septum from the left ventricle to the right ventricle (18, 19). Findings may be supported by the presence of increased T2 weighted signal indicative of regions with increased edema and potentially reflecting reversible inflammation. Pathology reports identifying non-caseating granulomas in myocardial tissue sample without evidence of alternative etiologies were considered positive for CS. Summary statistics were used to evaluate the frequency of different cardiac manifestations, extracardiac organ involvement, comorbid conditions, and management including pharmacotherapy and ICD implantation. Categorical variables are expressed as numbers and percentages (%). Continuous variables are expressed as median (IQR). Patients' vital status were ascertained using Social Security Death Index. The overall survival of patients was presented from diagnosis date to date of death or last follow up date in their electronic health record. Prognostic odds ratio of disease manifestations and treatment were adjusted for age. We applied univariate logistic regression analysis to calculate the prognostic odds ratio of disease manifestations and treatment. We also used age as a covariate to calculate the age adjusted odds ratio. Hazard ratio of statistically significant predictors in logistic models were also calculated using a multivariable Cox regression analysis. Data analysis was performed with Stata 16.2 (StataCorp, College Station, TX).

Results

We identified 44 unique patients with evidence of CS out of 246 CMR reports and 9 endomyocardial biopsy pathology reports. The median age of the cohort was 55 years, 60% were male and 25% were Black. The majority of patients (73%) had pulmonary manifestations classified according to Scadding stage (Table 1). The next most common extracardiac manifestations were hepatitis or elevated transaminases (27%), cutaneous involvement (23%), urolithiasis (20%), hypercalcemia (16%), joint involvement (18%), and peripheral neuropathy (16%). Heart failure was the most common cardiac manifestation affecting 59% of patients. Of these, 39% had a documented left ventricular ejection fraction (LVEF) of $< 50\%$ on their diagnostic CMR. The median (IQR) of LVEF was 56% (40–63).

The observed cardiovascular manifestations in our patients are displayed in Figure 1. Fifty eight percent of patients had a conduction disease, including 5% with some degree of AV nodal block, 39% with dysfunction in the His-Purkinje system as manifested as bundle branch blocks, and 14% had a combination of both AV nodal block and bundle branch block. Heart failure was diagnosed in 59% of patients, 65% of whom had an ejection fraction of $< 50\%$. Forty-four percent of patients had documented ventricular arrhythmias. One third of patients had a documented history of pulmonary hypertension in addition to their other cardiac findings. More than a quarter of patients (27%) had a history of coronary artery disease, which was based on history of myocardial infarction or the presence of coronary calcifications on imaging.

TABLE 1 Demographic and clinical characteristics of patients with cardiac sarcoidosis ($N = 44$).

Characteristic	Results
Age, median (IQR)	55 (46–61)
Male, *n* (%)	26 (59)
Black, *n* (%)	11 (25)
Initial lung Scadding stage, *n* (%)	
0	12 (27)
1	7 (16)
2/3	19 (43)
4	6 (14)

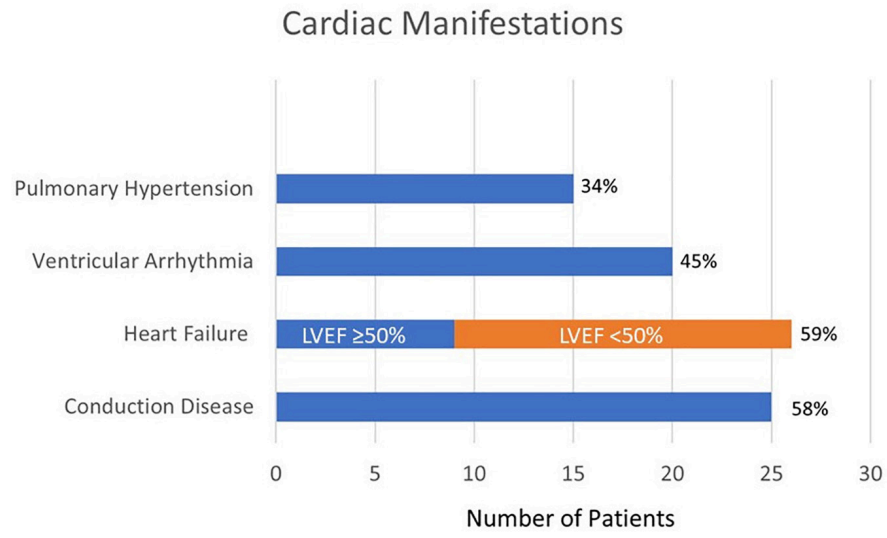

FIGURE 1
Cardiac manifestations of patients with cardiac sarcoidosis (N = 44).

Therapeutic interventions received by patients during the study period are detailed in **Table 2**. Nearly all patients (93%) were on prednisone at some point during their treatment course. The most commonly used steroid-sparing agent was methotrexate (75%). Forty three percent of patients underwent ICD implantation.

Patients were followed up for a median of 5.4 (IQR: 2.4–8.5) years. The 10-year survival was 70% (**Figure 2**). In addition to age, cutaneous involvement was associated with an increased risk of death (age adjusted OR = 8.47, 95% CI = 1.11–64.73, **Table 3**). LVEF < 50% (age adjusted OR = 1.1, p = 0.9) was not associated with significantly higher risk of mortality. In unadjusted analysis the presence of conductive disease was a risk factor of mortality which was not statistically significant after adjusting for age. Prednisone treatment was also a protective factor in unadjusted analysis (**Table 3**). In a survival analysis using a Cox regression model, both age (HR = 1.15 per year, 95% CI = 1.04–1.27, p = 0.007) and cutaneous involvement (age adjusted HR = 6.90, 95% CI = 1.33–35.95, p = 0.02) were associated with poor survival.

Discussion

Our single center case series describes a heterogenous population of patients with biopsy-proven extracardiac sarcoidosis who were diagnosed with CS by CMR and/or endomyocardial biopsy with a median follow up of 5 years. Our overall 10-year survival rate was 70%. This is lower than reported range of 70–93% for 10-year survival rates (20–22). It is challenging to directly compare these rates due to differences in methodology such as inclusion criteria, statistical analysis, and outcomes of interest. The retrospective study by Kandolin et al. selected patients based on the development of cardiac symptoms who were found to have sarcoidosis rather than patients with an established diagnosis of sarcoidosis (20). This may have resulted in a significant proportion of their patients (65%) having clinically isolated CS, which may have detected CS at an earlier stage. Additionally, the outcome of interest was 10-year cardiac survival, whereas our study focused on overall survival. In a study by Zhou et al. the 10-year survival rate was 93.4% (21). Only a small proportion of patients (31.5%) had LGE on CMR in contrast to the majority of patients in our study, which may suggest detection of disease at an earlier stage or lead time bias. Furthermore, nearly 60% of patients had an ICD implanted compared with only 43% in our study. This difference in mortality suggests a protective effect of ICD implantation. Furthermore, while ICD implantation was not an independent risk factor for mortality in our patients, the unadjusted OR of conduction abnormalities suggest that it is a prognostic factor in patients with CS. Furthermore, the median age at diagnosis in this cohort was 46, nearly a decade younger than the patients in our study. A similar survival rate was reported by Cacoub et al. in patients with a median age of 40 (22). The impact of other comorbid conditions, which are not consistently reported in other studies, such as hypertension, obesity, diabetes, and smoking may further help to explain differences in 10-year survival among these populations. The significant difference in age as well as stage of disease at diagnosis may account for some of the differences observed in 10-year survival.

Conduction disease is highly prevalent in patients with CS, which may be due to a high frequency of disease manifestation in the basal septum (11, 17). The prevalence of AV nodal

TABLE 2 Pharmacological treatment of patients with cardiac sarcoidosis (N = 44).

Medication	n (%)
Prednisone	42 (93)
Methotrexate	33 (75)
Infliximab	14 (32)
Azathioprine	5 (11)
Mycophenolate mofetil	5 (11)
Adalimumab	3 (7)

conduction disease was slightly lower in our population than in other published studies (19% vs. 25–30%). The prevalence for His-Purkinje system disease in our study was slightly higher at 39% compared to the 12–32% reported in other studies. It is possible that the increased prevalence of His-Purkinje disease in our population is attributable to a high proportion of patients (93%) having coexisting CAD and may not be directly due to CS.

Ventricular arrhythmias were more common in our case series at 44% than reported in previous studies at 23–40%. Prior studies have noted that ventricular arrhythmias may be the first presentation in up to 40% of patients with CS (23, 24). As the ability to diagnose CS improves, it is likely that the incidence, and therefore, prevalence of ventricular arrhythmias attributed to CS will increase. Ventricular arrhythmias may also be secondary to ischemia from CAD and not CS, which can be challenging to discern and will vary among populations. Studies show that patients suffering from ventricular arrhythmias secondary to CS may be less responsive to antiarrhythmic pharmacotherapies (25). As a result, these patients often require ICD implantation.

ICD implantation appears to be protective from sudden cardiac death in patients with CS regardless of whether they meet the typical indications for placement, such as ventricular tachycardia/fibrillation and/or low LVEF (26, 27). As ICD placement is a shared decision between the patient, cardiologist and pulmonologist, not all patients who meet criteria for ICD implantation will receive one (28, 29). Only 43% of patients in our study received an ICD, which is lower than in the study by Zhou et al. (21).

The prevalence of heart failure in this study is consistent with reported prevalence of 25–75% among other case series (30, 31). Left ventricular remodeling occurs in the setting of granulomatous inflammation and scarring. The degree of left ventricular systolic dysfunction, as measured by LVEF, is reported to be the most powerful predictor of mortality (13, 32), however, our study did not demonstrate this. Instead, our study showed that patients with conduction disease were nearly 19 times less likely to survive than those without such manifestations. More studies are needed to elucidate the use of advanced imaging as a prognostic factor in CS and its relationship to clinical cardiovascular manifestations.

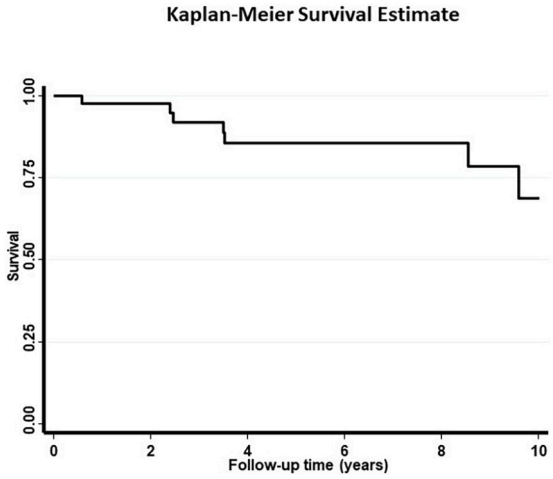

FIGURE 2
The 10-year survival curve for 44 patients with cardiac sarcoidosis.

While 33% of the cases in our study have documented pulmonary hypertension, reported prevalence has been highly variable. Depending on the population studied, pulmonary hypertension ranges from 6% in outpatient study to over 70% in patients listed for lung transplantation (33, 34). Furthermore, 40–60% of patients with sarcoidosis related pulmonary hypertension do not have evidence of overt lung parenchymal involvement such that Scadding stage may be discordant with disease severity and risk of death (35, 36). In this population, pulmonary hypertension may instead be caused by sarcoidosis related left ventricular systolic dysfunction, extrinsic compression of the pulmonary vasculature by mediastinal lymphadenopathy, chronic hypoxic vasoconstriction leading to cor pulmonale, and/or remodeling of the pulmonary vasculature (33, 34). In our cohort, 20 underwent RHC of whom 7 were diagnosed with pulmonary hypertension. However, there were 8 additional patients who were suspected to have pulmonary hypertension based on transthoracic echocardiography or stigmata on computed tomography of the chest. Only 3 of the 7 patients diagnosed with pulmonary hypertension *via* right heart catheterization were prescribed pulmonary artery vasodilators due to the presence of precapillary pulmonary hypertension. The remainder were managed through treatment of their underlying disease process, most commonly left-sided heart disease in the setting of sarcoidosis.

The frequency of extracardiac manifestations in our case series was in line with other studies. We were unable to capture isolated CS based on our inclusion criteria. Over 70% of patients in our cohort with CS have concomitant pulmonary sarcoidosis, which is consistent with other studies (9, 10). Cutaneous manifestations of sarcoidosis, which occur in 25–33% of patients, are heterogeneous with prognosis varying by subtype (37, 38). Specifically, lupus pernio and plaques have

TABLE 3 Association between important baseline factor and mortality (N = 44).

	Unadjusted		Age adjusted	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age	**1.11 (1.01–1.22)**	**0.033**	NA	
Male	2.09 (0.42–10.33)	0.4	2.82 (0.50–15.84)	0.24
Initial lung Scadding stage >1	2.33 (0.47–11.50)	0.3	2.16 (0.39–11.84)	0.37
Skin involvement	4.69 (0.99–22.26)	0.052	**8.47 (1.11–64.73)**	**0.040**
Heart failure	4.49 (0.69–29.06)	0.12	4.28 (0.62–29.41)	0.14
Conduction abnormalities	**18.94 (1.02–352.73)**	**0.049**	15.92 (0.83–306.01)	0.07
Pulmonary hypertension	3.97 (0.89–18.11)	0.08	3.62 (0.71–18.32)	0.12
Prednisone	**0.11 (0.01–0.98)**	**0.048**	0.14 (0.02–1.31)	0.09

Bolded results have a p-value < 0.05.

been associated with increased mortality, whereas erythema nodosum, Lofgren syndrome, maculopapular, and nodular lesions are associated with low mortality risk. Of the 10 patients with cutaneous manifestations in our study, only one had plaques and none had evidence of lupus pernio. As a result, it is challenging to explain the increased mortality risk in this subset of patients based on the presence of cutaneous manifestations alone.

Early administration of corticosteroids is the mainstay of treatment with subsequent tapering to tolerance, despite the lack of randomized control trials (22, 39). The majority of patients in our study received steroids (93%). Many transitioned to a steroid-sparing agent later in their disease course since maintaining adequate levels of corticosteroids to suppress inflammation and scarring is difficult over the long term due to its side effects (22). As studies exploring the use of steroids are retrospective, their effect on the natural history of CS is unknown. It is challenging to draw conclusions about steroid dosing or duration due to the heterogeneous nature of observational studies. A study by Kandolin et al. that evaluated transplant-free cardiac survival suggested that long-term outcomes are independent of initial steroid dosing and timing (20). The long-term benefits of steroid use, however, may depend on the presence and severity of left ventricular systolic dysfunction, one of the strongest prognostic indicators for survival (40). Retrospective studies have demonstrated that even with corticosteroid therapy, there is limited evidence of improvement when the LVEF is less than 30% (31, 32). Thus, corticosteroid treatment may be of benefit in early-to-mid stage disease prior to the development of fibrosis. Steroid sparing agents are increasingly used for long term therapy, but their safety and efficacy has yet to be established. The Cardiac Sarcoidosis multi-center randomized controlled trial (CHASM CS- RCT) is currently underway to compare different regimens (41).

It is unclear if we can distinguish survival benefit from the use of steroids in patients who are also receiving guideline directed medical therapy for heart failure. The management of ventricular tachyarrhythmias secondary to CS is difficult as inducibility may vary between active and quiescent phases of the disease and there are no studies evaluating the use of antiarrhythmics in CS (39). Other studies demonstrate improvement in AV nodal conduction with steroid therapy (13, 20). One study of symptomatic cardiac sarcoidosis patients concluded that the combination of steroids plus other immunosuppressive therapies may reduce the risk of relapse compared with steroids alone (42). In our study, the presence of an ICD did not significantly impact mortality risk.

The generalizability of the results from our case series is limited as it is from a single Sarcoidosis Center. The majority of the patients were diagnosed with CS *via* CMR; however, no FDG-PET images were available for comparison as this imaging modality is not currently available at our institution. Based on the inclusion criteria of our case series, namely biopsy-proven extracardiac sarcoidosis, we were unable to capture cases of isolated CS, for which the prevalence remains unknown. Currently, no protocol exists to screen for CS in the absence of cardiovascular symptoms in patients with sarcoidosis, although it may remain clinically silent for some time. Similarly, the value of repeat imaging in patients with cardiovascular symptoms with a negative initial scan has yet to be evaluated.

This case series focused on cardiac manifestations and extracardiac organ involvement in patients with CS to better understand the presentation of disease, the therapies most commonly prescribed, and frequency of advanced therapy with ICD implantation. Additional studies are necessary to develop robust criteria for the diagnosis of CS with advanced imaging techniques, to directly compare CMR and FDG-PET, assess the benefit of serial imaging for disease monitoring, and evaluate the effect of immunosuppressive therapies on disease progression. To date, it has been challenging to develop these types of studies given the low prevalence of disease, the multitude of confounding factors associated with treatment, and the reluctance to modify or discontinue treatment once remission is achieved.

Data availability statement

The datasets presented in this article are not readily available because due to IRB restrictions, data is not available for outside investigators. Requests to access the datasets should be directed to SN.

Ethics statement

The University of Pittsburgh IRB reviewed and approved "STUDY20010219". Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

TB, MS, SN, and KG contributed to the conception and design of the study. MS organized the database. TB abstracted electronic medical records and wrote the first draft of the manuscript. SN performed the statistical analysis. SN and KG wrote sections of the manuscript. All authors contributed to the manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Challenges of cardiac sarcoidosis

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Sarcoidosis is a multisystem granulomatosis of unknown origin, which can involve almost any organ. Most frequently the disease involves the lungs and mediastinal lymph nodes, but it can affect the skin, the eyes, nervous system, the heart, kidneys, joints, muscles, calcium metabolism, and probably any other anecdotical organ involvement. Cardiac sarcoidosis is one of the most challenging involvements, as it can lead to cardiac mortality and morbidity, and also because the diagnosis may be difficult. With no specific symptoms, cardiac sarcoidosis may be difficult to suspect in a patient with no previous extra-cardiac sarcoidosis diagnosis. This manuscript reviews the current knowledge of the diagnosis and decision to treat cardiac sarcoidosis, and illustrates the information with a case presentation of a young adult with no risk factors, no previous diagnosis of sarcoidosis, and with cardiac symptoms impairing his quality of life.

KEYWORDS

sarcoidosis, cardiac sarcoidosis, cardiac MRI, guidelines, corticosteroids

Introduction

Sarcoidosis is a multisystem granulomatosis of unknown origin, characterized by the formation of epithelioid granulomas in multiple organs in susceptible individuals.

Sarcoidosis involves most frequently the lungs and mediastinal lymph nodes, and about 90% of diagnosed patients have this type of intrathoracic involvement (1).

Sarcoidosis can affect almost any organ in the body, as an isolated involvement, or associated to lung or other organs disease. It can involve lymph nodes.

The heart is one of the organs that can develop the disease and is generally considered as one of the involvements that can be associated to high risk for premature death or irreversible heart conditions. This makes cardiac sarcoidosis one of the biggest concerns for severe disease.

Still, the heart involvement in sarcoidosis may be difficult to recognize in clinical practice, and the prevalence of the disease may be underestimated.

This article reviews the knowledge on the subject and illustrates it with the presentation of a case.

Prevalence of cardiac sarcoidosis

Sarcoidosis affects about 60 people per 100,000 in the United States, and has variations of prevalence over the world, with Northern Europe and African Americans, having a higher incidence, but with variation of incidence in regions of the same country (2).

Cardiac sarcoidosis was found in pathology studies in up to 30% of patients with sarcoidosis (3), but because in many cases it may be asymptomatic, it is noted typically in about 7% of sarcoidosis patients, with or without involvement of other organs (4).

Cardiac sarcoidosis is associated to mortality rates between 1 to 8%, most of them caused by cardiac involvement (3).

Heart involvement in sarcoidosis

Sarcoid granulomas may involve any area of the heart (5). Most frequently, the lesions are found in the interventricular septum, especially at the base. Other preferred sites are the left ventricle free wall and right ventricle, the atria being less commonly involved (6).

In patients requiring heart transplantation or who die from sudden death due to cardiac sarcoidosis, extensive scarring can be found, as a consequence of chronic disease (5).

Cardiac structural changes and presence of granulomas may induce the clinical manifestations of the disease: arrhythmias, conductance disturbances, dilated cardiomyopathy, right heart failure and peripheral edema, or left heart failure with pulmonary edema and hypertension, valvular disfunctions, or sudden cardiac death (4).

Clinical presentation includes most commonly: atrioventricular block, ventricular arrhythmias, and heart failure (7). Patients may also present less commonly with valvular abnormalities, bundle branch block, pericardial effusion (8). Pericardial involvement is uncommon, may be an incidental discovery and it may be associated to myocardial lessions in about 25% of cases (9). It is not clear if it is a distinct sarcoidosis serositis (similar to pleural effusion), it is secondary to ventricular failure or it is accompanying the heart involvement (10).

Cardiac sarcoidosis should be suspected in individuals presenting with new and unexplained atrial or ventricular arrhythmias, atrioventricular block, or with left ventricular dysfunction. Sometimes the presentation is less specific, with palpitations or presyncope as the only presenting symptoms. Cardiac sarcoidosis can be suspected particularly in patients who have already a diagnosis of sarcoidosis involving other organs. The real problem are the patients presenting with nonspecific symptoms like palpitations and syncope or presyncope, without being previously diagnosed with sarcoidosis. In these individuals, the diagnosis may be significantly delayed (8, 11).

Diagnosis of cardiac sarcoidosis

Diagnosis of cardiac sarcoidosis can be challenging from several perspectives.

Probably the most challenging issue is the suspicion of CS with initial cardiac manifestations in a patient with no previous diagnosis of sarcoidosis, or no other clinical apparent organ involvement. The sarcoidosis as the cause of heart rhythm disturbances in such a patient may not be suspected by the cardiologist, and diagnosis can be delayed.

Another difficulty in diagnosis is the absence of a golden standard for diagnosis.

Histologic proof of the epithelioid granulomas is part of the definition of sarcoidosis. For the heart involvement, the biopsy of endomyocardial tissue has a high specificity, but sampling errors and variable histology reduce the sensitivity down to 20 to 30% (12). Also, endomyocardial biopsies are usually obtained from the right side of the interventricular septum, while granulomas are typically located in the left ventricle (13).

An initial electrocardiogram may show a variety of abnormalities that are not specific to cardiac sarcoidosis: atrioventricular block type I, II or III, atrial and ventricular arrhythmias, including ventricular premature beats or ventricular tachycardia (8). Holter monitoring, used for detecting arrhythmias or heart block, has a sensitivity of 59 to 67% and a specificity of 58 to 80% (14). Heart block is often an early sign of cardiac involvement and it may be the manifestation with the best chance of responding to corticosteroids (15).

Heart ultrasound is accessible and non-invasive, can be easily performed in patients presenting with cardiac symptoms, but it has little specificity for early or localized cardiac sarcoidosis, and patients with cardiac sarcoidosis may have a normal echocardiogram (16).

When present, abnormal findings include abnormal myocardial wall thickness, possibly due to presence of granulomas, wall motion abnormalities, or diastolic dysfunction. In later stages of the disease, thinning of the myocardial wall, left ventricular dilatation and left ventricular systolic dysfunction may be noticed. These are considered predictors of mortality for cardiac sarcoidosis (16).

Cardiac magnetic resonance study has become the standard of care for the diagnosis of cardiac sarcoidosis. The findings may include regional wall motion abnormalities in a patchy distribution, and regional increased signal intensity on T2 and delayed gadolinium enhancement. The areas most frequently involved for enhancement are the basal interventricular septum. Though, any myocardial segment of the left or right ventricle can be involved: subepicardial, transmural or midmyocardial regions (17, 18).

PET-CT scan can be useful for the diagnosis of sarcoidosis, the whole-body scan being able to locate multiple organ involvements of the disease and allows the choice of the most accessible site for performing a biopsy (19).

PET-CT is useful for the diagnosis of cardiac sarcoidosis, with the use of a cardiac dedicated protocol including both FDG PET (to image the inflammation) and a scan to assess resting myocardial perfusion images with ^{82}Rubidium or ^{13}N-Ammonia, and using a protocol for preparing the patient with a high fat/low carbohydrate diet before the examination (11). Focal areas of FDG increased uptake may appear, corresponding with areas of resting perfusion defects. The overlap of regions of high FDG uptake and resting perfusion defects can support the diagnosis of cardiac sarcoidosis (11).

The sensitivity of PET-CT for the diagnosis of CS is 89% with a specificity of 78% (20).

Due to the difficulty to use a single golden standard test for the diagnosis of cardiac sarcoidosis, sets of major and minor criteria for the diagnosis were proposed in consensus statements.

The Japanese Ministry of Health and Welfare consensus states that, in the absence of a diagnostic endomyocardial biopsy, a patient with known extra-cardiac sarcoidosis may be diagnosed with cardiac sarcoidosis by fulfilling at least two major criteria or at least one major plus two minor criteria. Major criteria include advanced atrioventricular block, thinning of the basal interventricular septum, positive gallium-67 uptake, or diminished ejection fraction. Minor criteria include abnormal ECG findings, perfusion defects on nuclear imaging, delayed enhancement of the myocardium by cardiac MRI, or endomyocardial biopsy showing interstitial fibrosis or monocyte infiltration (21).

In 2014, the Heart Rhythm Society released a similar consensus statement, adding as criteria the response to corticosteroid or immunosuppressive therapy and the reasonable exclusion of other cause (s) for the cardiac manifestations (22).

The WASOG criteria for the diagnosis of cardiac sarcoidosis, classified according to the organ assessment instrument developed by this society, include (23):

- *Highly probable:* biopsy with granulomatous inflammation of no alternate cause.
- *Probable:*
  - Treatment responsive cardiomyopathy.
  - Atrio-ventricular block.
  - Reduced left ventricle EF in the absence of other clinical risk factors.
  - Spontaneous or inducible sustained ventricular tachyarrhythmia with no other risk factor.
  - Mobitz type II or 3rd degree heart block.
  - Patchy uptake on dedicated cardiac PET.
  - Delayed enhancement on CMR.
  - Positive gallium uptake.
  - Defect on perfusion scintigraphy or SPECT scan.
  - T2 prolongation on CMR.

- *Possible:*
  - Reduced LVEF in the presence of other risk factors (e.g., systemic hypertension, diabetes mellitus).
  - Atrial dysrhythmias.

- *No consensus:*
  - Frequent ectopy (>5% QRS).
  - Bundle branch block.
  - Impaired right ventricle function with a normal pulmonary vascular resistance.
  - Fragmented QRS or pathologic Q waves in ≥2 anatomically contiguous leads.
  - At least one abnormal signal averaged ECG domain.
  - Interstitial fibrosis or monocyte infiltration.

Prognosis of cardiac sarcoidosis

Cardiac sarcoidosis can be life threatening, as shown by a study in which over a 2.6 years follow-up, 8% of patients were recorded with death, aborted sudden cardiac death, or intra-cardiac device therapy (24). The MRI changes can be considered predictors for the risk of sudden death, with higher risk for patients who have proven late gadolinium enhancement (11). Moreover, it has been shown that extensive late gadolinium enhancement is associated to poor outcome, higher risk of death and severe arrhythmias and lack of improvement of left ventricular function, despite corticosteroid therapy (25).

When to treat cardiac sarcoidosis?

Cardiac sarcoidosis is potentially an important threat for mortality and long-term cardiac consequences. In the view of the classic "Wells postulate," to treat sarcoidosis if there is risk or impairment of the quality of life, cardiac sarcoidosis should be considered "risk" and treatment should be started.

Recently, a European Respiratory Task Force committee composed of clinicians, methodologists and patients developed eight specific questions, used to formulate specific evidence-based recommendations. These were developed based on the GRADE methodology (15).

Question 5 refers to cardiac sarcoidosis: "*In patients with clinically relevant cardiac sarcoidosis, should glucocorticoids with or without other immunosuppressives* versus *no immunosuppression be used?"*

The proposed answer is that in patients with evidence of functional cardiac abnormalities, including heart block, dysrhythmias, or cardiomyopathy, it is recommended the corticosteroid therapy (with or without other immunosuppressives) (strong recommendation, very low quality of evidence) (15). The treatment should be recommended only in patients with significant clinical consequences of cardiac involvement, and not to all patients with imaging changes suggestive of cardiac sarcoidosis.

The committee notes that the evidence supporting the use of corticosteroids in cardiac sarcoidosis is indirect. Still, corticosteroid treatment was associated with improvement in left ventricular ejection fraction (26), so the danger associated to cardiac sarcoidosis favors the corticosteroid treatment for clinically relevant cardiac sarcoidosis (15).

The features found to be associated with increased risk of mortality or morbidity that may influence treatment decisions for cardiac sarcoidosis are (15):

- Age greater than 50.
- Left ventricular ejection fraction less than 40%.
- NYHA functional class 3 or 4.
- Increased left ventricular end-diastolic diameter.
- Late gadolinium enhancement on cardiac MRI.
- Ventricular tachycardia.
- Cardiac inflammation identified by FDG-PET scan.
- Interventricular septum thinning.
- Elevated troponin or brain natriuretic peptide.

Treatment of cardiac sarcoidosis

The current evidence suggests that corticosteroids are effective in reducing the granulomas in the heart, diminishing the dysrhythmias and blocks and improving the ventricular function (26). Still, patients with severe impairment of the ejection fraction (< 30%) may not respond to treatment, probably due to irreversible fibrotic changes in these patients (8).

The current trend to reduce the initial dose of corticosteroids for sarcoidosis treatment is applicable also for cardiac involvement. A retrospective analysis suggested that prednisolone doses higher than 0.5 mg/kg were not more effective than a starting dose of 0.5 mg/kg (27). The duration of treatment was mentioned in several studies between 3 and 168 months (28).

Steroid-sparing drugs most frequently mentioned are methotrexate, azathioprine, mycophenolate mofetil, leflunomide, and cyclophosphamide. These drugs were used also as alternatives in patients refractory to or with major contraindications to corticosteroids (29, 30). The efficiency of these agents, used in combination with low-dose corticosteroids or alone, is similar to corticosteroid treatment, with similar rates of relapse (33–35%), but more dedicated trials to define the optimal combination are needed (31). Recently, TNF-α antagonists (inflizimab, adalimumab) showed promising results in refractory cardiac sarcoidosis patients (32, 33).

Screening for cardiac sarcoidosis

The current knowledge supports the screening for cardiac sarcoidosis in asymptomatic patients with extra-cardiac sarcoidosis (22). There is still debate regarding the best way to screen for cardiac involvement. Physical examination and medical history specifically asking about palpitations, syncope, and chest pain should be performed in all patients. The American Thoracic guidelines recommend performing routine ECG to screen for the possible cardiac involvement. The routine echocardiography or 24-h continuous ambulatory ECG are not recommended in patients without cardiac symptoms or signs, but could be used on a case-by-case basis (34). Routine echocardiography, mentioned in Heart Rhythm Society, may overlook the cardiac involvement (35). The use of cardiac MRI, rather than cardiac PET, should be recommended for patients with extra-cardiac sarcoidosis that exhibit symptoms of heart involvement, and also to patients with no previous diagnosis of sarcoidosis who present with unexplained Mobitz II or third-degree AV block and sustained monomorphic ventricular tachycardia of unknown cause (8, 34).

A young adult with palpitations

A 32 years old nonsmoker male patient had a 2 years history of palpitations occurring in episodes, apparently unrelated to any triggering event. The episodes were sometimes accompanied by presyncope, and he presented repeatedly in emergency department during these events. The electrocardiograms were repeatedly showing ventricular arrhythmia, with frequent ventricular premature beats with monophocal pattern, sometimes organized in bigeminism or trigeminism (Figure 1). Heart ultrasound performed on these occasions did not show any change in ventricular function. He consulted a cardiologist specialized in heart rhythm disturbances, and received several antiarhythmic drugs (bisoprolol, sotalol, and propaphenone), which did not prevent the occurrence of new palpitations episodes, accompanied by the same lipotimic state. Continuous 24-h ECG recording showed the presence of premature ventricular beats also outside the clinically manifest episodes (Figure 2). The premature beats had the same morphology, mimicking a right bundle branch block.

A cardiac MRI with gadolinium enhancement was performed, which showed increased thickness of left ventricle during systole and contrast enhancement in the middle of cardiac wall at the base of the heart, and was considered by the radiologist who evaluated it initially as non-obstructive hypertrophic cardiomyopathy.

As the patient was not satisfied with the outcome of the investigations and the treatment, he sought a second opinion. On this occasion, the same cardiac MRI images were analyzed by a cardiologist specialized in MRI imaging of the heart. He interpreted the same images differently, describing the presence of focal edema at the base of the septum in T2 and granulomas with homogenous uptake of gadolinium at the base of the interventricular septum (Figure 3). He concluded that the image changes are highly suggestive for cardiac sarcoidosis. He also referred the patient to the pulmonology unit for further evaluation. The CT scan of the thorax showed multiple micronodules distributed mostly in the upper parts of the lungs, with a perilymphatic distribution, as well as moderately enlarged mediastinal lymph nodes (Figure 4). A bronchoscopy was performed, with a transbronchial biopsy of the mediastinal lymph nodes. The histology did not overtly confirm the presence of typical sarcoid granulomas, describing lymphocytic inflammation and some giant cells. Broncho-alveolar lavage proved a lymphocytic alveolitis with 34.4% lymphocytes in the BAL fluid, with normal CD4/CD8 ratio. The serum angiotensin-converting enzyme was highly elevated, three-fold the normal upper limit.

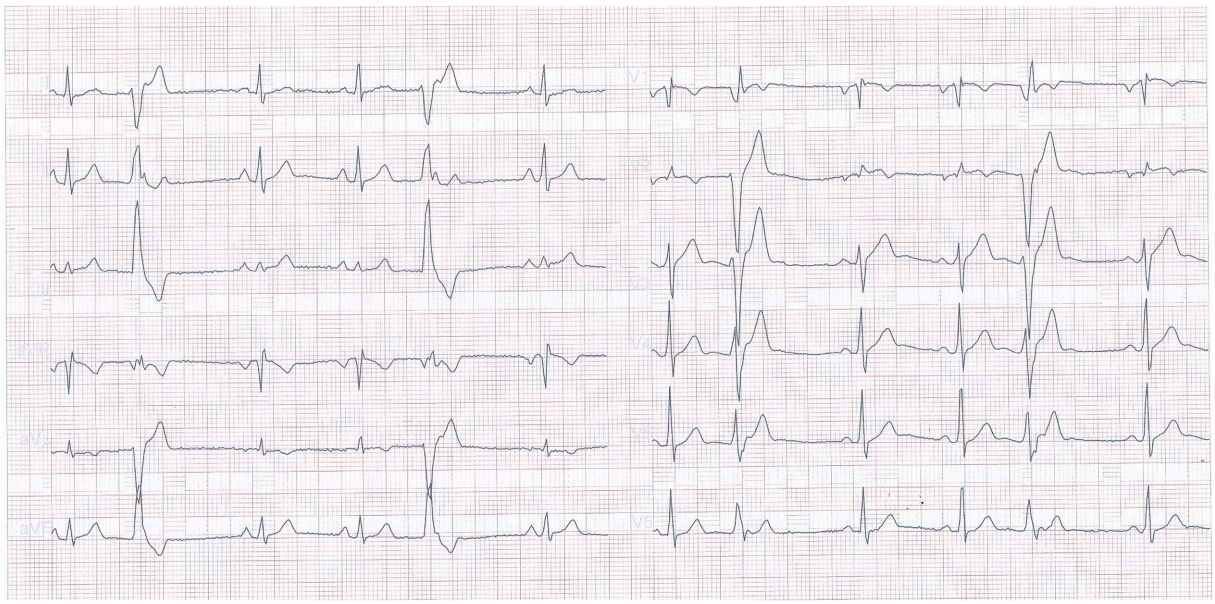

FIGURE 1
Electrocardiogram recorded during an emergency presentation, showing frequent premature ventricular beats.

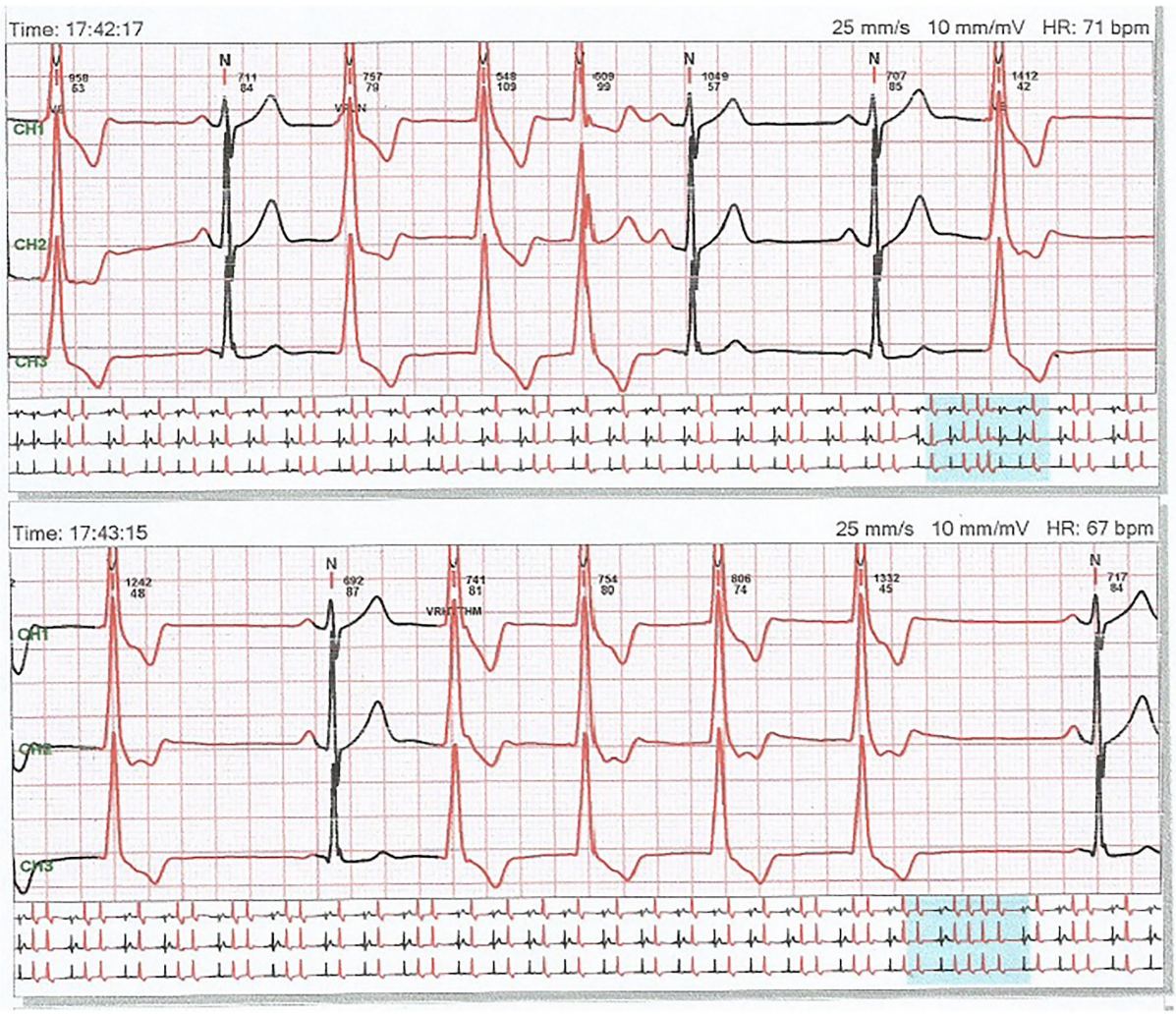

FIGURE 2
A 24h recording of electrocardiogram showing frequent premature ventricular beats with unique morphology, mimicking a right bundle branch block.

The patient did not complain of any respiratory symptoms, and the lung function tests were normal, including the diffusion capacity. He did not show signs of any other organ involvement: he had normal renal function, normal calcium level in the blood and urine, had no neurological symptoms, no eye ailment, no skin changes, no peripheral lymph node enlargement, and no joint pain.

He was diagnosed with sarcoidosis involving the lungs, mediastinal lymph nodes and heart. The diagnosis was considered as such even in the absence of a clear histologic confirmation. The BAL lymphocytosis was compatible with sarcoidosis (36). The normal CD4/CD8 ratio does not exclude the diagnosis, as it has a limited value (37). In this patient, even in the absence of a typical biopsy, applying the clinical sarcoidosis diagnosis score (SDS clinical) would have brought enough elements in favor of the diagnosis: highly probable: HRCT imaging changes, at least probable: BAL lymphocytic alveolitis, TBNA showing lymphoid aggregates and giant cells, spontaneous ventricular tachyarrhythmia in the absence of other risk factor, delayed enhancement on cardiac MRI (38, 39).

Oral corticosteroid treatment was started at a dose of 32 mg of methylprednisolone per day, tapered to 8 mg per day over 6 months. Low dose treatment was continued up to 36 months. The clinical improvement was noticed during the first month of treatment. The frequency of the palpitation episodes decreased, and the events were no longer accompanied by presyncope. The arrhythmic events disappeared completely during the first year of treatment. Cardiological examinations and chest ultrasound showed no impairment of cardiac function. The corticosteroid treatment was well tolerated, with minimal muscle weakness during the first months of treatment.

This case illustrates the difficulty of diagnosis of cardiac sarcoidosis in a patient that is not previously diagnosed with the disease, based on other organ involvements. Our patient had highly suggestive changes on the HRCT of the lungs, but this was not performed previously as he did not complain of any respiratory symptoms. The frequent visits to the emergency department and the occurrence of severe arrhythmia in a young patient with no risk factors and no other heart problems should have triggered the suspicion of a cardiac sarcoidosis earlier. The patient displayed the late gadolinium enhancement on cardiac MRI, which is considered a risk factor for severe outcome, and had potentially severe

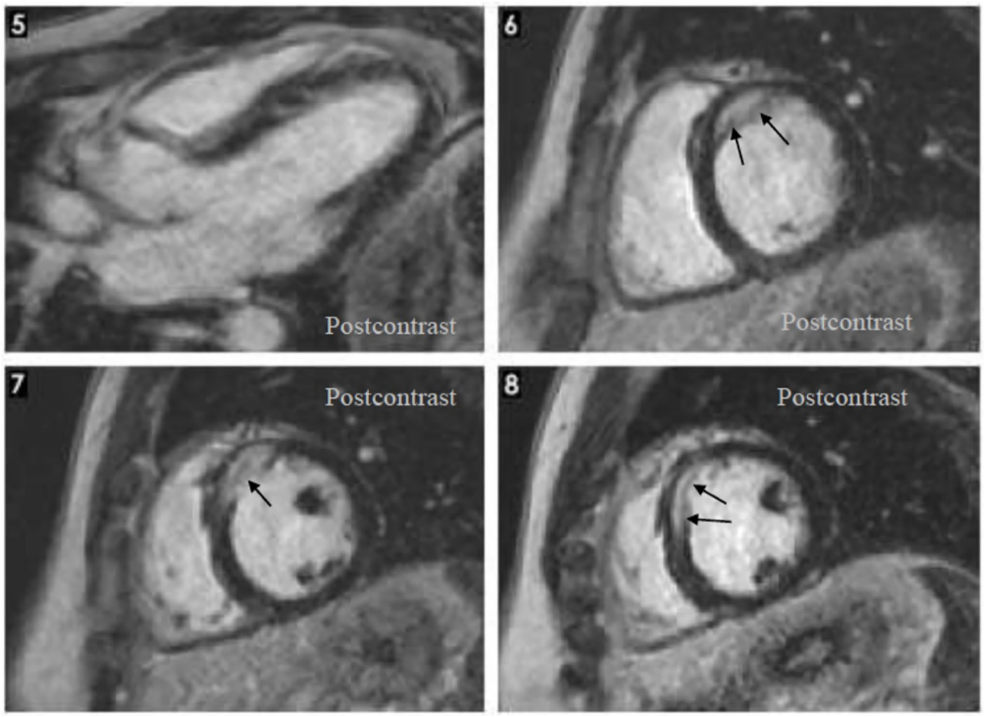

FIGURE 3
Cardiac magnetic resonance: Hypertrophy localized at the base of the septum, T2 focal edema at the base of the septum, late gadolinium enhancement: homogenous late uptake of gadolinium in the granuloma.

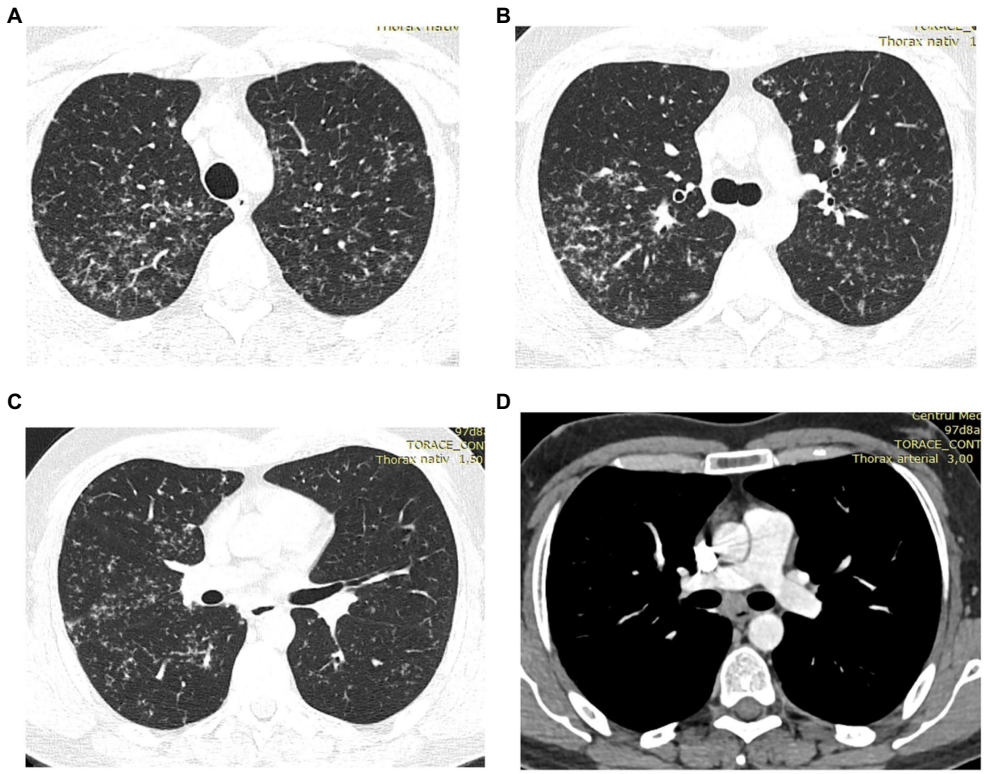

FIGURE 4
(A–D) HRCT of the lungs: multiple bilateral micronodules predominant in the upper lobes, increased infracarinal lymph nodes.

ventricular arrhythmia, but fortunately the function of the ventricle was not impaired and responded well to corticosteroid treatment.

Cardiac sarcoidosis can represent a life-threatening condition, the risk being augmented by the lack of suspicion of this disease in patients with no previous diagnosis of sarcoidosis.

Author contributions

IS contributed to the review of literature, editing the manuscript, medical care of the illustrative case, and choice of the figures. The author confirms being the sole contributor of this work and has approved it for publication.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Sex differences in the genetics of sarcoidosis across European and African ancestry populations

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Background: Sex differences in the susceptibility of sarcoidosis are unknown. The study aims to identify sex-dependent genetic variations in two clinical sarcoidosis phenotypes: Löfgren's syndrome (LS) and non-Löfgren's syndrome (non-LS).

Methods: A meta-analysis of genome-wide association studies was conducted on Europeans and African Americans, totaling 10,103 individuals from three population-based cohorts, Sweden (n = 3,843), Germany (n = 3,342), and the United States (n = 2,918), followed by an SNP lookup in the UK Biobank (UKB, n = 387,945). A genome-wide association study based on Immunochip data consisting of 141,000 single nucleotide polymorphisms (SNPs) was conducted in the sex groups. The association test was based on logistic regression using the additive model in LS and non-LS sex groups independently. Additionally, gene-based analysis, gene expression, expression quantitative trait loci (eQTL) mapping, and pathway analysis were performed to discover functionally relevant mechanisms related to sarcoidosis and biological sex.

Results: We identified sex-dependent genetic variations in LS and non-LS sex groups. Genetic findings in LS sex groups were explicitly located in the extended Major Histocompatibility Complex (xMHC). In non-LS, genetic differences in the sex groups were primarily located in the MHC class II subregion and *ANXA11*. Gene-based analysis and eQTL enrichment revealed distinct sex-specific gene expression patterns in various tissues and immune cell types. In LS sex groups, a pathway map related to antigen presentation machinery by IFN-gamma. In non-LS, pathway maps related to immune response lectin-induced complement pathway in males and related to maturation and migration of dendritic cells in skin sensitization in females were identified.

Conclusion: Our findings provide new evidence for a sex bias underlying sarcoidosis genetic architecture, particularly in clinical phenotypes LS and non-LS. Biological sex likely plays a role in disease mechanisms in sarcoidosis.

KEYWORDS

sarcoidosis, genetics, genome-wide association study (GWAS), meta-analysis, single nucleotide polymorphisms, immunogenetics and HLA

Background

Sarcoidosis is a multi-system disease with unknown etiology, characterized by non-caseating granulomas (1). In 90% of cases, sarcoidosis affects the lungs and lymphatic system (2).

Sarcoidosis is prevalent worldwide, commonly affecting individuals between 20 and 50 years of age, with males diagnosed earlier than females (3, 4). On a global scale, sarcoidosis incidence and prevalence vary based on sex, age, geography, and ethnicity (4). In the United States, the prevalence is 141 per 100,000 in African–Americans and 49.8 per 100,000 in Caucasians (5). In Nordic countries such as Sweden, the prevalence of sarcoidosis was reported between 152 and 215 per 100,000 (6), whereas in Denmark was reported at 77 per 100,000 (7).

The disease is heterogeneous, as different manifestations and clinical outcomes have been observed in clinical settings, particularly among sex groups (3, 8, 9). Interestingly, sarcoidosis is more common in females, has a late-onset, has a variable disease course depending on the affected organ, and has a higher mortality rate than men (3). Sex differences are also seen in extra-pulmonary phenotypes (10, 11), including cardiac (12, 13) and skin sarcoidosis.

Sex hormones have been shown to play a role in sarcoidosis (14, 15). For instance, sarcoidosis has a low disease activity in pregnancy or goes into remission. However, the ameliorating effect is lost as flares occur after delivery (16, 17).

In the lungs, sarcoidosis is characterized by Löfgren's syndrome (LS) and non-Löfgren's syndrome (LS). LS is an acute form of the disease characterized by periarticular swelling around the ankles, erythema nodosum, and bilateral hilar lymphadenopathy (18). Typically, females with LS exhibit erythema nodosum (EN), while males with LS have symptoms of bilateral ankle arthritis. Patients with LS usually have a good prognosis (19). LS often occurs in individuals of European ancestry and is relatively rare in individuals of African origin. In non-LS, sarcoidosis is characterized by a heterogeneous disease course, often with an insidious onset, unrelenting disease course, and a high risk for clinical organ impairment, predominantly pulmonary fibrosis (18, 20). Patients with fibrotic sarcoidosis have markedly decreased survival compared with the general population (21, 22).

The causes for clinical differences and disease course in sarcoidosis among sex groups are unknown. However, evidence-based factors, including genetics, epigenetics, and environmental exposures, have been postulated. Regarding genetics, few studies have addressed sex differences in the genetic variation in disease, despite most studies adjusting for sex in their analyses (23). An exception is a genome-wide admixture scan conducted in African Americans stratified by sex, identifying several genetic variants associated with sarcoidosis exclusively in females (24).

In this work, we sought to investigate the genetic associations in the sex groups and identify differences and commonalities in European and African population ancestries by a meta-analysis approach and high-density mapping.

Materials and methods

Samples and study design

The samples were from three independent sarcoidosis population-based cohorts from Sweden, Germany, and the United States. All participants provided written informed consent for the study. Study protocols of all studies had been approved by respective local institutional boards.

The Swedish cohort

The case-control study consisted of 4,133 individuals. The study was approved by the local institutional review board in Stockholm, Sweden. All participants provided written informed consent and were permitted to use their DNA for research purposes. Baseline clinical and demographic characteristics were measured when the participants were enrolled in the study.

Patients were enrolled at the time of disease investigation at the Sarcoidosis Centrum, Karolinska University Hospital Solna, Sweden. The diagnosis was established on radiographic manifestations, findings at bronchoscopy with bronchoalveolar lavage (BAL), including an elevated CD4/CD8 ratio > 3.5, and positive biopsies and in accordance with the criteria outlined by the World Association of Sarcoidosis and Other Granulomatous Disorders [WASOG, Statement on sarcoidosis (27)]. Sarcoidosis patients were further characterized into two clinical phenotypes, LS and non-LS. LS was defined by typical clinical manifestations with an acute onset of the disease, including fever, bilateral hilar lymphadenopathy on chest X-ray, bilateral ankle arthritis, and/or erythema nodosum. Non-LS was defined as a heterogeneous disease course with an insidious onset, unrelenting disease course, and a

Abbreviations: LS, Löfgren's syndrome; Non-LS, non-Löfgren's syndrome; SNPs, single nucleotide polymorphisms; eQTL, expression quantitative trait loci; UK Biobank, United Kingdom Biobank; *ANXA11*, Annexin A11 gene; GWAS, Genome-wide association study; US-AA, United States African Americans; PC, Principal component; PCA, principal component analysis; IVW, inverse variance weighting; OR, odds ratio; CI, confidence interval.

high risk for clinical organ impairment, such as developing fibrosis in the lung. Healthy controls included 3,085 individuals who were recruited from two large-scale epidemiological cohorts. Mainly, 2,025 individuals were from the Environmental Investigation of Rheumatoid arthritis (EIRA) study described in Klareskog et al. (25) and 1,060 individuals were from the Epidemiological investigation of risk factors for Multiple Sclerosis (EIMS) study described in Hedstrom et al. (26).

The German cohort

The case-control study consisted of 4,975 individuals, of which 413 were non-LS (27). The description and inclusion of these patients are described elsewhere (28, 29). Briefly, German control subjects (n = 4,498) were derived from Popgen (n = 2,485) (30), and the Heinz Nixdorf RECALL (HNR) study (n = 1,499) (31). Additionally, 304 control individuals of South German origin were recruited from the Bavarian Red Cross, and 210 control individuals were recruited from the Charité - Universitätsmedizin Berlin. The mean age and male percentage were 62.5 years (SD = 12.3) and 40% male in non-LS and 57.8 years (SD = 12.2), and 51% male in controls.

The USA African American cohort

The case-control study consisted of 1,657 individuals. The sample included 781 sarcoidosis cases characterized as non-LS and 876 healthy controls. All individuals were taken from an extensive cohort of African-American (AA) sarcoidosis patients and controls assembled from various studies. Further details are available in Refs. (32–35).

Genotyping and quality control

Genotyping for sarcoidosis patients in the Swedish cohort was performed using the Illumina Immunochip platform and was performed at The SNP&SEQ Technology Platform in Uppsala University, Sweden, with Illumina Infinium assay using the Immuno Bead Chip (Immunochip version 1) as described in Rivera et al. (23). Healthy controls in the EIRA cohort (n = 2,086) were genotyped on the same platform at the Genome Institute of Singapore, as described previously (36, 37). Healthy controls in the EIMS cohort (n = 1,060) were genotyped with the same SNP array described elsewhere (26). Briefly, quality control filtering thresholds were applied using tools implemented in PLINK v1.09b software (38, 39). SNPs at call rate < 95% with minor allele frequency (MAF) > 1% and call rate < 99% with MAF ≤ 5% were filtered out. Moreover, SNPs that had Hardy–Weinberg Equilibrium (HWE) $P < 1 \times 10^{-7}$ (in the control group) were also excluded. Individuals with missing genotypes < 97% were also removed. Quality control (QC) resulted in 141,151 SNPs and 3,842 individuals on the Illumina Immunochip array.

Genotypes for the German cohort were quality controlled, as described in Fischer et al. (40). Genotyping for the African American cohort (US-AA) was performed at the OMRF using the Illumina HumanOmni1-Quad array for ∼1.1M variants across the genome. Of these, 121,988 were in common with the Immunochip platform and carried forward for analysis. Further details on genotyping and quality control filtering are described in Adrianto et al. (41).

Statistical analysis

Association analysis of LS and non-LS in sex groups

In each sex group, we examined the association of single nucleotide polymorphisms (SNPs) in LS and non-LS. For assessing the association in LS, we applied an additive model using logistic regression adjusted for age and four principal components (PCs). PCs were derived from principal component analysis (PCA) performed using EIGENSTRAT (42) software on a pruned genotyped data set. The pruning of genotypes was performed using the pruning function and default parameters implemented in PLINK 1.90 beta software (39). For assessing the association in non-LS, we applied an additive model using logistic regression without covariate adjustment due to the lack of age information across the cohorts. In LS and non-LS, a significance threshold was defined by genomic control-corrected $P < 3.5 \times 10^{-7}$ (0.05/141,151 quality-controlled SNPs) considering Bonferroni correction. A suggestive $P < 5 \times 10^{-5}$ was also considered to identify potential signals (43–46). Furthermore, to account for high linkage disequilibrium (LD) ($r^2 \geq 0.8$) among associated SNPs, LD pruning was performed using PriorityPruner version 0.01.4 software[1] with default parameters, which identified and prioritized SNPs in genomic loci and thus referred as tag-SNPs.

As an exploratory analysis, to assess the effect of sex on genetics, we implemented an interaction model where the disease outcome was regressed on SNP adjusted for sex and the interaction term (SNP × sex) as covariates. A significant threshold for the interaction term was set at $P < 0.05$. The interaction analysis was performed using the glm function implemented in PLINK 2.0 software (39).

Additionally, we also performed a heterogeneity test to assess the effects of association between males and females. Heterogeneity statistics i.e., Cochran's Q-statistic, I^2 statistic (the percentage of variability in the effect sizes), and tau^2 (the between-study variance in our meta-analysis) were computed applying a random effects model and using the rma function implemented in the metafor R package (47).

Meta-analysis of significant SNPs

Meta-analysis was performed using SNP association results in non-LS. Herein, the meta-analysis was conducted using the inverse variance weighting (IVW) method implemented in the METAL software (48). For each SNP, the combined genetic effect size (defined by Beta), standard error (SE), meta-P-value (P_{meta}), total variability in effect size, also known as the heterogeneity index (I^2), and heterogeneity P-value were calculated. SNPs at $P_{meta} < 5 \times 10^{-8}$ were defined as genome-wide significant. Two meta-analyses were conducted, (1) a meta-analysis considering SNP associations in the European ancestry cohorts (Sweden and Germany) and (2) a meta-analysis considering SNP associations in the multi-ethnic (Sweden, Germany, and US African American).

Besides METAL computing heterogeneity index (I^2), we also performed an independent heterogeneity test applying the random effects model using the rma function implemented in the metafor

1 http://prioritypruner.sourceforge.net/

R package (47). Summary of heterogeneity statistics include Cochran's Q-statistic, I^2 statistic (the percentage of variability in the effect sizes), and tau^2 (the between-study variance in our meta-analysis).

Lookup of significant SNPs in the UK Biobank

Using the UKB resource, we examined SNP associations at $P < 5 \times 10^{-5}$ using summary statistics of a genome-wide association study (GWAS) on "doctor-diagnosed sarcoidosis" (Data-Field ID: 22133), consisting of 91,787 individuals (395 cases and 91,392 controls) in the UKBB (49). Chiefly, sex-stratified summary statistics GWAS on males (189 cases and 41,045 controls) and females (206 cases and 50,347 controls) were obtained from https://github.com/Nealelab/UK_Biobank_GWAS. GWAS analysis was conducted using an additive model and logistic regression adjusted by the first 20 PCs, age, and age^2. A lookup significance threshold was set at $P < 5 \times 10^{-8}$.

Gene-based analysis

A gene-based analysis was conducted using SNP associations at $P < 5 \times 10^{-5}$ and MAGMA (50). Gene-based significance was set to $P < 2.0 \times 10^{-6}$ based on Bonferroni correction (0.05/24,769 genes). The number of genes was adopted from the VEGAS2 software definition, where 24,769 unique genes on the 22 autosomes were identified (51).

SNP enrichment analysis via expression quantitative trait loci (eQTLs) and pathway analysis

Expression quantitative trait loci enrichment was performed using SNP associations at $P < 5 \times 10^{-5}$ and gene expression data from 53 human tissues from the Genotype-Tissue Expression (GTEx) project version 8 available on the Functional Mapping and Annotation of genetic associations (FUMA) web tool (52). Additional eQTL enrichment using gene expression of lung tissue, whole blood, and immune cell types was conducted using EUGENE software (53, 54). A significant threshold for n SNP correlated with gene expression (defined as eQTL-SNP or eSNP) was set at $P < 0.05$.

Pathway analysis was conducted based on SNP associations at $P < 5 \times 10^{-8}$ to elucidate disease mechanisms using MetaCore^{TM} software.

Results

The summary of phenotypes and the number of samples in the sex groups for cohorts investigated are shown in Table 1.

Association results

The genome-wide association analysis using high-density mapping Immunochip array was conducted in males and females independently in Swedish, German, and US-African American cohorts.

TABLE 1 Sample size in association analysis in Sweden, Germany, and United States (A); sample size in replication lookup in the UK Biobank (B).

(A)				
Cohorts	LS		Non-LS	
	Males	Females	Males	Females
Swedish	166 cases vs. 849 HC	132 cases vs. 2,236 HC	263 cases vs. 855 HC	181 cases vs. 2,245 HC
German			470 cases vs. 1,750 HC	732 cases vs. 1,692 HC
USA-AA			322 cases vs. 497 HC	951 cases vs. 1,148 HC

(B)	
Males	Females
344 cases vs. 166,644 controls	361 cases and 193,792 controls

HC, healthy controls.

LS sex groups

In the Swedish cohort (Supplementary Tables 1, 2), 542 SNPs in LS males and 617 SNPs in LS females were identified at $P < 3.5 \times 10^{-7}$. Rs2187668 (OR = 4.715, SE = 0.1983, $P = 6.02 \times 10^{-14}$) located in *HLA-DQA1* was the top signal identified in males, and rs9268219 (OR = 3.667, SE = 0.1586, $P = 3.33 \times 10^{-15}$) located in *C6orf10* was the top signal identified in females. After considering linkage disequilibrium (LD) among associated signals, 77 SNPs in males and 101 SNPs in females remained, which were defined as tag-SNPs. The top results for LS sex groups (Table 2) are illustrated in the forest plot (Figure 1). Manhattan and QQ plots are shown in Supplementary Figure 1. Heterogeneity statistics are available in Supplementary Table 2X.

Conditioning for the top SNP rs2187668 in males identified 12 SNPs at $P < 5 \times 10^{-5}$. The most significant SNP was rs9296079 (OR = 7.476, SE = 0.410, $P = 9.51 \times 10^{-7}$), located at 7.8 kb 5′ of *HLA-DPB2*. Similarly, conditioning for rs9268219 in females identified 8 SNPs at $P < 5 \times 10^{-5}$. The most significant was rs29243 (OR = 10.980, SE = 0.514, $P = 6.04 \times 10^{-6}$) located in *GABBR1*. Non-MHC SNPs at $P < 5 \times 10^{-5}$ were identified near *LRRTM4* in males and *CAST, LNPEP, TNIP1, CSMD1, B4GALNT1, SBNO2,* and *ABCG1* in females (Supplementary Table 3).

Non-LS sex groups

In the Swedish cohort (Supplementary Tables 4, 5), 5 SNPs in males at $P < 3.5 \times 10^{-7}$ and 1 SNP in females at $P = 6.73 \times 10^{-7}$ were identified. The top signals were rs1049550 (OR = 0.5231, SE = 0.1126, $P = 1.82 \times 10^{-8}$) located in *ANXA11* in males and rs1964995 (OR = 0.5314, SE = 0.1198, $P = 6.73 \times 10^{-7}$) located at 36 kb of the 3′ of *HLA-DRB5*, in females. After LD assessment, 3 SNPs in males defined as tag-SNPs remained.

In the German cohort (Supplementary Tables 6, 7), rs1964995 (OR = 0.5452, SE = 0.08223, $P = 3.40 \times 10^{-11}$) in males and rs4502931 (OR = 0.5899, SE = 0.06549, $P = 4.36 \times 10^{-14}$) in females were identified as the top signals. In the US African American cohort (Supplementary Tables 8, 9), rs9271640 (OR = 1.771, SE = 0.1097, $P = 3.62 \times 10^{-7}$) in males and rs1964995 (OR = 0.5452, SE = 0.08223, $P = 3.40 \times 10^{-11}$) in females were identified as the top signals.

TABLE 2 The top tag-SNPs ($P_{GC} < 3.5 \times 10^{-7}$) associated with LS males and females in the Swedish cohort.

SNP	CHR	BP (hg19)	Alleles	RefSeq genes	Sex group	CA	CAF	OR	95% CI	P_{GC}
rs2187668	6	32,605,884	A/G	*HLA-DQA1*	LS-M	A	0.3557	4.7150	(3.1966, 6.9547)	6.02E-14
					LS-F	A	0.3557	3.4630	(2.5643, 4.6768)	6.57E-15
rs3130288	6	32,096,001	A/C	*ATF6B*	LS-M	A	0.3456	4.4560	(3.0489, 6.5124)	1.29E-13
					LS-F		0.3456	3.5090	(2.58, 4.7725)	1.39E-14
rs2105902	6	32,395,698	A/T	*12kb 5′ of HLA-DRA*	LS-M	A	0.4010	4.2330	(2.9272, 6.1214)	1.87E-13
					LS-F		0.4010	3.1160	(2.32, 4.1851)	3.76E-13
rs3135382	6	32,383,441	C/A	*8.5kb 5′ of BTNL2*	LS-M	C	0.4446	3.9120	(2.749, 5.567)	3.47E-13
					LS-F		0.4460	2.9310	(2.2128, 3.8823)	5.50E-13
rs3131643	6	31,442,782	A/G	*2.6kb 3′ of HCG26*	LS-M	A	0.3788	3.9640	(2.7471, 5.7201)	1.59E-12
					LS-F		0.3788	2.8890	(2.1476, 3.8864)	1.57E-11
rs4143332	6	31,348,365	A/G	*19kb 5′ of MICA*	LS-M	A	0.3389	4.1600	(2.8303, 6.1145)	3.32E-12
					LS-F		0.3389	3.3970	(2.5095, 4.5985)	2.66E-14
rs2071278	6	32,165,444	G/A	*NOTCH4*	LS-M	G	0.3725	3.8000	(2.6308, 5.4888)	8.55E-12
					LS-F		0.3725	3.1160	(2.3032, 4.2156)	1.36E-12
rs3094049	6	30,359,360	A/G	*45kb 3′ of RPP21*	LS-M	A	0.2903	4.1510	(2.7873, 6.1819)	1.78E-11
					LS-F		0.2903	3.2150	(2.3198, 4.4557)	1.53E-11
rs2524069	6	31,244,789	T/A	*4.9kb 5′ of HLA-C*	LS-M	T	0.3540	3.6090	(2.5168, 5.1752)	2.11E-11
					LS-F		0.3540	2.7470	(2.0481, 3.6845)	8.72E-11
rs2736157	6	31,600,820	G/A	*PRRC2A*	LS-M	G	0.4161	3.4380	(2.4292, 4.8657)	2.22E-11
					LS-F		0.4161	2.7580	(2.0587, 3.6949)	6.19E-11
rs9268219	6	32,284,108	C/A	*C6orf10*	LS-M	C	0.3356	4.4090	(3.0003, 6.4792)	4.10E-13
					LS-F		0.3356	3.6670	(2.6872, 5.004)	3.33E-15
rs1634721	6	30,977,680	A/G	*PBMUCL1*	LS-M	A	0.3221	3.4810	(2.372, 5.1085)	9.45E-10
					LS-F		0.3221	3.5930	(2.6382, 4.8934)	6.08E-15
rs3132449	6	31,626,013	A/G	*25bp 3′ of APOM*	LS-M	A	0.3418	3.9780	(2.7203, 5.8173)	8.08E-12
					LS-F		0.3418	3.4680	(2.5569, 4.7038)	1.47E-14
rs1064627	6	30,698,541	G/A	*FLOT1*	LS-M	G	0.354	2.9360	(2.0263, 4.2541)	4.61E-08
					LS-F		0.354	3.3110	(2.4618, 4.4532)	2.64E-14
rs9266669	6	31,348,077	A/G	*19kb 5′ of MICA*	LS-M	A	0.3625	3.3530	(2.3232, 4.8393)	5.56E-10
					LS-F		0.3625	3.3570	(2.4872, 4.531)	2.73E-14
rs886423	6	30,782,205	G/C	*70kb 5′ of DDR1*	LS-M	G	0.3322	2.8220	(1.9374, 4.1107)	2.11E-07
					LS-F		0.3322	3.2850	(2.4358, 4.4303)	6.69E-14
rs3129963	6	32,380,208	G/A	*5.3kb 5′ of BTNL2*	LS-M	G	0.4513	3.7770	(2.6521, 5.3791)	1.53E-12
					LS-F		0.4513	3.0010	(2.2666, 3.9734)	1.61E-13
rs2233974	6	31,080,016	C/G	*C6orf15*	LS-M	C	0.3793	3.3470	(2.325, 4.8184)	4.47E-10
					LS-F		0.3793	3.1680	(2.3592, 4.2542)	1.66E-13
rs3130477	6	31,428,920	G/A	*2kb 5′ of HCP5*	LS-M	G	0.3389	4.0890	(2.7858, 6.0019)	5.00E-12
					LS-F		0.3389	3.2220	(2.3816, 4.359)	2.91E-13

SNP, single nucleotide polymorphism; BP, chromosome base pairs are based on human genome assembly version 19 (hg19); CA, coded allele; CAF, coded allele frequency; OR, odds ratio; SE, standard error of odds ratio; P_{GC}, genomic controlled *P*-value.

The top association results for each cohort are summarized in Table 3. Manhattan and QQ plots for non-LS sex groups are shown in Supplementary Figures 2–4.

Interaction analysis with sex

Results from the interaction analysis in the LS and non-LS sex groups in the Swedish cohort showed significant SNPs interacting with the sex variable. In LS sex groups (Supplementary Table 10), the most significant interacting SNPs were rs2853973 ($P_{int} = 3.8 \times 10^{-4}$) in males and rs1470410 ($P_{int} = 5.44 \times 10^{-4}$) in females. In non-LS sex groups (Supplementary Table 11), the most significant interacting SNPs were rs10940422 ($P_{int} = 4.18 \times 10^{-4}$) in males and rs12432418 ($P_{int} = 4.85 \times 10^{-5}$) in females.

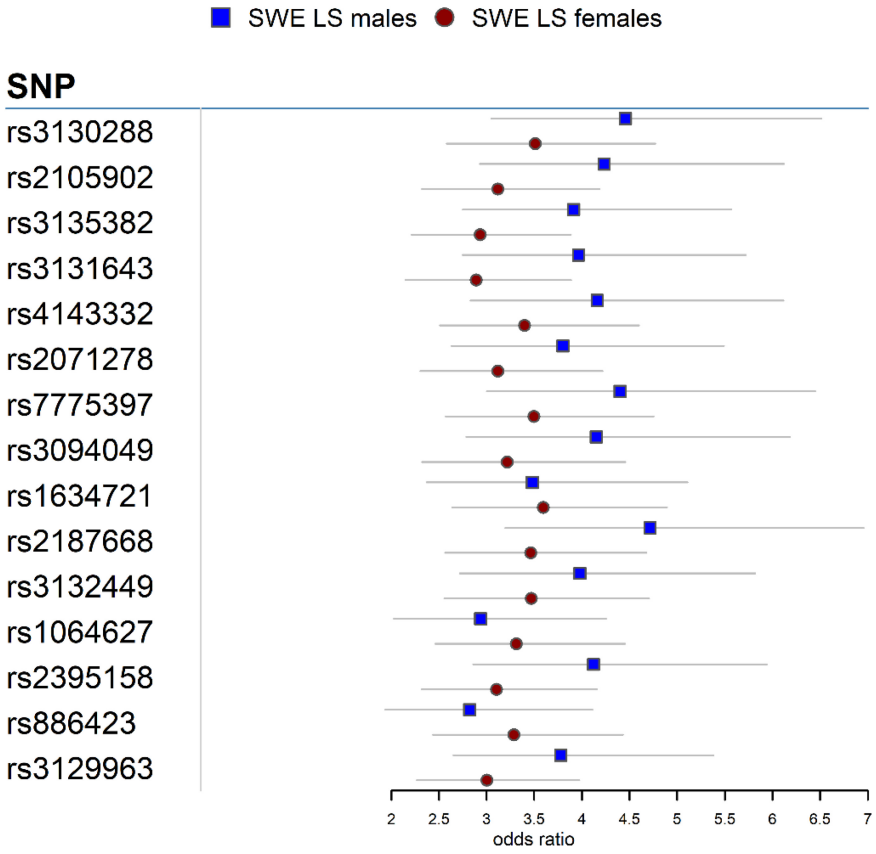

FIGURE 1
Forest plot for the 15 top common SNPs in the LS sex groups in the Swedish cohort.

Meta-analysis in non-LS

Non-LS sex groups

Meta-analysis in the European cohorts (Sweden and Germany) (Supplementary Tables 12, 13) identified 57 SNPs in males and 112 SNPs in females at $P_{meta} < 5 \times 10^{-8}$. Top signal rs1964995 located 36 kb from the 3′ of *HLA-DRB5* was the same in males (OR = 1.715, SE = 0.0656, $P_{meta} = 3.92 \times 10^{-18}$) and females (OR = 1.7304, SE = 0.0598, $P_{meta} = 5 \times 10^{-20}$). A forest plot illustrating the top findings is shown in Figure 2. Manhattan and Q-Q plots of the meta-analysis are shown in Supplementary Figure 5. Heterogeneity statistics in the SWE-GER are shown in Supplementary Table 13X.

A comparison of associations at $P_{meta} < 5 \times 10^{-8}$ showed 44 SNPs shared between non-LS males and females, 17 SNPs exclusively associated with non-LS males, and 215 SNPs exclusively associated with non-LS females. Most SNPs were in the MHC region. Non-MHC SNPs include rs694739 (OR = 1.3626, SE = 0.063, $P_{meta} = 9.21 \times 10^{-07}$) located at 7.9 kbp from the 3′ of *PRDX5* associated with non-LS males, and rs2573346 (OR = 0.7712, SE = 0.0564, $P_{meta} = 4.19 \times 10^{-06}$) in *ANXA11* associated with non-LS females.

Results from a meta-analysis in the multi-ethnic cohorts (Sweden, Germany, and US-African American) (Supplementary Tables 14, 15) identified 12 SNPs in non-LS males and 49 in non-LS females (Table 3). Top SNPs were rs1964995 (OR = 1.7736, SE = 0.0587, $P_{meta} = 1.54 \times 10^{-22}$) located 36 kb from 3′ of *HLA-DRB5* in males, and rs2395153 (Beta = 0.6565, SE = 0.0477, $P_{meta} = 1.11 \times 10^{-18}$) located at 5.9 kb from 5′ of *C6orf10* in females. The top meta-SNPs are shown in Table 4. Forest plots (Figure 2) illustrate the top common meta-SNPs in the European and multi-ancestry cohort groups. Manhattan plots of meta-analysis in multi-ethnic groups are shown in Supplementary Figure 6. Heterogeneity statistics in SWE-GER-USA are shown in Supplementary Table 15X.

A comparison analysis at $P_{meta} < 5 \times 10^{-8}$ showed a shared SNP between non-LS males and females, 11 SNPs exclusively associated with non-LS males, and 48 SNPs exclusively associated with non-LS females. Most associated SNPs were in the MHC region. Non-MHC SNPs were observed in non-LS females and included rs7813186, rs1049550, and rs7133604 (Data not shown).

Lookup of meta-SNPs in the UK Biobank

Genome-wide association study of "Doctor diagnosed sarcoidosis" in the UKB was used as a validation step. SNP-lookup showed several sex-specific SNPs in LS and non-LS at $P < 5 \times 10^{-5}$. Chiefly, 223 SNPs associated with LS males and 209 SNPs associated with LS females were validated at $P < 5 \times 10^{-8}$ (Supplementary Tables 16, 17). In non-LS, 11 non-LS males and 31 SNPs non-LS females in the SWE-GER cohorts were validated

TABLE 3 The top tag-SNPs ($P_{GC} < 3.5 \times 10^{-7}$) associated with non-LS males and females in Swedish, German, and USA-AA cohorts.

						Males				
SNP	CHR	BP (hg19)	Alleles	RefSeq genes	Cohort	CA	CAF	OR	95% CI	P_{GC}
rs1049550	10	81,926,702	A/G	*ANXA11*	SWE	A	0.2962	0.5231	(0.4195, 0.6523)	1.82E-08
					GER	A	0.3845	0.7325	(0.6281, 0.8543)	3.63E-04
					USA-AA	A	0.1508	0.7724	(0.5841, 1.0215)	7.70E-02
rs2239802	6	32,411,846	C/G	*HLA-DRA*	SWE	C	0.3375	1.7790	(1.4295, 2.214)	4.52E-07
					GER	C	0.2283	1.4480	(1.2336, 1.6997)	4.75E-05
					USA-AA	C	0.3321	1.5140	(1.2288, 1.8655)	1.41E-04
rs7197	6	32,412,580	A/G	*HLA-DRA*	SWE	A	0.2885	1.7610	(1.4056, 2.2063)	1.50E-06
					GER	A	0.1901	1.4210	(1.1976, 1.6862)	2.96E-04
					USA-AA	A	0.2253	1.6350	(1.2895, 2.0731)	7.32E-05
rs1964995	6	32,449,411	G/A	*36kb 3′ of HLA-DRB5*	SWE	G	0.2995	0.6033	(0.4872, 0.747)	5.74E-06
					GER	G	0.3664	0.5452	(0.464, 0.6406)	3.40E-11
					USA-AA	G	0.2314	0.5568	(0.431, 0.7194)	1.21E-05
rs3830135	6	32,548,464	A/G	*HLA-DRB1*	SWE	A	0.0529	0.3543	(0.2278, 0.5512)	6.71E-06
					GER	A	0.0980	0.4374	(0.3212, 0.5956)	2.40E-06
					USA-AA	C	0.0702	0.5942	(0.3897, 0.906)	1.82E-02
rs3131283	6	32,119,898	A/G	*177bp 5′ of PRRT1*	SWE	A	0.1971	1.8100	(1.4054, 2.3312)	6.85E-06
					GER	A	0.1417	1.3790	(1.1382, 1.6707)	3.21E-03
					USA-AA	C	0.0256	1.7040	(0.9263, 3.1348)	9.41E-02
rs4530903	6	32,581,889	A/G	*23kb 5′ of HLA-DQA1*	SWE	A	0.0529	0.3590	(0.2308, 0.5585)	8.79E-06
					GER	A	0.0973	0.4282	(0.3133, 0.5853)	1.74E-06
					USA-AA	T	0.0702	0.5942	(0.3897, 0.906)	1.82E-02
rs1800684	6	32,151,994	T/A	*AGER*	SWE	T	0.1982	1.7920	(1.3917, 2.3076)	9.75E-06
					GER	A	0.1428	1.3810	(1.1401, 1.6729)	3.00E-03
					USA-AA	A	0.0293	1.6280	(0.9369, 2.8289)	9.13E-02
rs3104402	6	32,681,676	A/C	*27kb 5′ of HLA-DQA2*	SWE	A	0.1050	2.0860	(1.5013, 2.8984)	1.83E-05
					GER	T	0.0689	1.3750	(1.0539, 1.794)	3.50E-02
					USA-AA	G	0.0153	2.3790	(1.0553, 5.3629)	4.12E-02
rs1044506	6	32,172,065	A/C	*NOTCH4*	SWE	A	0.2016	1.7870	(1.3878, 2.3011)	1.08E-05
					GER	T	0.1441	1.3560	(1.1203, 1.6413)	4.97E-03
					USA-AA	G	0.0263	1.9640	(1.0695, 3.6067)	3.35E-02
						Females				
rs1964995	6	32,449,411	G/A	*36kb 3′ of HLA-DRB5*	SWE	G	0.2995	0.5314	(0.4202, 0.6721)	6.73E-07
					GER	G	0.3611	0.5942	(0.519, 0.6804)	1.65E-12
					USA-AA	G	0.2408	0.8051	(0.6963, 0.9309)	3.77E-03
rs3129727	6	32,679,690	A/G	*29kb 5′ of HLA-DQA2*	SWE	A	0.0474	3.0760	(2.0025, 4.7251)	1.37E-06
					GER	A	0.0268	1.9580	(1.3773, 2.7836)	4.53E-04
					USA-AA	T	0.0217	1.6270	(1.0686, 2.4773)	2.47E-02
rs3998158	6	32,681,992	G/A	*27kb 5′ of HLA-DQA2*	SWE	G	0.1700	0.5047	(0.3771, 0.6755)	1.49E-05
					GER	C	0.1990	0.5811	(0.4908, 0.6881)	3.67E-09
					USA-AA	C	0.1665	1.0890	(0.925, 1.2821)	3.10E-01
rs2395153	6	32,345,595	C/G	*5.9kb 5′ of C6orf10*	SWE	C	0.2748	0.5947	(0.4694, 0.7535)	5.05E-05
					GER	C	0.3391	0.6137	(0.5347, 0.7044)	7.74E-11
					USA-AA	G	0.2175	0.7410	(0.6372, 0.8618)	1.18E-04

(Continued)

TABLE 3 (Continued)

						Females				
SNP	CHR	BP (hg19)	Alleles	RefSeq genes	Cohort	CA	CAF	OR	95% CI	P_{GC}
rs2076529	6	32,363,955	G/A	*BTNL2*	SWE	G	0.3176	0.6105	(0.4859, 0.7672)	6.66E-05
					GER	C	0.3752	0.6458	(0.5659, 0.7371)	1.22E-09
					USA-AA	C	0.3056	0.8406	(0.7363, 0.9598)	1.11E-02
rs9268472	6	32,355,605	A/G	*6.9kb 3′ of BTNL2*	SWE	A	0.3176	0.6107	(0.486, 0.7674)	6.71E-05
					GER	T	0.3754	0.6446	(0.5648, 0.7357)	1.04E-09
					USA-AA	A	0.3063	0.8471	(0.7421, 0.967)	1.51E-02
rs9275580	6	32,679,462	G/A	*30kb 5′ of HLA-DQA2*	SWE	G	0.1791	0.5543	(0.4192, 0.7329)	9.63E-05
					GER	C	0.2102	0.5519	(0.4666, 0.6528)	7.77E-11
					USA-AA	G	0.2458	1.1700	(1.0138, 1.3503)	3.35E-02
rs3129882	6	32,409,530	G/A	*HLA-DRA*	SWE	G	0.5146	1.5580	(1.263, 1.922)	9.81E-05
					GER	G	0.4499	1.1700	(1.0356, 1.322)	1.85E-02
					USA-AA	A	0.4586	1.0660	(0.9446, 1.203)	3.05E-01

SNP, single nucleotide polymorphism; BP, chromosome base pairs are based on human genome assembly version 19 (hg19); CA, coded allele; CAF, coded allele frequency; OR, odds ratio; SE, standard error of odds ratio; P_{GC}, genomic controlled *P*-value.

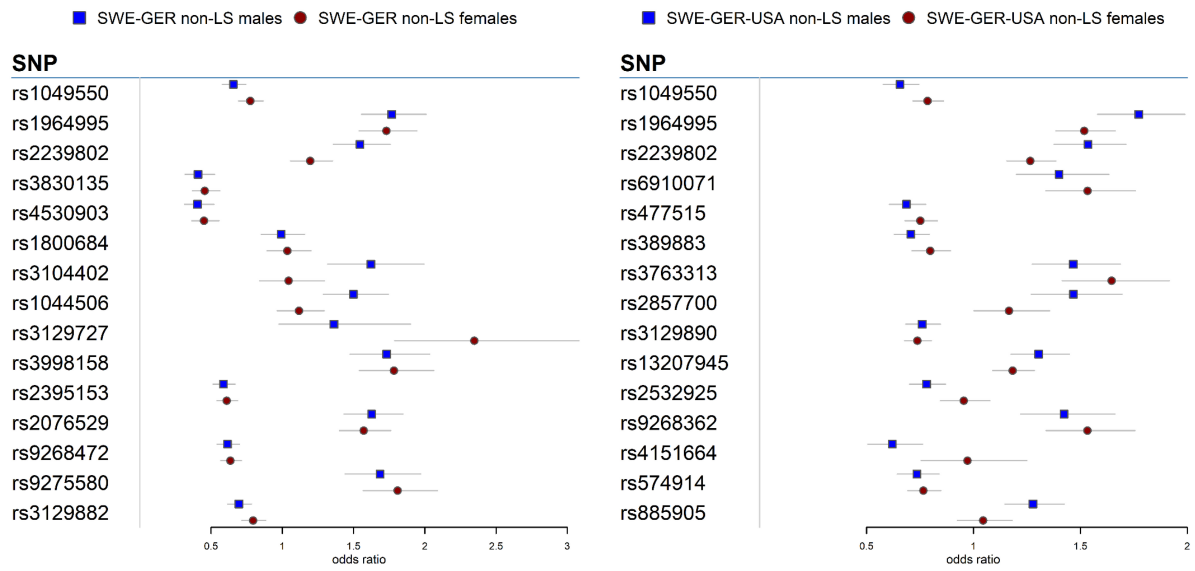

FIGURE 2
Forest plots for the 15 top common meta-SNPs in the non-LS sex groups in the European group (Swedish-German) and the multi-ethnic group (Swedish-German-United States-African American).

at $P < 5 \times 10^{-8}$ (Supplementary Tables 18, 19). In the multi-ethnic cohorts (SWE-GER-USA-AA), 2 SNPs in non-LS males and 8 SNPs in non-LS females were observed (Supplementary Tables 20, 21).

Gene-based analysis

The gene-based analysis revealed significant genomic loci associated with sex groups in LS and non-LS (Supplementary Tables 22, 23). The most significant genomic locus was *HLA-DRA* ($P = 4.36 \times 10^{-14}$) in LS males and *FKBPL* ($P = 1.39 \times 10^{-14}$) in LS females at a gene-based $P < 2.0 \times 10^{-6}$ (0.05/24,769 genes).

In the European cohorts (SWE-GER), 15 genes associated with non-LS males and 18 genes associated with non-LS females were identified, where the most significant genomic locus was *HLA-DRA* ($P = 5.16 \times 10^{-15}$) in non-LS males and *HLA-DRB1* ($P = 2.42 \times 10^{-13}$) in non-LS females. In the multi-ethnic cohorts (SWE-GER-USA-AA), 5 genes associated with non-LS males and 8 genes associated with non-LS females were identified. The top genes were *HLA-DRA* ($P = 1.88 \times 10^{-14}$) in non-LS males and *C6orf10* ($P = 6.47 \times 10^{-12}$) in non-LS females.

A comparison of gene-based analysis in LS and non-LS sex groups across cohorts (Supplementary Table 24) revealed differences in gene-based associations. In LS, gene-based associations showed 77 genes shared among sex groups, 11

TABLE 4 The top significant SNPs (P_{meta} < 5e-8) of GWAS meta-analysis on non-LS sex groups.

Sex group (Cohorts)	SNP	CHR	BP	Alleles	RefSeq_ genes	CA	OR	95% CI	*P*-value	Direction	Het-I^2	Het-PVal
Non-LS males (SWE-GER)	rs1964995	6	32,449,411	G/A	*36kb 3′ of HLA-DRB5*	A	1.7681	(1.5548, 2.0107)	3.92E-18	++	0	0.4583
	rs2395153	6	32,345,595	C/G	*5.9kb 5′ of C6orf10*	C	0.5861	(0.5134, 0.6692)	2.61E-15	–	0	0.5916
	rs2213585	6	32,413,150	G/A	*323bp 3′ of HLA-DRA*	A	0.6315	(0.5618, 0.71)	1.35E-14	–	0	0.3240
	rs7195	6	32,412,539	A/G	*HLA-DRA*	A	1.5830	(1.4082, 1.7795)	1.46E-14	++	14.5	0.2795
	rs9271588	6	32,590,953	G/A	*14kb 5′ of HLA-DQA1*	A	1.6153	(1.4291, 1.8258)	1.72E-14	++	0	0.5981
	rs2213586	6	32,413,094	A/G	*267bp 3′ of HLA-DRA*	A	1.5785	(1.4046, 1.7742)	1.93E-14	++	10	0.2918
	rs2227139	6	32,413,459	G/A	*632bp 3′ of HLA-DRA*	A	0.6335	(0.5637, 0.712)	1.93E-14	–	10	0.2918
	rs7192	6	32,411,646	A/C	*HLA-DRA*	A	1.5763	(1.4023, 1.7721)	2.54E-14	++	0.3	0.3165
	rs4373382	6	32,350,868	C/A	*11kb 5′ of C6orf10*	A	1.6291	(1.4337, 1.8512)	6.84E-14	++	0	0.6069
	rs2076529	6	32,363,955	G/A	*BTNL2*	A	1.6268	(1.4322, 1.8479)	7.03E-14	++	0	0.5625
Non-LS females (SWE-GER)	rs1964995	6	32,449,411	G/A	*36kb 3′ of HLA-DRB5*	A	1.7305	(1.5391, 1.9457)	5E-20	++	0	0.4192
	rs4502931	6	32,380,782	A/T	*5.9kb 5′ of BTNL2*	A	0.6033	(0.5401, 0.674)	3.82E-19	–	0	0.4968
	rs2395153	6	32,345,595	C/G	*5.9kb 5′ of C6orf10*	C	0.6088	(0.5405, 0.6859)	3.14E-16	–	0	0.8219
	rs9275582	6	32,680,070	A/G	*29kb 5′ of HLA-DQA2*	A	0.5499	(0.4757, 0.6358)	6.15E-16	–	0	0.7935
	rs2647012	6	32,664,458	A/G	*30kb 5′ of HLA-DQB1*	A	1.5502	(1.3899, 1.7291)	3.74E-15	++	0	0.5473
	rs9275393	6	32,669,439	A/G	*35kb 5′ of HLA-DQB1*	A	0.5890	(0.5156, 0.6728)	6.22E-15	–	0	0.3517
	rs2294878	6	32,367,795	A/C	*BTNL2*	A	0.6563	(0.5872, 0.7337)	1.27E-13	–	0	0.4173
	rs4530903	6	32,581,889	A/G	*23kb 5′ of HLA-DQA1*	A	0.4500	(0.364, 0.5564)	1.68E-13	–	0	0.5299
	rs2858332	6	32,68,1161	A/C	*28kb 5′ of HLA-DQA2*	A	0.6747	(0.6043, 0.7535)	2.83E-12	–	0	0.6205
	rs2858867	6	32,575,325	G/A	*18kb 5′ of HLA-DRB1*	A	0.6810	(0.6105, 0.7597)	5.54E-12	–	0	0.7201
Non-LS males (SWE-GER-USA-AA)	rs1964995	6	32,449,411	G/A	*36kb 3′ of HLA-DRB5*	A	1.7736	(1.5809, 1.9899)	1.54E-22	+++	0	0.7552
	rs2239802	6	32,411,846	C/G	*HLA-DRA*	C	1.5360	(1.3764, 1.7143)	1.88E-14	+++	19.6	0.2883
	rs4530903	6	32,581,889	A/G	*23kb 5′ of HLA-DQA1*	A	0.4478	(0.36, 0.5571)	5.40E-13	—	27.7	0.2506
	rs477515	6	32,569, 691	A/G	*12kb 5′ of HLA-DRB1*	A	0.6866	(0.607, 0.7767)	2.21E-09	—	0	0.4141
	rs389883	6	31,947,460	C/A	*STK19*	A	0.7073	(0.6302, 0.7939)	4.02E-09	—	0	0.3934

(Continued)

TABLE 4 (Continued)

Sex group (Cohorts)	SNP	CHR	BP	Alleles	RefSeq_ genes	CA	OR	95% CI	*P*-value	Direction	Het-I^2	Het-PVal
	rs3763313	6	32,376,471	C/A	*1.6kb 5′ of BTNL2*	A	1.4667	(1.2747, 1.6877)	9.03E-08	+++	21.6	0.2794
	rs9268835	6	32,428,115	A/G	*15kb 3′ of HLA-DRA*	A	0.7131	(0.6293, 0.8081)	1.13E-07	—	0	0.6909
	rs2857700	6	31,572,481	A/G	*11kb 5′ of AIF1*	A	1.4676	(1.27, 1.696)	2.02E-07	+++	16.1	0.3036
	rs3129890	6	32,414,273	G/A	*1.4kb 3′ of HLA-DRA*	A	0.7603	(0.6831, 0.8464)	5.58E-07	—	64.4	0.0604
	rs2269425	6	32,123,639	A/G	*PPT2*	A	0.6415	(0.538, 0.765)	7.69E-07	—	0	0.9529
Non-LS females (SWE-GER-USA-AA)	rs2395153	6	32,345,595	C/G	*5.9kb 5′ of C6orf10*	C	0.6565	(0.598, 0.7209)	1.11E-18	—	50.8	0.1311
	rs7195	6	32,412,539	A/G	*HLA-DRA*	A	1.4402	(1.3254, 1.5651)	7.42E-18	+++	0	0.8684
	rs2294878	6	32,367,795	A/C	*BTNL2*	A	0.7053	(0.6471, 0.7689)	2.06E-15	—	57.1	0.0971
	rs3129890	6	32,414,273	G/A	*1.4kb 3′ of HLA-DRA*	A	0.7374	(0.6772, 0.8031)	2.61E-12	—	20.5	0.2843
	rs6913309	6	32,339,840	A/T	*183bp 5′ of C6orf10*	A	0.7060	(0.6397, 0.7792)	4.25E-12	—	0	0.5575
	rs2269425	6	32,123,639	A/G	*PPT2*	A	0.6160	(0.5359, 0.7082)	9.74E-12	—	5.5	0.3471
	rs3129727	6	32,679,690	A/G	*29kb 5′ of HLA-DQA2*	A	2.1069	(1.6768, 2.6473)	1.62E-10	+++	56.6	0.1000
	rs6910071	6	32,282,854	G/A	*C6orf10*	A	1.5340	(1.3392, 1.7573)	6.61E-10	+++	0	0.4492
	rs3115572	6	32,220,484	C/G	*29kb 5′ of NOTCH4*	C	1.2871	(1.1875, 1.3951)	8.48E-10	+++	14.3	0.3113
	rs3817963	6	32,368,087	G/A	*BTNL2*	A	1.3835	(1.2453, 1.5371)	1.53E-09	+++	0	0.8790

SNP, single nucleotide polymorphism; CHR, chromosome; BP, base pairs; hg19, human genome assembly version 19; OR, odds ratio; 95% CI, 95% confidence interval; P_{meta}, metal *P*-value; Direction: (–) reversed strand; (++) forward strand; Het-I^2, heterogeneity I-squared; Het-PVal, heterogeneity I-squared *P*-value.

genes exclusively associated with males, and 14 genes exclusively associated with females. In non-LS sex groups, in the European cohorts (SWE-GER), 18 genes were shared among non-LS sex groups, and 8 genes were exclusively associated with non-LS males. In the multi-ethnic cohorts (SWE-GER-USA-AA), 4 genes were shared, one gene was exclusively associated with non-LS males, and 4 were exclusively associated with non-LS females.

Figure 3 shows a circos plot depicting gene-based associations among LS and non-LS sex groups across cohorts.

Gene expression and expression quantitative trait loci (eQTL) enrichment

Results from tissue expression analysis using 54 tissue types in GText version 8 and FUMA revealed significant sex-specific gene expression at Bonferroni *P*-value ($P_{Bon} < 0.05$).

Löfgren's syndrome males and females (Supplementary Figure 7), significant differential gene expression was observed in the spleen, small intestine, lung, and blood.

In the European cohorts (SWE, GER) (Supplementary Figure 8), differential gene expression was observed in the spleen, small intestine terminal Ileum, lung, and blood in non-LS males. In contrast, significant differential gene expression was observed in the brain hippocampus, and lung in non-LS females. In the multi-ethnic cohorts (SWE, GER, USA-AA) (Supplementary Figure 8), differentially expressed genes were observed in the spleen, small intestine terminal ilium, lung, and blood in non-LS males and females.

Expression quantitative trait loci enrichment using different eQTL databases across different tissues and cell types (Supplementary Table 25) revealed several significant eQTLs SNPs (eSNPs) in sex groups.

In LS sex groups, 311 eSNPs in 134 genes in males and 354 eSNPs in 140 genes in females were identified using the GText v8 database. In SWE-GER cohorts, non-LS sex groups, 32 eSNPs in 77 genes in males and 59 eSNPs in 89 genes in females were identified using the GText v8 database. In the SWE-GER-US-AA cohorts, non-LS sex groups, two eSNPs in males and 23 eSNPs in females were identified using the GText v8 database.

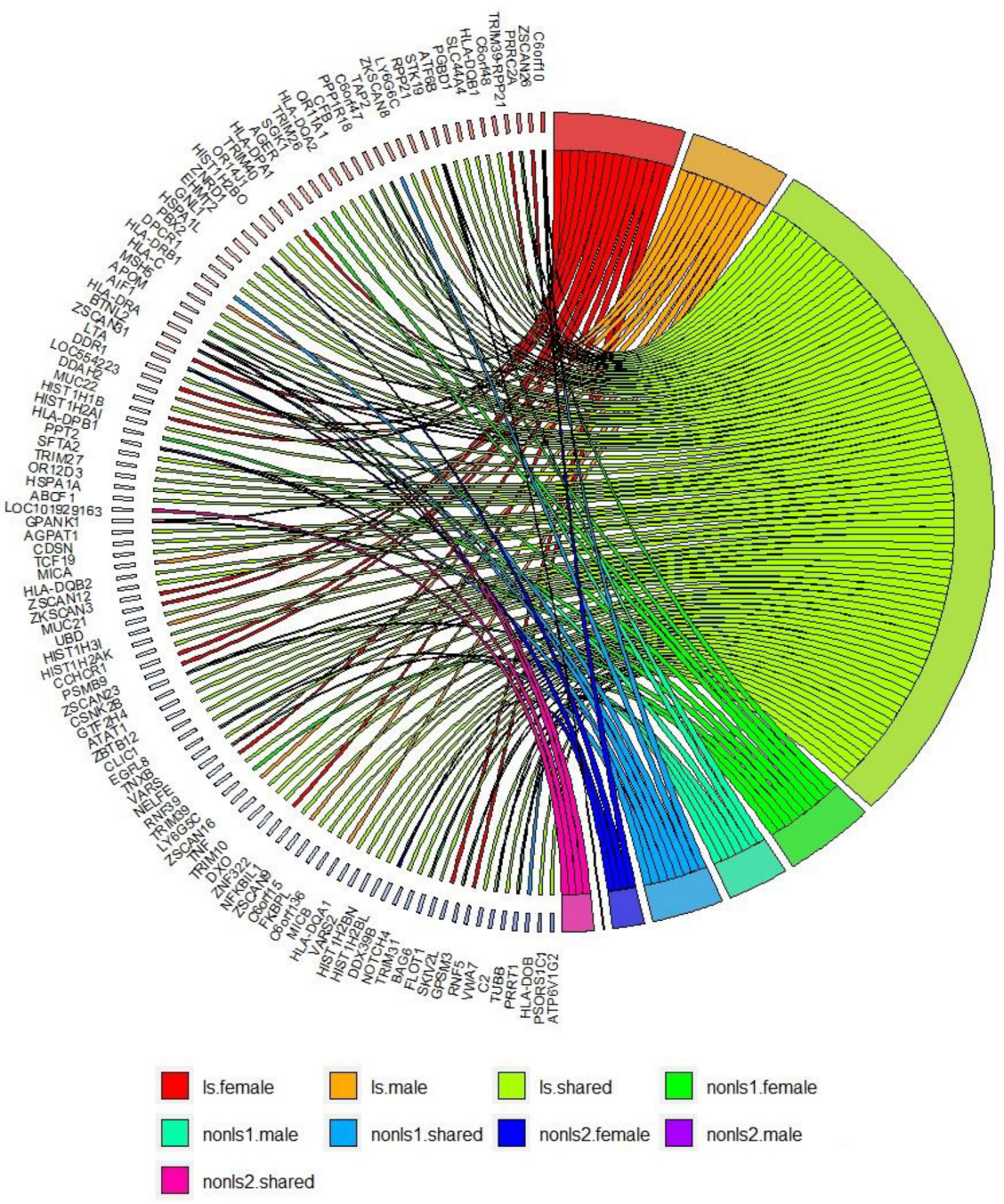

FIGURE 3
Gene-based analysis in LS and non-LS sex groups across all cohorts at $p < 2e\text{-}6$. ls, LS in SWE; non-ls1, non-LS in SWE and GER; non-ls2, non-LS in SWE, GER, and USA-AA; shared, genes shared by sex groups; male, genes associated with males only; female, genes associated with females only.

Similar observations were also observed using eQTLGen and eQTLcatalogue databases.

Moreover, eQTL mapping using immune cell eQTLs (Supplementary Table 26) available in the EUGENE database identified significant eSNPs in relevant immune cell types. In LS sex, significant eSNPs of B cells, T cells, CD4, and CD8 T cells, granulocytes, monocytes, macrophages, neutrophils, and PBMCs were identified. In the SWE-GER-USA-AA non-LS sex groups, eQNPS of T cells, CD4 T cells, fibroblasts, monocytes, macrophages, and neutrophils were observed.

Gene enrichment and pathway analysis

In LS sex groups (Supplementary Table 27), a pathway map related to immune response induction of the antigen presentation machinery by IFN-gamma (FDR = 1.530e-8 in males; FDR = 1.926e-11 in females) were identified. In the SWE-GER non-LS sex groups (Supplementary Table 27), pathway maps related to maturation and migration of dendritic cells in skin sensitization (FDR = 2.085e-9) in males and related to immune response

induction of the antigen presentation machinery by IFN-gamma (FDR = 1.998e-10) in females were identified. In the SWE-GER-USA-AA non-LS sex groups (**Supplementary Table 27**), pathway maps related to immune response lectin-induced complement pathway (FDR = 1.571e-5) in males and related to maturation and migration of dendritic cells in skin sensitization (FDR = 1.824e-6) in females were noted.

Discussion

The present study investigated sex differences in sarcoidosis, particularly in LS and non-LS phenotypes. This work is the first investigation for independently characterizing the genetic architecture of sarcoidosis in males and females. In brief, we analyzed genetic associations between LS and non-LS males and females conducted in European and multi-ethnic cohorts. The multi-ethnic cohorts comprised three population-based cohorts from Sweden, Germany, and the United States. The cohorts included different ancestries, i.e., white European and black African American.

For both LS and non-LS sex groups, a genetic analysis based on a genome-wide association study was conducted, followed by a meta-analysis, an SNP lookup in the UKB, gene-based analysis, differential gene expression analysis using the GText version 8 resource, eQTL tissue/cell mapping enrichment, and pathway analysis.

Löfgren's syndrome, an acute form of sarcoidosis, is highly prevalent in Sweden (55). However, due to insufficient LS cases in the German cohort and being rare in the United States, genetic analyses in the LS sex groups were only conducted in the Swedish cohort. In contrast, genetic investigations in the non-LS sex groups were performed across all three cohorts.

Association analysis in LS sex groups identified distinct genetic associations clustering in the extended MHC (xMHC) (56), highlighting the role of genes located in this region as main component of LS susceptibility, as previously reported (23). The top LS findings were associations in *HLA-DQA1* in males and *C6orf10* in females. Although these genomic loci are on the same chromosome region (classical class II) and many variants in these genes are in linkage disequilibrium (LD $r^2 > 0.8$), we observed significant differences in the genetic effects (beta estimates) among variants associated with LS males and females (**Figure 1**). Interestingly, after conditioning for the top MHC signals, we identified non-MHC loci worth investigating in future studies of LS. For instance, a nearby signal close to *LRRTM4* associated with LS males is worth looking into, as *LRRTM4* is a synapse-organizing molecule involved in protein-protein interactions that regulate the nervous and immune systems (57). Another interesting non-MHC association is *LNPEP* associated with LS females. *LNPEP* is an aminopeptidase that regulates hormones (i.e., arginine-vasopressin and oxytocin), and is involved in trimming peptide antigens for cross-presentation to T cells in autoimmune diseases (58).

In the European cohorts (SWE and GER), meta-analysis in non-LS sex groups identified mainly associations in the MHC classical class II subregion and a few non-MHC signals, chiefly in *ANXA11, TMEM163,* and nearby *PRDX5* in both sex groups. As in LS, the differences among these signals were the genetic effects (beta estimates) in the sex groups, as illustrated in the forest plot (**Figure 2**). Similar findings were observed in the multi-ethnic cohorts (SWE, GER, and USA-AA).

To further understand the genetic differences, the comparison of genetic signals of LS and non-LS sex groups showed that some signals were associated with a specific sex, whereas other signals were common to both males and females; however, their genetic effects differed. These observations were then validated using the UKB resource, using the "doctors diagnosed sarcoidosis" phenotype. Based on this evidence, it is compelling to suggest that genetic differences in the sex groups could be linked to the observed differences in clinical manifestations in sarcoidosis male and female patients, as documented in various works (59–61). Additionally, it is essential to highlight that ancestry also played a role in the differences in genetic effects in the sex groups, as the effects changed when multi-ethnic ancestries were considered.

Results from gene-based analysis showed patterns of genomic associations in the sex groups, such as shared and sex-specific (**Figure 3**). Notably, the genomic patterns of associations in the LS sex groups were more extensive since the LS susceptibility spanned across the xMHC, which harbors 252 genes and 139 pseudogenes. In the non-LS sex groups, on the contrary, the genomic patterns were limited to a few genes located in the MHC class II region. As expected, genomic patterns decreased as more ancestries were included, stressing the role of ancestry as a modifier of sarcoidosis susceptibility. Ancestry plays a significant role in the genetics of complex diseases (62, 63), which is also relevant in sarcoidosis.

Insights from tissue gene expression and eQTL enrichment showed that SNPs associated with biological sex play a role in gene expression variability across various tissues and cell types. For instance, gene expression in the spleen, small intestine, lung, and whole blood differed in LS and non-LS sex groups. Furthermore, SNP associations correlated with eQTLs of tissues and cell types, particularly immune cells eQTLs (e.g., B cells, T cells, CD4 T cells, CD8 T cells, macrophages, and monocytes), were identified. These findings underscore the functional role of genetic variants on gene expression of immune cells, thus shaping the immunopathogenesis of sarcoidosis in the sex groups. eQTL studies in complex diseases showed that eQTL SNPs act as master regulators of gene expression in several tissues and cell types (64, 65), influencing the expression of multiple genes and acting as gene regulators (66).

Expression quantitative trait loci findings in various immune cell types (**Supplementary Table 26**) further supports our hypothesis that sarcoidosis is an immune-regulated disease, as previously proposed (67–69).

Indeed, differences in SNP associations in HLA genes (70–75) may result in different immune responses and inflammatory disease course as seen in male and female patients with sarcoidosis. Thus, it is worth keeping in mind that HLA genes play a crucial role in autoimmunity and that their function has a profound sex bias on disease phenotype, as more females are affected by autoimmune (or chronic inflammatory) disorders than the counterparts. Understanding the molecular mechanisms underlying sex selection in the development of autoimmune disorders is an area of active research (76).

Compiling the evidence shown in this work, with a particular focus on the strong presence of HLA genetic associations in sarcoidosis, the involvement of various immune cells types, and the differences in immune response and clinical course in sex groups

(67, 68, 77), the possibility of sarcoidosis being an autoimmune disorder cannot be ignored.

Besides HLA genes, we also identified signals in the *ANXA11* gene in non-LS sex groups with different genetic effects, as denoted by rs1049550 (**Figure 2**). SNP associations in *ANXA11* were first reported by Hofmann et al. (78) and have been linked to sarcoidosis risk, radiographic phenotype (Scadding stage IV), and to interact with HLA-DRA (79). SNPs in *ANXA11* have also been reported to be associated with autoimmune diseases (80). Indeed, the functional role of *ANXA11* in cell division and apoptosis (81) and the regulation of inflammatory cells (82) make *ANXA11* an attractive biomarker for sarcoidosis.

Moreover, evidence for a pathway related to immune response induction of the antigen presentation machinery by IFN-gamma in LS sex groups offers an incentive to examine the role of IFN-gamma in sarcoidosis (**Supplementary Table 27**). IFN-gamma is a pro-inflammatory cytokine produced by immune cells and has immune effector functions on many genes involved in tissue homeostasis, immune/inflammatory responses, and tumor immunosurveillance (83). In the non-LS groups, pathway maps identified mainly were related to immune response but not specific to a sex group. Thus, more data is needed to understand the underlying mechanisms of non-LS as this sarcoidosis phenotype is heterogeneous and polygenic.

In summary, we provide new insights into a sex bias underpinning the genetic architecture of sarcoidosis. The differences observed in the genetic susceptibility in the sex groups in LS and non-LS add further information which may explain the well-recognized observations between sex groups, incidence, prevalence, age of onset, symptoms, severity of the disease, and drug reactions observed in male and female sarcoidosis patients (23, 84–87).

It goes without a doubt that further genetic and omics studies ought to be conducted to broaden the characterization of the genetic architecture of sarcoidosis among sex groups. Such efforts, of course, shall consider the inclusion of multi-ancestry populations with large sample sizes and balanced sex ratios. An ongoing endeavor to meet such requirements is the MESARGEN consortium,[2] an international framework aimed to include multiple ancestry populations and large sample sizes for genetics and genomics studies of sarcoidosis.

Limitations of the study

We used the Illumina Immunochip version 2 platform in our analysis—a customized design based on investigating major autoimmune and inflammatory diseases (88). Therefore, we limited our investigations to immune-mediated genomic regions, leaving out other potential loci implicated in sarcoidosis susceptibility. Despite this limitation, we offer substantial evidence that the genetic susceptibility of sarcoidosis and LS and non-LS phenotypes in males and females shared common genomic loci but also differed in their genetic architectures given the sex group. We observed these differences in gene-based analysis, eQTLs enrichment across different tissues, and pathway analysis. Further investigations with multiple ancestry populations and large sample sizes are needed to unveil discoveries.

Perspectives and significance

In this work, we revealed sex differences in the genetic architecture of sarcoidosis, particularly in clinical phenotypes LS and non-LS. Our work provides further knowledge that the genetic architectures of LS and non-LS are distinct, and that biological sex plays a role in determining the underlying genetic architecture.

Data availability statement

Access to genomic data is limited due to GPDR restrictions because the data contain personal or other sensitive information and cannot be deposited in public databases. All summary statistics from the analyses are accessible via DOI: 10.6084/m9.figshare.21785273.

Ethics statement

This study was approved by the Stockholm Ethical Review Board, Stockholm, Sweden. The patients/participants provided their written informed consent to participate in this study.

Author contributions

NR and LP designed and implemented the study. YX conducted all the genetic analyses in the Swedish cohort and a meta-analysis among all cohorts. YX and NR drafted the manuscript. SK, JG, and AE recruited patients, obtained samples, and clinically evaluated patients for phenotype characterization in the Swedish cohort. DE conducted genetic analyses in the German cohort. SS and JM-Q recruited patients, obtained samples, and clinically evaluated patients for phenotype characterization in the German cohort. BR and MI recruited patients and obtained samples. CM provided genotype data and oversaw analyses conducted by LG and NP of the US-AA cohort. All authors revised the manuscript and provided feedback.

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2 http://mesargen.org

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1132799/full#supplementary-material

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Differential diagnosis of pulmonary sarcoidosis: a review

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Diagnosing pulmonary sarcoidosis raises challenges due to both the absence of a specific diagnostic criterion and the varied presentations capable of mimicking many other conditions. The aim of this review is to help non-sarcoidosis experts establish optimal differential-diagnosis strategies tailored to each situation. Alternative granulomatous diseases that must be ruled out include infections (notably tuberculosis, nontuberculous mycobacterial infections, and histoplasmosis), chronic beryllium disease, hypersensitivity pneumonitis, granulomatous talcosis, drug-induced granulomatosis (notably due to TNF-a antagonists, immune checkpoint inhibitors, targeted therapies, and interferons), immune deficiencies, genetic disorders (Blau syndrome), Crohn's disease, granulomatosis with polyangiitis, eosinophilic granulomatosis with polyangiitis, and malignancy-associated granulomatosis. Ruling out lymphoproliferative disorders may also be very challenging before obtaining typical biopsy specimen. The first step is an assessment of epidemiological factors, notably the incidence of sarcoidosis and of alternative diagnoses; exposure to risk factors (e.g., infectious, occupational, and environmental agents); and exposure to drugs taken for therapeutic or recreational purposes. The clinical history, physical examination and, above all, chest computed tomography indicate which differential diagnoses are most likely, thereby guiding the choice of subsequent investigations (e.g., microbiological investigations, lymphocyte proliferation tests with metals, autoantibody assays, and genetic tests). The goal is to rule out all diagnoses other than sarcoidosis that are consistent with the clinical situation. Chest computed tomography findings, from common to rare and from typical to atypical, are described for sarcoidosis and the alternatives. The pathology of granulomas and associated lesions is discussed and diagnostically helpful stains specified. In some patients, the definite diagnosis may require the continuous gathering of information during follow-up. Diseases that often closely mimic sarcoidosis include chronic beryllium disease and drug-induced granulomatosis. Tuberculosis rarely resembles sarcoidosis but is a leading differential diagnosis in regions of high tuberculosis endemicity.

KEYWORDS

sarcoidosis, differential diagnosis, granuloma, tuberculosis, thoracic computed tomography, pathology, microbiology

> *How often have I said to you that when you have eliminated the impossible, whatever remains, however improbable, must be the truth?*
> Sir Arthur Conan Doyle (Sherlock Holmes, *The Sign of Four*, 1890).

1. Introduction

Sarcoidosis is challenging to diagnose given the broad range of presentations, many of which mimic other conditions (1). No single criterion providing a definite diagnosis of sarcoidosis with 100% accuracy is available. Current guidelines indicate that the diagnosis of sarcoidosis should rely on three criteria, two positive (compatible presentation and evidence of noncaseating granulomas) and one negative (exclusion of all alternative diagnoses whose presentation and/or histopathology are similar) (1, 2). However, not all patients who meet these three criteria have sarcoidosis. The easiest criterion to assess is probably the presence of noncaseating granulomas, which can be sampled using highly efficient tools such as endobronchial ultrasound-guided transbronchial needle aspiration, transbronchial lung and bronchial biopsy, and biopsy of peripheral lesions (e.g., involving the skin or peripheral lymph nodes) (3). In contrast, assessing compatibility of the presentation and ruling out alternative diagnoses can be laborious. These two criteria must be evaluated concomitantly, since the presentation governs the likelihood of each alternative diagnosis. Determining whether the presentation is compatible with sarcoidosis relies heavily on expertise in the field of sarcoidosis. However, the diverse and nonspecific initial manifestations of sarcoidosis often lead patients to visit general practitioners or emergency physicians, who have limited experience with the disease. This point probably explains the often long diagnostic delays, which may exceed 6 months, and the need for several physician visits before the diagnosis is established (4).

Several recent articles provide useful information for diagnosing sarcoidosis. Development of the American Thoracic Society (ATS) diagnostic guidelines involved using a Delphi methodology to classify several manifestations as indicating a highly probable or probable diagnosis of sarcoidosis, based on the World Association of Sarcoidosis and Other Granulomatosis (WASOG) Sarcoidosis Organ Assessment Instrument (1, 5). Moreover, two Sarcoidosis Diagnostic Scores, with and without biopsy data, respectively, have been validated in confirmation cohorts, in the USA (6) and in a multicontinental study (7). The main strength of these studies is assessment of the scores under real-life conditions, with both sarcoidosis and a panel of alternative diseases mimicking sarcoidosis, to address both presentation compatibility and differential diagnoses.

This review focuses on the differential diagnosis of pulmonary sarcoidosis. Our aim was to provide practical diagnostic help to physicians who have limited experience with sarcoidosis. The first section presents useful diagnostic tools (epidemiology, clinical presentation, thoracic imaging, pathology, and microbiology) and provides examples of imaging and pathology findings. In the second section, we describe specific settings in which alternative diagnoses must be considered and discuss the best tools for differentiating these diagnoses from sarcoidosis.

2. Methods

We performed a comprehensive literature search for publications relevant to the differential diagnosis of sarcoidosis. To this end, we searched PubMed using the terms "sarcoidosis" OR "pulmonary granulomatosis" OR "tuberculosis" OR "histoplasmosis AND "epidemiology" OR "thoracic imaging" OR pathology" OR "microbiology" OR "occupational-induced" OR "environmental-induced" OR "drug-induced" OR "immunodeficiency" OR "genetic" OR "vasculitis" OR "Crohn's disease." We considered original articles and reviews published in English between January 2010 and January 2023, as well as other articles that did not meet these criteria but were of specific interest.

3. Results

3.1. How can epidemiology, clinical presentation and imaging, pathology, and microbiology studies help differentiate sarcoidosis from alternative diseases?

Table 1 lists the pulmonary granulomatous diseases to consider in the differential diagnosis of sarcoidosis, categorized according to their cause or mechanism. The table does not include granulomatous diseases that do not involve the lungs, such as cat scratch disease.

3.1.1. Ratio of incidences of sarcoidosis over alternative diagnoses according to the epidemiological setting

The ratio of the incidence of sarcoidosis over the incidence of each alternative diagnosis varies significantly across geographic areas and across sub-populations in a given area. These variations dramatically impact the probabilities of different diagnoses, according to Bayes' theorem. A comparison of the incidences of sarcoidosis and tuberculosis illustrates this point. In some countries, tuberculosis is the main differential diagnosis of sarcoidosis (8, 9). Sweden has a high incidence of sarcoidosis (11.5/100,000/y) (10) and a very low incidence of tuberculosis (3.6/100,000/y), Italy a low incidence of sarcoidosis (1.2–3/100,000/y) and higher incidence of tuberculosis (6.6/100,000/y), and Russia a low incidence of sarcoidosis (1.1–3.8/100,000/y) and high incidence of tuberculosis (46/100,000/y). The probability that a patient will have sarcoidosis rather than tuberculosis therefore varies widely across these three countries, the incidence ratios being 3.2 in Sweden, 0.18 in Italy, and 0.02 in Russia; the value in Russia is 160 times smaller than that in Sweden. In some countries, both diseases are very common: in India, the prevalence of sarcoidosis is 61.2–150/100,000 (incidence unknown) and the incidence of tuberculosis is 188/100,000/y. As discussed below, the diagnosis of sarcoidosis is considerably more difficult in regions where tuberculosis is endemic. Moreover, in a given area, significant epidemiological differences may exist across ethnic groups, as shown by a study done in a Paris suburb, where the odds ratio for having sarcoidosis was 2.97 in black people from the Caribbean or subsaharian Africa, with European Caucasians as the reference (11). Similarly, in high-income countries, tuberculosis is far more common in migrants from low-income countries than in locals.

Contrary to tuberculosis, pulmonary histoplasmosis is not ubiquitous but instead occurs in well-delineated areas such as the Ohio River and Mississippi River valleys in the USA and specific regions of Central and South America, Africa, Asia, and Australia. This disease is very rare in Europe, with the exception of Italy (12). Patients must be asked about travel to endemic areas. In the USA, nontuberculous mycobacterial infections are more common than tuberculosis and must be included in the differential diagnosis (13).

TABLE 1 Differential diagnoses of pulmonary granulomatous diseases that must be ruled out before diagnosing sarcoidosis.

Diseases	Clinical importance	Proportion of cases mimicking sarcoidosis (clinical and CT)	Circumstances and presentations
Infections			
o Tuberculosis	++++	Low (multiple presentations)	TB-endemic countries; high individual risk
o NTM infection	+	Low (only in rare presentations)	Individual risk; nodular pattern at imaging
o Histoplasmosis	+	Low (only in rare presentations)	Living in or visiting endemic areas; nodular pattern or PH
o Other infections	+/−	Very low	Vary across settings
Occupational/Environmental			
o CBD	+++	High	Exposure history +++, few extrapulmonary manifestations
o Other metal-induced granulomatosis	Very few data	Can mimic	Exposure Very rare
o HP	+	Almost never	Causal antigen often identified
o Hot-tub lung	+	Almost never (but pathology often similar)	Hot tub use
o Granulomatous talcosis	+	High	Drug abuse (inhalation or intravenous injection)
Drug-induced granulomatosis			
o TNF-a antagonists	+++	High	Treatments known to induce sarcoidosis-like reactions; with BCG therapy, the presentation can be different (miliary)
o Checkpoint inhibitors			
o Targeted therapies			
o Intravesical BCG			
o Interferons			
o Other drugs			
Immunodeficiency			
o G- CVID	+	Low	Recurrent infections, autoimmunity, hypogammaglobulinemia
o CGD	+/−	Never	History of infections in infancy
Genetic disease			
o Blau syndrome	+/−	Never	Onset before the 3–4 years of age; no lung involvement; familial history in 40%
Vasculitis, CTD, inflammatory disease			
o GPA	+	Rare (in particular presentations)	multiple consolidation; nasosinusal manifestations
o EGPA	−	Never	Asthma; eosinophilia
o NSG	+++	High	Main issue is nosological
o ILD in Sjögren's syndrome	+	Rare	In exceptional cases, sarcoidosis mimics NSIP
o Crohn's disease	+	Almost never	Consider drug-induced granulomatosis or association with sarcoidosis
Proliferative disorders			
o Cancer	++	Rare (in particular presentations)	Lymphadenopathy and lung nodules
o Lymphoma	++	Rare (with an expert radiologist)	Possible association or succession of sarcoidosis and lymphoma
o Lymphomatoid granulomatosis	+	Rare (in particular presentations)	Lung nodules

NTM: nontuberculous mycobacteria; TB: tuberculosis; PH: pulmonary hypertension; CBD: chronic beryllium disease; HP: hypersensitivity pneumonitis; G-CVID: granulomatosis-associated common variable immune deficiency; CGD: chronic granulomatous disease; CTD: connective tissue disease; GPA: granulomatosis with polyangiitis; EGPA: eosinophilic granulomatosis with polyangiitis; NSG: necrotizing sarcoid granulomatosis; ILD: interstitial lung disease; NSIP: nonspecific interstitial pneumonia.

In a biopsy study comparing the causes of pulmonary granulomatosis in the USA and in other countries (Austria, Brazil, India, Japan, Scotland, and Turkey), pulmonary histoplasmosis was demonstrated in 18 patients in the USA vs. none in the other countries, whereas tuberculosis was found in a single patient in the USA vs. 18 in the other countries (14). Thus, region-specific epidemiological factors weigh heavily on the differential diagnosis.

Chronic beryllium disease and other occupational mineral-induced granulomatous diseases, hypersensitivity pneumonitis, and drug-induced granulomatosis occur in specific settings. Blau syndrome, which very rarely involves the lungs, is usually diagnosed at a younger age compared to pediatric sarcoidosis (15).

3.1.2. Clinical presentation

Symptoms and signs, particularly those observed in extra-pulmonary manifestations which may be observed in up to 50% of sarcoidosis patients, may be useful to differentiate sarcoidosis from alternative diseases. For example, some manifestations were classified supportive of sarcoidosis diagnosis with a high probability (erythema nodosum, lupus pernio or uveitis) or with probability (seven cranial nerve paralysis) according to official American Thoracic Society Clinical Practice Guidelines (1) Recently, a Sarcoidosis Diagnosis Score Clinical could be validated in a large multicontinental sarcoidosis population compared to controls with multiple other diseases made possible to assess the probability of sarcoidosis

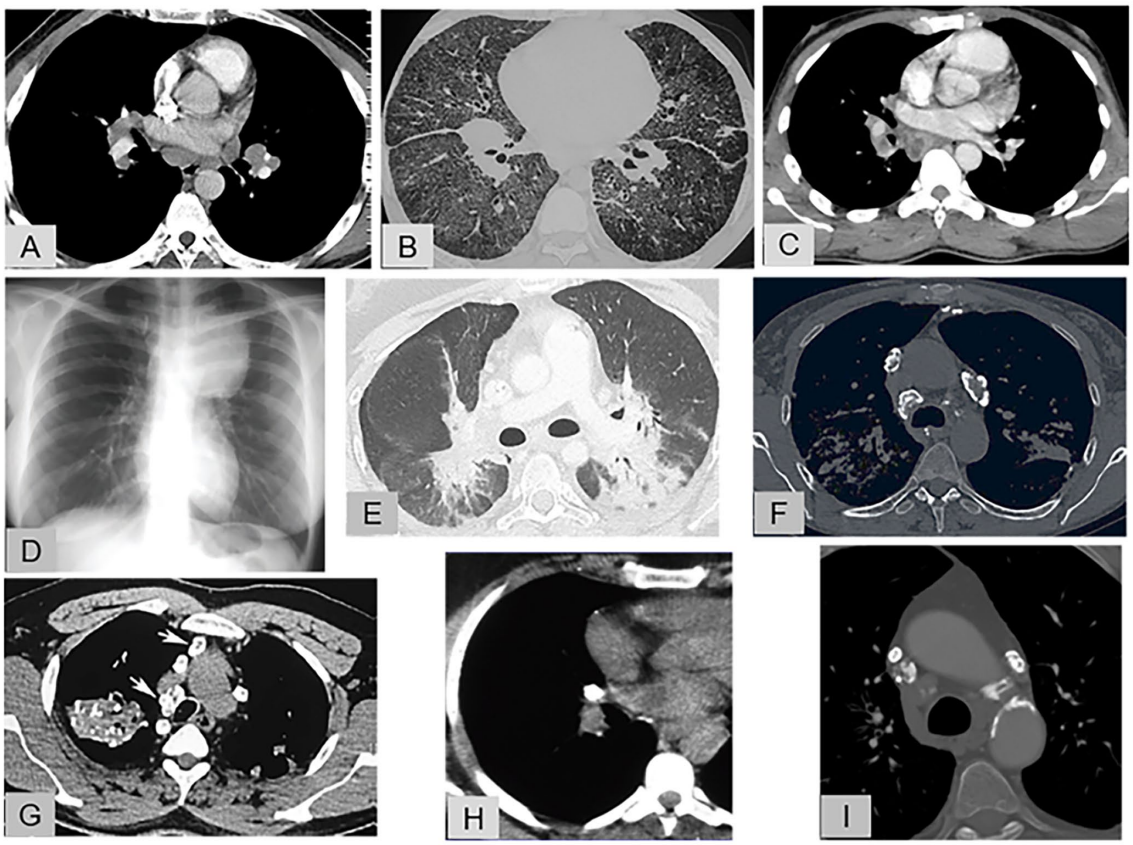

FIGURE 1
Representative features of lymphadenopathy. **(A)** Enhanced chest CT (mediastinal window): Sarcoidosis with typical, symmetric, hilar and mediastinal lymphadenopathy that does not compress the adjacent pulmonary vessels, **(B)** Chest CT (pulmonary window): Sarcoidosis with a rare and atypical lymphadenopathy pattern combining unilateral hilar and mediastinal lymphadenopathy, thickened fissures, diffuse ground-glass opacities, and sparse parenchymal micronodules. **(C)** Enhanced chest CT (mediastinal window): Tuberculosis with unilateral hilar and mediastinal lymphadenopathy exhibiting central necrosis. **(D)** Chest radiography: Hodgkin's disease with voluminous, left, anterosuperior, mediastinal lymphadenopathy. **(E)** Enhanced chest CT (pulmonary window): Immune checkpoint inhibitor-induced sarcoid-like reaction: bilateral hilar lymphadenopathy with blurred contours extending as ground-glass opacities into the posterior lung parenchyma. **(F)** Chest CT (mediastinal window): Sarcoidosis: mediastinal lymphadenopathy with eggshell calcifications. **(G)** Chest CT (mediastinal window): Silicosis: asymmetric, calcified, mediastinal lymphadenopathy, with eggshell calcification of some nodes; calcified mass in the right upper lung lobe and small, scattered calcifications in the left lung. **(H)** Chest CT (mediastinal window): Tuberculosis: completely calcified unilateral hilar lymph node. **(I)** Chest CT (mediastinal window): Amyloidosis: small, bilateral, calcified, mediastinal lymphadenopathy.

diagnosis before obtaining typical granulomas on biopsy specimens (7).

3.1.3. Thoracic imaging

The likelihood of specific findings (e.g., lymphadenopathy, lung infiltration with or without fibrosis, nodules, pulmonary hypertension) differs between sarcoidosis and the alternative diagnoses.

3.1.3.1. Hilar and mediastinal lymphadenopathy

Figures 1A–I shows representative features of lymphadenopathy by chest computed tomography (CT), from the most typical to the most atypical, in sarcoidosis and in the alternative diagnoses.

3.1.3.1.1. Bilateral hilar lymphadenopathy with or without mediastinal lymphadenopathy

In 70% of patients with sarcoidosis, chest radiographs show typical lymphadenopathy, with or without pulmonary infiltrates. Parenchymal disease is evident in only 45% of patients at the time of maximal lymphadenopathy. In patients without parenchymal infiltrates, the lymphadenopathy is hilar and bilateral (98%). In a study of 62 patients, the most common mediastinal locations were the aortopulmonary (76%) and right paratracheal (71%) regions and the most common combination was bilateral hilar, right paratracheal, and aortopulmonary (37%) (16). Symmetrical hilar lymphadenopathy is a characteristic finding (Figure 1A) and can help distinguish sarcoidosis from other conditions responsible for hilar and mediastinal lymphadenopathy (17). Typically, sarcoid lymphadenopathy does not compress the adjacent airways and vessels.

Other conditions whose features can include symmetrical bilateral hilar lymphadenopathy are chronic beryllium disease (*cf infra*) in which the node enlargement is usually moderate, and drug-induced granulomatosis (18, 19) (Figure 1E).

By contrast, symmetrical bilateral hilar lymphadenopathy was a presentation feature in only 3.8% of lymphomas, 0.8% of bronchogenic carcinomas, and 0.2% of extrathoracic carcinomas (20). In granulomatosis-associated common variable immunodeficiency, bilateral hilar lymphadenopathy indistinguishable from sarcoidosis was seen in only 10% of patients (21).

In IgG4-related disease, the chest CT presentation very rarely suggests sarcoidosis, with lymphadenopathy, peribronchovascular thickening, and nodules. The risk of confusion is greatest in patients who have symmetrical swelling of the lacrimal, parotid, and submandibular glands. However, findings of auto-immune pancreatitis, bilateral orbital pseudo-tumor, and retroperitoneal fibrosis with no granulomas but typical features of IgG4-related disease upon histological examination provide the correct diagnosis (22, 23).

3.1.3.1.2. Unilateral or asymmetrical hilar lymphadenopathy, or mediastinal lymphadenopathy without hilar lymphadenopathy

Asymmetric or unilateral hilar or mediastinal lymphadenopathy occurs in less than 5% of patients with sarcoidosis overall (24) but is more common after 50 years of age (Figure 1B) (24).

Unilateral hilar lymphadenopathy is more often seen in tuberculosis, histoplasmosis, lymphoma, bronchogenic carcinoma, and metastatic carcinoma than in sarcoidosis (25).

Tuberculosis must be considered routinely (26). Features that strongly suggest active tuberculosis include unilateral hilar/paratracheal lymphadenopathy (Figure 1C), mediastinal lymphadenopathy with peripheral rim enhancement (due to central necrosis) or heterogeneous enhancement (Figure 1C), and lymph-node conglomeration or obscuration of perinodal fat with or without ipsilateral parenchymal lesions.

In the acute form of pulmonary histoplasmosis in immunocompetent patients, lymphadenopathy may be present (20% of patients), and small nodules (<3 mm) are usually seen (80% of patients) (27).

Large lymphadenopathy in the anterosuperior mediastinum suggests lymphoma (Figure 1D) (20).

3.1.3.1.3. Calcified lymph nodes

Eggshell calcifications can be seen in sarcoidosis (Figure 1F), some fungal infections, and amyloidosis but are more common in silicosis (Figure 1G) and coal miner's pneumoconiosis (28). In a retrospective CT study, nodal calcifications were present in 53% of 49 patients with sarcoidosis and 46% of 28 patients with tuberculosis (29). Focal calcification was more common in sarcoidosis (58%) than in tuberculosis (23%), whereas the opposite was true for complete calcification (62 and 27%, respectively) (Figure 1H). When the nodes were calcified, involvement was far more often bilateral in sarcoidosis than in tuberculosis (65 and 8%, respectively) (Figures 1F,H) (29). In patients with systemic amyloidosis, lymphadenopathy was the single most common abnormality (75%) and contained punctate calcifications in 33% of cases (Figure 1I).

3.1.3.2. Lung involvement

3.1.3.2.1. Micronodular parenchymal pattern

Figures 2A–H illustrates this pattern.

The typical radiographic manifestation of parenchymal lung involvement by sarcoidosis consists in diffuse micronodules predominating in the upper and middle parts of the lungs (16).

By chest CT, perilymphatic micronodules are the most common abnormality in pulmonary sarcoidosis (77% of cases) (30). These opacities predominate in the upper and middle lungs (68%). Clusters of micronodules and nodules are often visible around the peripheral bronchovascular bundles (Figure 2A) (30). The micronodules may be so profuse as to make their distribution difficult to assess. However, micronodule predominance along the fissures suggests a perilymphatic distribution (Figure 2B). At the level of the secondary pulmonary lobules, the interlobular septa are thickened or nodular and the centrilobular interstitium is thickened (31). In a study of pulmonary sarcoidosis, 15 of the 25 patients had nodular lesions 1 to 5 mm in diameter (32). These nodules predominated along the bronchovascular bundles in 17 patients and, to a lesser extent, in the subpleural regions in 19 patients and along the interlobular septa, with a beaded appearance. Thickening of the interlobular septa was seen in 10 patients. The nodule contours were irregular in 17 patients.

A limited number of sarcoidosis-like granulomatous diseases can closely mimic sarcoidosis, including chronic beryllium disease and drug-induced granulomatous diseases (19, 33). In 28 patients with chronic beryllium disease, the chest CT findings were similar to those reported in sarcoidosis, with nodules predominating in the peribronchovascular regions or along the interlobular septa (57%), interlobular septal thickening (50%), hilar and mediastinal lymphadenopathy (39%), and other findings such as ground-glass opacities (32%) (Figure 3B) (33).

Granulomatosis induced by checkpoint-inhibitor cancer immunotherapy is becoming increasingly common due to the expanding indications for these drugs (34). The imaging features resemble those of sarcoidosis, with perilymphatic nodules predominating in the upper lungs and with mediastinal and hilar lymphadenopathy (35). The immune checkpoint inhibitor most often associated with a sarcoid-like reaction is ipilimumab. Melanoma was initially the most common underlying malignancy (34). The main other drugs that can induce sarcoid-like reactions (TNFa antagonists and biosimilars, interferons and pegylated interferons, and targeted therapies against cancer) can also produce chest CT lung abnormalities mimicking sarcoidosis.

By contrast, in tuberculosis, the most typical lesions in the event of airway dissemination are nodules, centrilobular nodules (notably tree-in-bud nodules), and clustered nodules predominating in the upper lobes, right middle lobe, lingula, and superior segment of the lower lobes (Figure 2C); and consolidation in these same regions with ipsilateral lymph node enlargement. Hematogenous dissemination produces a typical miliary pattern (Figure 2D); thick-walled cavities; cavities with surrounding consolidation, especially in the upper and middle parts of the lungs; pleural effusion; and the split pleura sign with separation of the two pleural leaflets by an effusion or empyema (26).

The most common radiographic abnormality in acute symptomatic histoplasmosis consists of multiple nodular opacities usually smaller than 3 mm in diameter, generally in a diffuse distribution (36). Pleural effusions are rare (2%).

In hypersensitivity pneumonitis, profuse, minute, diffuse micronodules in a centrilobular distribution sparing the subpleural parenchyma are frequently described (Figure 2E).

Several nongranulomatous lung diseases may mimic sarcoidosis, such as silicosis and coal miner's pneumoconiosis, in which micronodules predominate in the upper lungs (78%), in a centrilobular and subpleural, bilateral, and symmetrical distribution, sometimes with confluence and posterior predominance (38%) (Figure 3L) (37, 38). Granulomatous talcosis is a pulmonary disease secondary to

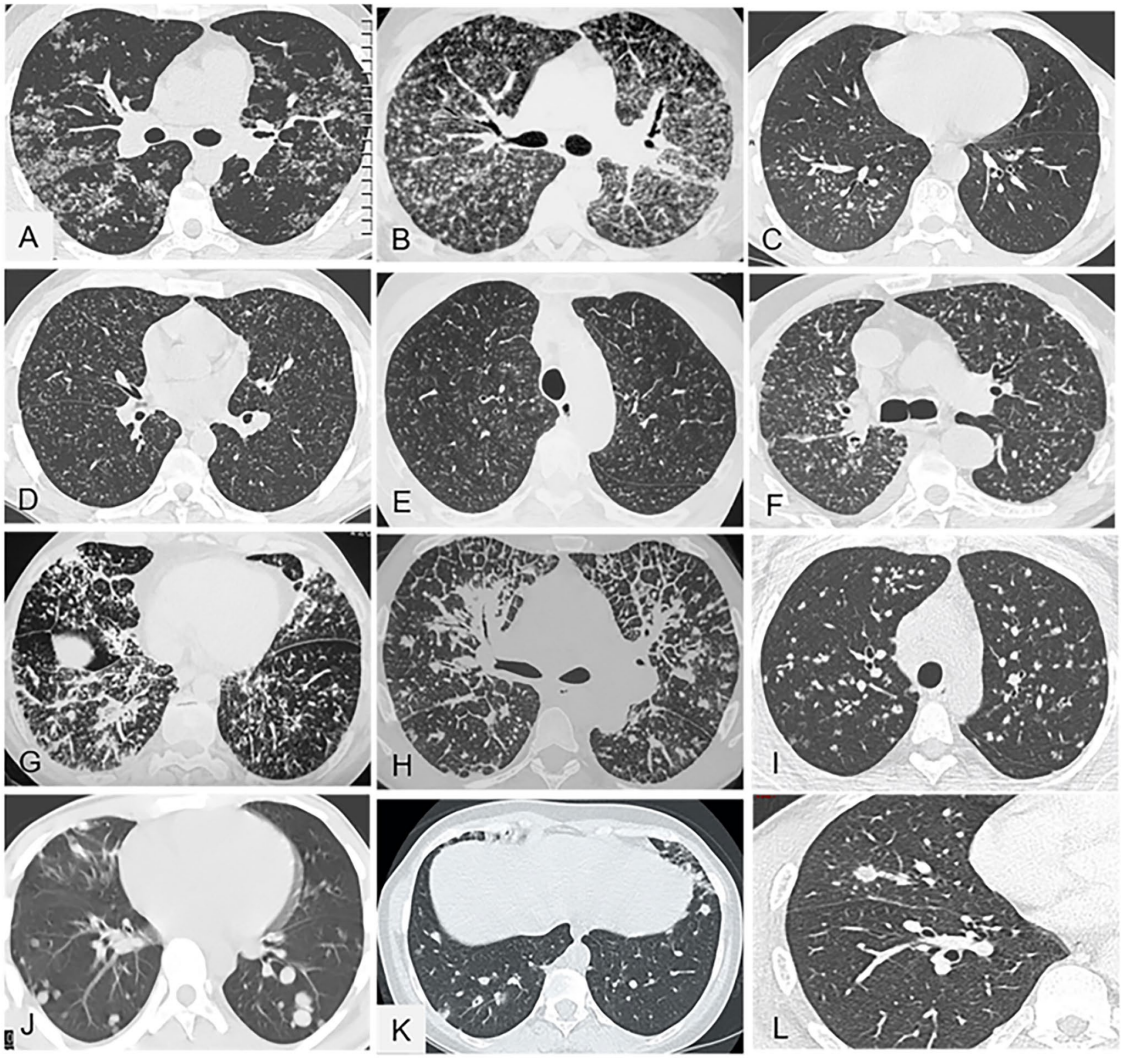

FIGURE 2
Representative of micronodular parenchymal pattern (A-H) and nodular pattern (I-L) in chest CT (parenchymal window). **(A)** Sarcoidosis with typical diffuse micronodules aggregated in small clusters and predominating along the fissures (lymphatic distribution). **(B)** Sarcoidosis: less typical presentation with profuse micronodules whose distribution is more difficult to determine; nevertheless, the predominance of micronodules along the fissures is suggestive of lymphatic distribution. **(C)** Bronchogenic tuberculosis with micronodules in a unilateral and centrilobular distribution that spares the pleural surface. **(D)** Typical, hematogenous, miliary tuberculosis with minute micronodules that are all the same size and are distributed uniformly (i.e., at random) throughout both lungs. **(E)** Hypersensitivity pneumonitis with profuse, minute, diffuse micronodules in a centrilobular distribution that spares the subpleural parenchyma, leaving a 2–3cm uninvolved border. **(F)** Neoplastic miliary: the distribution is not symmetrical and the nodules are larger and more irregular than in miliary tuberculosis. **(G)** Systemic amyloidosis with diffuse pulmonary involvement and no lymphadenopathy; the micronodules are diffuse with no particular distribution and are combined with several irregular lines and thickened interlobular septa. **(H)** Lymphangitis carcinomatosa: thickened peribronchovascular interstitium, nodules, and nodular septal reticulations without distortion. **(I)** Pulmonary sarcoidosis: multiple nodules with irregular and well-defined contours. **(J)** Pulmonary metastases from renal carcinoma: sharply contoured nodules in a more peripheral distribution. **(K)** Granulomatosis-associated common variable immunodeficiency: nodules, some of which exhibit the halo sign. **(L)** Chronic histoplasmosis: sparse pulmonary nodules.

inhaled or intravenous drug abuse. Diffuse bronchiolar micronodular lesions are visible, sometimes in combination with masses containing areas of amorphous density (39).

In lymphangitis carcinomatosa, the nodules share with sarcoidosis a predominance in the peribronchovascular regions (11 of 18 cases) (40). However, nodular polygonal lines (51%) or thickened undistorted septal lines (66%), pleural fluid (60%), and unilateral predominance of small opacities (38%) strongly suggest lymphangitis carcinomatosa (Figure 2H) (40). Thus, the predominant lesions often differ from those found in sarcoidosis (41). The beaded septum sign consisting in nodular thickening of the interlobular septa is common in lymphangitis carcinomatosa and less so in sarcoidosis (42). In neoplastic miliary, the nodules are not symmetrically distributed and are larger and more irregular than in miliary tuberculosis (Figure 2F).

Pulmonary amyloidosis may produce misleading imaging-study findings such as diffuse micronodules in a predominantly perilymphatic distribution (41, 43, 44). In a retrospective study, 12 of 19 patients with proven amyloidosis had the systemic form of the disease, including six with both lymphadenopathy and diffuse parenchymal involvement and two with only the latter manifestation

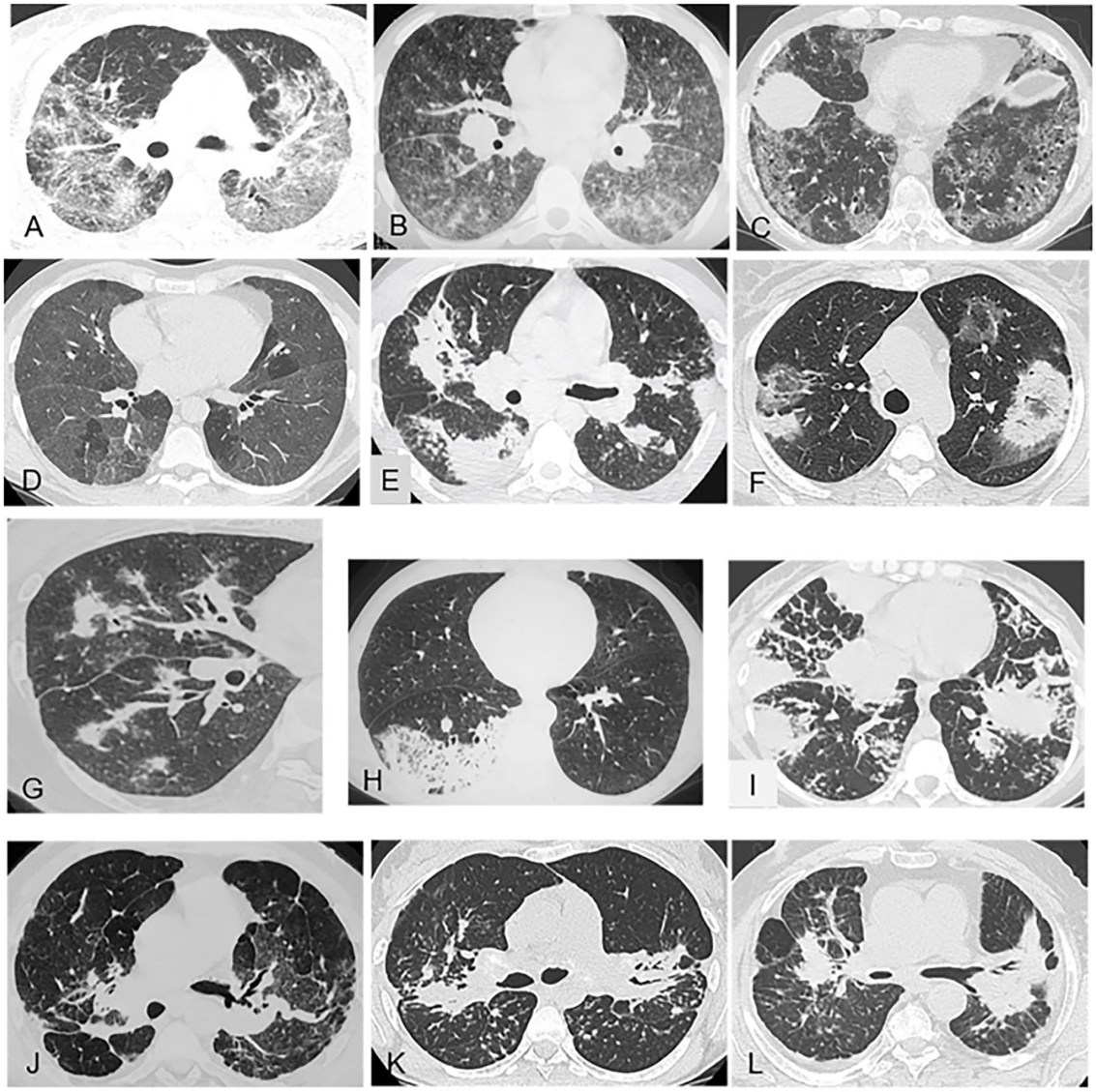

FIGURE 3
Representative of gound-glass opacities **(A–D)** and alveolar consolidations **(E–I)** and masses **(J–L)** on chest CT (pulmonary window). **(A)** Pulmonary sarcoidosis with predominant, diffuse, ground-glass opacities combined with lymphatic micronodules. **(B)** Chronic beryllium disease: heterogeneous ground-glass opacities with bilateral hilar and mediastinal lymphadenopathy. **(C)** Nonspecific interstitial pneumonia: diffuse ground-glass opacities and fine reticulations sparing the subpleural lung. **(D)** Hypersensitivity pneumonitis: diffuse ground-glass opacities and multiple clear lobules trapped in expiration. **(E)** Pulmonary sarcoidosis with bilateral alveolar consolidations and lymphatic micronodules. **(F)** Cryptogenic organizing pneumonia: bilateral peripheral consolidations with lower-density (ground-glass) centers. **(G)** Granulomatosis with polyangiitis: alveolar consolidation related to a pulmonary vessel. **(H)** Lepidic-growth carcinoma: pneumonic form with a unilateral focus. **(I)** Lymphoma: bilateral alveolar consolidations with a limited number of septal reticulations and micronodules. **(J)** Fibrosing pulmonary sarcoidosis: typical presentation with bilateral hilar masses and bronchial distortion. **(K)** Sarcoidosis with bilateral masses in the posterior segments of the upper lobes and micronodules. **(L)** Coal miner's pneumoconiosis: bilateral mass-like lesions in the upper lobes.

(44). Of the eight patients with diffuse parenchymal involvement, six had pulmonary nodules (Figure 2G), four diffuse irregular lines or interlobular septal thickening, three honeycombing, and three patchy ground-glass opacities.

3.1.3.2.2. Less typical patterns of lung infiltration in sarcoidosis

These patterns are illustrated in Figures 3, 4.

In about 10% of patients with sarcoidosis, the predominant chest CT finding consists in ground-glass opacities (Figure 3A) or alveolar consolidations (Figure 3E).

Predominant ground-glass opacities in patients without immunodepression occur chiefly in connective tissue diseases, drug-induced pneumonitis, alveolar proteinosis, nonspecific interstitial pneumonia (Figure 3C), and smoking-related diseases. However, the main differential diagnosis with sarcoidosis is hypersensitivity pneumonitis, in which the ground-glass opacities are usually extensive or combined with other findings consisting chiefly in centrilobular micronodules with no tree-in-bud appearance nor involvement of the pleura or fissures (Figure 3D). The opacities are diffuse and symmetrically distributed. Their contours are blurred and their

density usually low. Other signs of hypersensitivity pneumonitis are air trapping, the three-density pattern, and fibrosis. Foci of lobular air trapping on expiratory scans have been reported to be common (Figure 3D). Cystic cavities are visible in a minority (15%) of patients with hypersensitivity pneumonitis. Of note, although predominant ground-glass opacities are rare in sarcoidosis, a study demonstrated that the logical analysis of CT data established the diagnosis of sarcoidosis with high accuracy in patients with predominant ground-glass lesions, due to the additional presence of either lymphadenopathy in 79% of cases or fissural micronodules in 55% of cases (45).

Predominant multiple **consolidations** are more common in organizing pneumonia (Figure 3F), granulomatosis with polyangiitis (Figure 3G), lymphoma (Figure 3I), and lepidic-growth carcinomas (Figure 3H) than in sarcoidosis. However, as with ground-glass opacities, co-existing abnormalities can help to establish the diagnosis of sarcoidosis (46).

3.1.3.2.3. Nodules

Sarcoidosis can also manifest as multiple nodules (defined as >3 mm in diameter) (Figure 2I). Alternative diagnoses include other granulomatous diseases, notably infections [e.g., tuberculosis, nontuberculous mycobacterial infections, and histoplasmosis (Figure 2L)], drug-induced granulomatosis, granulomatosis with polyangiitis, and eosinophilic granulomatosis with polyangiitis. In granulomatosis-associated common variable immunodeficiency (Figure 2K), chest CT findings seen more often than in sarcoidosis were nodules, which were sometimes multiple, with the halo sign (30% vs. 3.5%) and smooth margins; air bronchograms; and bronchiectasis (65% vs. 23%) (21). The distribution of micronodules was perilymphatic in 42% of patients with granulomatosis-associated common variable immunodeficiency vs. 100% of patients with sarcoidosis. Nongranulomatous diseases such as metastases (Figure 2J), lymphoma, lymphoid granulomatosis, and amyloidosis should also be considered.

3.1.3.2.4. Cysts and other cavities

True primary pulmonary sarcoid cavitation, in which the walls of the cavities are formed by characteristic noncaseating lesions, has been reported in 2.2% of patients with sarcoidosis (47). However, even in patients with sarcoidosis, cavitation is more often due to necrotizing pyogenic, mycobacterial, mycotic, or parasitic infections (Figure 4E) (25).

Other interstitial lung diseases can cause cavities. Thus, thin-walled cystic air-filled spaces can develop in lymphocytic interstitial pneumonia (Figure 4F) and thick-walled cavities in granulomatosis with polyangiitis (Figure 4G).

3.1.3.3. Pulmonary fibrosis

Figures 3, 4 show the chest CT features of pulmonary fibrosis.

3.1.3.3.1. Progressive massive fibrosis

Progressive massive fibrosis often manifests as mass-like lesions, usually in a bilateral upper-lobe distribution, not only in sarcoidosis (Figures 3J,K) but also in silicosis dusts (Figure 3L), coal miner's pneumoconiosis with heavy exposure to inorganic dusts, and granulomatous talcosis (48, 49). Background nodular opacities are associated with pneumoconiosis, with or without emphysematous destruction adjacent to the massive fibrosis (48).

3.1.3.3.2. Irregular septal thickening and irregular linear opacities from hilum to subpleural lung

A common chest CT finding in sarcoidosis is interlobular septal thickening, which is often irregular or associated with marked distortion of the lung structures (49). Linear opacities extending from the hilum to the lung periphery, with distortion, are also common in sarcoidosis (Figure 4A) and are much rarer in nonspecific interstitial pneumonia (Figure 4B).

Several signs of fibrosis strongly suggest sarcoidosis. In a study of 27 patients with sarcoidosis, nine patients exhibited varying degrees of parenchymal distortion consistent with fibrosis (50) and in another, lobular distortion was a feature in 13 of 25 patients with sarcoidosis (32).

3.1.3.3.3. Honeycombing

Fibrosing pulmonary sarcoidosis can produce honeycomb cavities (Figure 4C). Honeycombing predominates in the upper lobes, and the cavities are usually large. In usual interstitial pneumonia, the joined cavities are smaller than in sarcoidosis and located in the lower lobes in contact with the pleura (Figure 4D).

3.1.3.4. Pulmonary hypertension

In pulmonary hypertension, signs of lobular distortion (Figure 4I), fibrosing mediastinitis, and pulmonary-artery compression by lymph nodes (Figure 4H) help to suggest sarcoidosis as the cause (51). When these signs are absent and the disease is recognized very late, it may be extremely difficult to eliminate other causes of pulmonary hypertension such as histoplasmosis or nongranulomatous diseases, notably interstitial lung disease-associated connective vascular diseases (52).

3.1.4. Pathology

A careful examination of biopsy specimens can help with the differential diagnosis, since several microscopic features differ between sarcoidosis and the alternative diseases. We highlight the most typical and most atypical findings in sarcoidosis and in most alternative diseases which are summarized in Table 2. Figure 5 illustrates several histopathological findings.

3.1.4.1. Pathological features of pulmonary sarcoidosis

Pulmonary sarcoidosis is defined as a systemic disease manifesting chiefly as granulomatous interstitial pneumonia. Consequently, the identification of characteristic noncaseating epithelioid granulomas is essential to the diagnosis.

In sarcoidosis, the epithelioid-cell granulomas are well-formed structures whose compact core is composed of macrophages and macrophage-derived cells (epithelioid and giant cells), closely associated with CD4+ T lymphocytes. The peripheral component contains CD8 lymphocytes, CD4+ FOXP3 + Treg cells, Th17 cells, B lymphocytes, and IgA-producing plasma cells (53–55) (Figure 5A). A feature that is common but not specific of sarcoidosis is the presence of cytoplasmic inclusions, chiefly within multinucleate giant cells. These inclusions may be Schaumann bodies (conchoidal bodies) made of iron and calcium salts (Figure 5B), crystalline bodies composed of calcium oxalate and carbonate, and/or asteroid bodies made of cholesterol (Table 2). Focal coagulative necrosis is occasionally seen in the center of the granulomas. Importantly, sarcoid granulomas are particularly florid and tend to coalesce. They are observed in 54–90%

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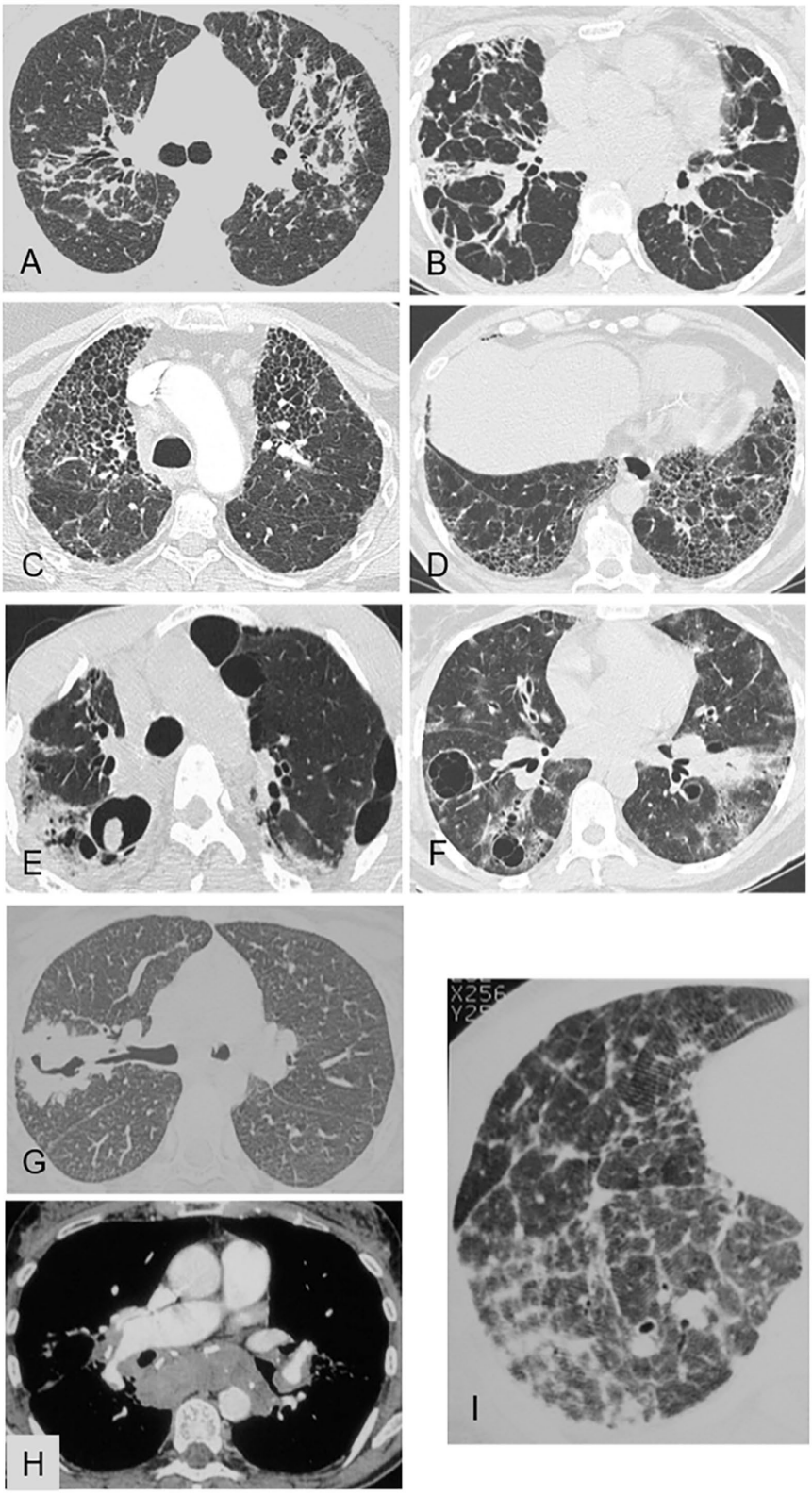

FIGURE 4
Representative of linear opacities **(A,B)**, honeycombing **(C,D)** cysts and cavities **(E–G)** pulmonary hypertension **(H,I)** on chest CT [pulmonary window **(A–G; I)** and mediastinal window **(H)**]. **(A)** Pulmonary sarcoidosis: bilateral linear opacities extending from the hilum to the subpleural area, with distortion. **(B)** Nonspecific interstitial pneumonia: bilateral linear opacities extending from the hilum to the subpleural area, with distortion. **(C)** Fibrosing pulmonary sarcoidosis: honeycombing with joined cavities predominating in the upper lobes. **(D)** Usual interstitial pattern in idiopathic pulmonary

(Continued)

FIGURE 4 (Continued)
fibrosis: honeycombing with joined cavities that are located in the lower lobes in contact with the pleura and are smaller than in sarcoidosis. **(E)** Sarcoidosis with chronic pulmonary aspergillosis: fibrotic lung lesions with cavities and an aspergilloma within a right upper-lobe cavity. **(F)** Granulomatosis-associated common variable immunodeficiency: thin-walled cystic air spaces, patchy areas of ground-glass opacities in both lungs, thickened peribronchovascular interstitium, and bilateral hilar lymphadenopathy. **(G)** Granulomatosis with polyangiitis: consolidation with a thick-walled cavity in the right upper lobe. **(H)** Sarcoidosis with pulmonary hypertension: compression of the right branch of the pulmonary artery by lymphadenopathy. **(I)** Sarcoidosis with pulmonary hypertension: septal reticulation and distortion; note that the pulmonary arteries are wider than the bronchi.

of **bronchial mucosal** biopsies, whose number strongly affects the diagnostic yield (56). The bronchi may be more severely affected, and classic endobronchial sarcoidosis is characterized by waxy, yellow, nonuniform, mucosal nodules 2 to 4 mm in diameter that are more profuse in the lobar and segmental bronchi and mimic a malignant mass (57, 58). A key feature of sarcoidosis is the presence in the **hilar and mediastinal lymph nodes** of variously sized, noncaseating, epithelioid granulomas (Figure 5A). At the early phase, small epithelioid-cell nodules develop in the cortex. Subsequently, well-demarcated granulomas are visible throughout the lymph node and may coalesce. At the late phase, fibrosis and hyalinization develop (Figure 5C) (53). Needle aspiration of intrathoracic lymph nodes under ultrasonographic guidance, either from the airways (transbronchial needle aspiration) or from the esophagus (transesophageal needle aspiration), is now the method of choice for mediastinal-node sampling in patients with presumed stage 1 or 2 sarcoidosis (59, 60). In the **lung**, collections of granulomas may produce macroscopically visible but small white nodules (micronodules) or large masses (macronodules), with relative sparing of the intervening lung. The nodules predominate along the lymphatics (collecting lymphatics in the pleural interstitium, interlobular septa, bronchovascular interstitium, and intralobular lymphatics). This topographic distribution is a very strong argument for pulmonary sarcoidosis. It suggests a critical role for lymphatics in nodule development and, consequently, supports the involvement of airborne particles in the pathogenesis of pulmonary sarcoidosis. In addition, given the presence of granulomas near the small airways, peribronchiolitis with bronchiole-lumen narrowing is common. Furthermore, the blood vessels are often involved in areas of granulomatous inflammation. Granulomatous vasculitis may affect all levels from the large elastic pulmonary arteries to the venules, although the lesions are more marked in the veins than in the arteries. Necrotizing sarcoid granulomatosis, first reported in 1973, is characterized by extensive noncaseating granulomatous inflammation with foci of parenchymal necrosis, combined with marked vasculitis affecting both the arteries and the veins (61). The granulomas may resolve without sequelae or leave fibrotic changes. Concentric fibrosis surrounding the granulomas is nearly always found. In addition, progressive pulmonary fibrosis with interstitial thickening by hyalinized fibrous tissue may be responsible for interstitial parenchymal fibrosis, which ultimately results in end-stage lung disease.

3.1.4.2. Pathological features in the alternative diseases

Epithelioid granulomas develop in many infectious and non-infectious diseases (62–66). For example, the granulomas in chronic beryllium disease closely resemble sarcoid granulomas (Figure 5D). Inorganic agents (e.g., aluminum) can be identified by polarized light examination (Figures 5E,F) or special methods (67). The pathologist must consider all the lung diseases in which the organization of macrophages and macrophage-derived cells, with variable numbers of lymphocytes, resembles that seen in sarcoidosis. A granulomatous reaction *per se* is not specific of sarcoidosis, as shown in Table 2, and may be an immunological and inflammatory response to factors such as bacteria (e.g., *Mycobacterium tuberculosis* (Figures 5G,J) or nontuberculous mycobacteria). Controversial examples are hot-tub lung (68, 69) and Whipple's disease (70) (Figures 5M,N). Fungal infections (e.g., *Histoplasma capsulatum, Cryptococcus, Pneumocystis*) may be misdiagnosed (Figures 5K,L) (71). Parasites (e.g., *Schistosoma mansoni*) may produce arterial granulomas responsible for pulmonary arterial hypertension (72). Exposure to organic airborne agents can cause hypersensitivity pneumonitis characterized by poorly formed granulomas that are often difficult to identify (65). In addition, granulomas develop in various systemic diseases including common variable immune deficiency, granulomatosis with polyangiitis, eosinophilic granulomatosis with polyangiitis, Sjögren's syndrome, and Crohn's disease. Figure 5O illustrates the various granuloma patterns, with the characteristics and extent of necrosis, and lists the methods for identifying causative agents (e.g., special stains and polarized light examination). These distinctive features are summarized in Table 2.

3.1.4.3. Bronchoalveolar lavage

BAL is a safe and minimally invasive procedure that has been widely performed for nearly 40 years (73). Its relevance for diagnosing diffuse interstitial lung disease remains debated (65, 74). However, in patients with sarcoidosis, BAL allows the demonstration of CD4+ T-cell lymphocytic alveolitis (75). Although this finding is not specific, once infections have been ruled out, a lymphocyte count above 25% strongly suggests sarcoidosis, hypersensitivity pneumonitis, or drug toxicity (65, 76, 77). In sarcoidosis, moderate lavage-fluid lymphocytosis (20%–50%) is found in 80% of cases, and the CD4/CD8 T-cell ratio is higher than 3.5 in 50% of cases. The CD4/CD8 T-cell ratio is of controversial diagnostic relevance and, in practice, should be considered informative only when above 3.5, when its specificity is 93%–96% but its sensitivity only 53%–59% (78). Studies that used integrated differential analyses of lavage-fluid cells achieved either by a computer program based on a logistic model (79) or by Bayesian analysis (80) suggested that low percentages of lymphocytes, neutrophils, and eosinophils combined with a high CD4/CD8 T-cell ratio may raise the likelihood of sarcoidosis to more than 85% (80). BAL, although perhaps not decisive for the diagnosis, has a major role to play in research on sarcoidosis. Thus, recent data obtained using this investigation have generated new hypotheses about the possible role in sarcoidosis for B cells (81) and for causal agents such as *Aspergillus* antigens (82).

TABLE 2 Histological patterns of granulomatous interstitial lung diseases.

Diseases	Main granuloma patterns	Main sites involved	Additional features	Special methods for causal-agent identification
	Sarcoidosis			
Sarcoidosis	Well-circumscribed, coalescent, epithelioid and giant-cell, noncaseating granulomas surrounded by lymphocytes, in a peripheral envelope of lamellar fibrosis Very rarely, minimal focal necrosis Granulomatous vasculitis	Mediastinal lymph nodes In the lung: - Along the lymphatics - Usually along the pulmonary vessels, lobular septa, visceral pleura, and large and small airways. - Granulomatous vasculitis predominantly involving the small pulmonary veins	Nonspecific cytoplasmic inclusions mostly in multinucleate giant cells: - Schaumann and crystalline bodies (iron and calcium salts) - Asteroid bodies (cholesterol) Usually with scant pulmonary inflammation Confluent granulomas with necrosis (NSG pattern)	None, except special staining or polarized light microscopy to exclude agents involved in other granulomatous diseases (minerals, mycobacteria, fungi).
	Most common granulomatous lung diseases that must be distinguished from sarcoidosis			
Chronic beryllium disease (CBD)	Granulomas mimicking sarcoidosis: well circumscribed, often coalescent granulomas, combined with poorly formed granulomas Fibrosis at the periphery of granulomas, coalescing into hyalinizing nodules	Mediastinal lymph nodes Lymphangitic, frequently associated with scattered small lobular granulomas	Interstitial fibrosis Deposits of various dusts depending on associated respiratory exposures Residual Schaumann bodies	None
Drugs	Granulomas mimicking sarcoidosis	Mediastinal lymph nodes Airway mucosa Pulmonary parenchyma		None
Tuberculosis (TB) (*Mycobacterium tuberculosis* infection)	Early stages: necrotizing granulomas containing mycobacteria; neutrophils Interstitial caseating and noncaseating granulomas At the late stage, palisades of epithelioid histiocytes delineating geographic-shaped necrosis, cavitation, rare neutrophils Little or no fibrosis Granulomas are less well organized in immunocompromised patients	Mediastinal lymph nodes Aerogenic TB: early lesions centered on the bronchioles; later, randomly distributed lesions Hematogenous miliary TB: multiple scattered nodules made of groups of granulomas	Frequently, areas of organizing pneumonia Alveolar and interstitial acute inflammation Secondary vasculitis	Ziehl-Neelsen stain or fluorochrome stain using auramine O Special cultures PCR
Hypersensitivity pneumonitis (subacute)	Small and poorly structured nonnecrotizing noncoalescent granulomas Schaumann bodies Fibrosis at the periphery of granulomas	Airway-centered inflammation (bronchioles and alveolar ducts) Bronchiolocentric interstitial pneumonitis with sparse, poorly structured granulomas	Interstitial infiltration by lymphocytes with lymphoid aggregates Frequently, areas of bronchiolitis obliterans and organizing pneumonia Lymphoid follicles	None
Fungal infection by *Histoplasma capsulatum*	Transition from the acute phase: granulomas with central areas of necrosis replace the mononuclear infiltrate Chronic phase: - noncaseating well-structured granulomas (similar to sarcoidosis); - necrotizing granulomas; - concentric lamellar fibrosis (similar to reactivated TB)	Mediastinal lymph nodes Lung parenchyma Rarely, bronchocentric granulomatosis	Acute phase: acute fibrinous pneumonia without granulomas Pulmonary fibrosis Sclerosing mediastinitis Calcification of nodules	PAS Grocott methenamine silver stain
Pulmonary granulomatous-associated common variable immunodeficiency (CVID/GD)	Granulomatous and lymphocytic interstitial lung disease Clusters of alveolar and interstitial nonnecrotizing granulomas admixed with lymphocytic interstitial pneumonia	Mediastinal lymph nodes Peribronchiolar	Organizing pneumonia Lymphoid interstitial pneumonia/ Lymphoid hyperplasia Follicular bronchiolitis	
Granulomatosis with polyangiitis (GPA)	Necrosis and granulomatous vasculitis Poorly structured granulomas; clusters of giant cells; small suppurative granulomas Granulomas within the vessel wall Palisading granulomas	Focal granulomatous vasculitis affecting medium-sized and small veins and arteries Loosely scattered multinucleated giant cells	Association: - Parenchymal geographic basophilic necrosis - Vasculitis, endotheliolitis, capillaritis - Granulomatous inflammation with neutrophilic micro-abscesses - Collagen necrosis	
Eosinophilic granulomatosis with polyangiitis (EGPA)	Necrotizing vasculitis with fibrinoid necrosis Necrotizing granulomas with eosinophils in the center Eosinophilic inflammation		Eosinophilic pneumonia Eosinophilic small-vessel vasculitis (small arteries, veins, and capillaries)	
	Less common and rare granulomatous lung diseases that must be distinguished from sarcoidosis			
Granulomatous pneumonitis due to inorganic agents	- Aluminum: pseudo-sarcoid granulomas - Hard metal: giant-cell interstitial pneumonia, infarcted granulomas	- Aluminum: occasional scattered sarcoid-like granulomas - Hard metal: centrilobular nodules	-Aluminum: centri-acinar macules - Hard metal: Numerous multinucleate giant cells; diffuse giant-cell alveolitis	Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM/EDS)

(Continued)

TABLE 2 (Continued)

Diseases	Main granuloma patterns	Main sites involved	Additional features	Special methods for causal-agent identification
Intravenous drug abuse-related lung disease (intravascular talcosis)	Interstitial foreign-body granulomas Granulomatous vasculitis	Juxtavascular and perivascular interstitial granulomas	Associated vascular thrombotic lesions Emphysema Massive fibrosis	Polarized light examination: birefringent particles
Bacterial Infections Silico-tuberculosis	Epithelioid granulomas peripheral to silicotic nodules with central necrosis			
Bacterial Infections Nontuberculous atypical mycobacteria	Well-formed granulomas; less tendency to caseate than TB	Lung parenchyma (upper lobes)	Bronchiectasis: bronchocentric granulomas Necrosis with serpiginous borders and vasculitis (different from GPA)	Ziehl-Neelsen stain Fluorochrome but difficult to differentiate from *M. tuberculosis*
Bacterial Infections Hot-tub lung (*Mycobacterium avium intracellulare*)	Well-formed, nonnecrotizing, often coalescent granulomas	Bronchiolocentric and randomly distributed within airspaces Granulomas in the lumen of bronchioles Air-space granulomas		Organizing pneumonia Mild interstitial pneumonia
Bacterial Infections Melioidosis (tropical regions)	Granulomatous pattern: loose granulomas with necrosis Palisading histiocytes	Lung parenchyma	Necrotizing inflammation	Gram stain (*Burkholderia pseudomallei*: Gram-negative)
Bacteria Infections *Tropheryma whipplei*	Poorly formed granulomas composed of large clusters of histiocytes Foamy macrophages filled with bacteria or bacterial debris True granulomas in lymph nodes	Mediastinal lymph nodes Patchy, peribronchial and peribronchiolar granulomas Airway mucosa Perivascular Pleural		Gram-positive PAS-positive Grocott methenamine silver stain Thin sickle-shaped inclusions (*Tropheryma whipplei*)
Fungal infections *Cryptococcus* *Coccidioides* *Blastomyces* *Zygomycetes*	Nodular granulomas Necrotizing granulomas Giant cells containing yeasts	Lymph nodes Lung parenchyma Pleura	Coexisting areas of necrosis	Gram-positive PAS-positive Grocott methenamine silver stain Fontana-Masson stain
	Suppurative granulomas Granulomas with focal necrosis Granulomas with purulent exudate	Angioinvasive		
Fungal infections *Pneumocystis jirovecii*	Necrotizing granulomas (5–17%)	Lymph nodes: uncommon Lung parenchyma: collected cysts in subpleural areas	Lymphoplasmacytic interstitial infiltration	PAS Grocott methenamine silver stain
Parasites *Schistosoma mansoni*	Granulomatous vasculitis in association with eggs		Granulomatous vasculitis: arteritis	Observed eggs (measuring about or more than 100 mm)
Parasites *Paragonimus*	Granulomatous reaction associated with eggs	Adjacent to large bronchioles	Small cysts surrounding worms	Observed eggs (measuring less than 100 mm)
Sjögren's syndrome	Small nonnecrotizing granulomas	Sparse and randomly distributed over the pulmonary interstitium	Peribronchial lymphoid hyperplasia Narrowing of the small airways Occasional bullae	
Granulomatous pneumonitis associated with Crohn's disease	Crohn's: scattered tiny nonnecrotizing granulomas	Crohn's: rarely, randomly distributed	Crohn's: related to drugs used to treat the disease	

3.1.5. Microbiology

3.1.5.1. Microbiology of mycobacteria

The prompt and accurate diagnosis of pulmonary tuberculosis is essential. To establish a definite diagnosis of pulmonary tuberculosis, mycobacteria of the *M. tuberculosis* complex must be identified in pathological samples such as respiratory secretions (e.g., sputum), lymphadenopathy, other infected tissues, or pleural fluid. The microbiology laboratory must perform microscopic examinations and cultures according to standardized procedures (83, 84). The diagnosis of tuberculosis relies on the interpretation of findings from multiple tests including the microscopic examination of stained pathological samples, cultures, and genome-amplification tests designed to identify *M. tuberculosis* complex organisms.

The microscopic examination of smears takes less than 1 hour. Two methods are used routinely: (i) the auramine O fluorescent stain, usually chosen for screening, with a reading at X25 or X40 magnification and (ii) the Ziehl-Neelsen stain, used to confirm a positive auramine O test, with a reading at X100 magnification. Culturing is the reference test, since it identifies a bacterial strain belonging to the *M. tuberculosis* complex, but at least 10 to 30 days are required.

M. tuberculosis complex detection based on polymerase-chain-reaction (PCR) gene amplification of samples (PCR-TB test) is a routine investigation recommended for diagnosing tuberculosis (85, 86). When applied to samples positive by microscopy, this test can identify *M. tuberculosis* complex organisms within a few hours, thereby allowing appropriate patient management before the culture results are available (87–89).

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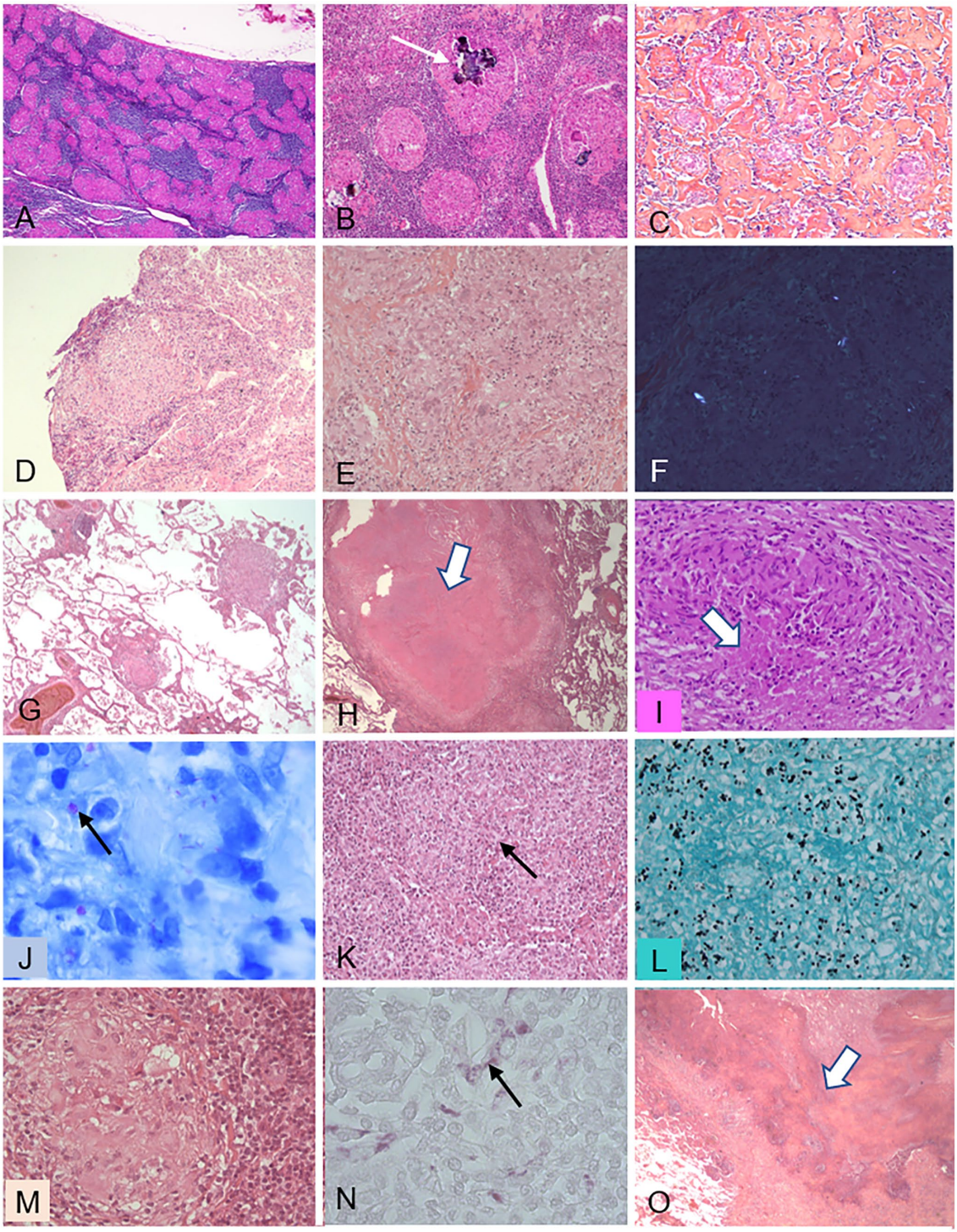

FIGURE 5
Histopathological features of granulomas in various diseases. This figure illustrates several features of granulomas observed in various granulomatous diseases, either in mediastinal lymph nodes **(A–C)** or in pulmonary biopsies **(D–O)**. **(A–C)** Sarcoidosis. **(A)** (X50): nonnecrotizing granulomas filling most of the node tissue; **(B)** (X100): Schaumann's bodies (arrow); **(C)** (X100): dense fibrosis intermingled with granulomas. **(D)** Chronic beryllium disease: nonnecrotizing granuloma in a transbronchial biopsy (X200). **(E,F)** Intravenous pulmonary talcosis. **(E)** (X300): granuloma mimicking sarcoidosis; F (X300) birefringent talc particles visible under polarized light. **(G–J)** Tuberculosis. **(G)** (X50): low-magnification view showing the perivascular distribution of granulomas in miliary tuberculosis; **(H)** (X50): large nodule composed of caseating necrosis (arrow); **(I)** (X300): granuloma with a limited focus of necrosis (arrow); **(J)** (X950): Ziehl-Neelsen stain showing tuberculous mycobacteria (arrow). **(K,L)** Histoplasmosis. **(K)** (X300): granuloma with a central necrotic focus (arrow); **(L)** (X500): Grocott stain showing numerous yeast forms of *Histoplasma capsulatum*. **(M,N)** Whipple's disease. **(M)** (X300): granuloma with foamy histiocytes; **(N)** (X500): periodic-acid Schiff-positive inclusions within the foamy histiocytes. **(O)** Granulomatosis with polyangiitis (GPA): low-magnification view (X50) showing geographic basophilic necrosis suggestive of GPA when observed within a granulomatous pneumonitis.

The microscopic examination of stained smears has two main limitations: (i) it does not reliably distinguish between *M. tuberculosis* complex and nontuberculous mycobacteria and (ii) its sensitivity is lower compared to cultures, as the results are positive only with loads above 5,000 to 10,000 bacilli/mL of sample compared to 1 bacillus/mL, in theory, for cultures. Smear examination can rapidly detect those patients with the highest bacillus loads and account for about 50–60% of patients with culture-positive pulmonary tuberculosis (89–91). Sensitivity increases with the number of samples examined with a sensitivity of 74%, 86%, and 92% with one, two, and three samples, respectively (92).

Culturing is the reference standard test for diagnosing tuberculosis. The samples are first decontaminated and centrifuged to kill nontuberculous bacteria (pyogenic and commensal bacteria), which grow faster than do mycobacteria and can contaminate cultures (93). The sample is then inoculated either onto solid Löwenstein-Jensen medium, which is incubated at 35°C–37°C and produces visible colonies within 21 to 28 days, or into liquid medium, which can produce colonies within 10 to 21 days. A microscopic examination is then performed to confirm the presence of acid-alcohol resistant bacteria. With solid medium, the results are expressed quantitatively as the number of colonies per tube. With liquid medium, the time to a positive detection signal correlates with the amount of bacilli, thus providing a semi-quantitative estimate. The culture is reported as negative after 42 days with liquid medium and 60 days with solid medium. The identification of mycobacteria recovered by culturing was classically based on several cultural and biochemical features. This method is being increasingly replaced by molecular techniques (RNA/DNA hybridization, amplification of specific gene regions or insertion sequences) or by an immunochromatographic test that detects the MPT64 antigen (83).

An alternative method relies on matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), which provides a characteristic mass spectral fingerprint of whole inactivated mycobacterial cells. This method is accurate for identifying bacteria. Its simplicity, speed, and affordability make it suitable as a routine technique for identifying mycobacteria (94, 95).

Many tests marketed since the 1990s use gene-amplification techniques (PCR-TB tests) to identify mycobacteria of the *M. tuberculosis* complex within a few hours (standard or real-time PCR, ligase chain reaction, transcription-mediated amplification, nucleic-acid sequence-based amplification, isothermal amplification) (87, 96–99). Automated PCR tests (notably the Xpert® MTB/RIF assay) can detect *M. tuberculosis* complex and rifampicin resistance simultaneously, generally within 2 hours. Overall, sensitivity of these tests is about 80%–90% with culture-positive respiratory samples but only 60%–70% with smear-negative samples (85). Specificity is 98%–99% (85, 86, 100).

With extrapulmonary samples, the larger the volume the greater the likelihood of identifying the bacteria. For example, for possibly tuberculous lymph nodes, needle aspiration alone has a limited diagnostic yield, except in countries where tuberculosis is uncommon or when a PCR test is performed on the aspirate (101). However, PCR requires special equipment, reagents, and technician time that may not be available in low-income countries.

3.1.5.2. Microbiology of nonmycobacterial microorganisms

Histoplasmosis is diagnosed either by identification of the organism upon microscopic examination and culturing of sputum or tissue samples or by PCR testing of whole blood. *Histoplasma capsulatum* is a dimorphic fungus. Urine can be tested for *Histoplasma* antigen.

Several bacteria other than mycobacteria may cause symptoms suggestive of sarcoidosis and should be sought using standard bacteriological tests before considering tests for organisms that are more challenging to identify. Most of these bacteria are easily recovered by culturing then identified by routinely available MALDI-TOF mass spectrometry. Unlike *M. tuberculosis*, these bacteria do not require any special precautions in the laboratory. If the standard cultures are negative, PCR tests for the 16S rRNA gene can be performed as they may, in some cases, identify the causative bacterial agent.

Syphilis and leprosy do not typically damage the lung parenchyma. A single case of syphilitic granuloma in the lung parenchyma has been reported (102). The diagnosis relies mainly on indirect methods, i.e., serology or PCR testing, which are performed only in selected cases.

To look for viruses such as the human immunodeficiency virus, varicella-zoster virus, and cytomegalovirus, specific tests must be ordered. The diagnosis is usually achieved by specific PCR or serological tests, which are selected based on the clinical presentation, country of residence, and local epidemiology.

Metagenomic next-generation sequencing may, in the future, prove a useful and rapid tool for detecting bacteria, fungi, or viruses in granulomatous tissue. Advantages of this technique include the simultaneous identification of multiple pathogens, detection of microorganisms that are present in small amounts or difficult to grow, and feasibility on formalin-fixed and paraffin-embedded tissue.

3.1.6. Other investigations

Increased serum angiotensin converting enzyme level and abnormal calcium and vitamin D metabolism are often observed in sarcoidosis. However, both abnormalities are neither sensitive nor specific. They can be abnormal in several other granulomatous conditions particularly tuberculosis and CBD and even in non-granulomatous conditions like lymphomas.

^{18F}Fluodeoxyglucose (FDG) positron emission tomography (PET) CT (PET/CT) is the most sensitive test to assess activity in involved organs in sarcoidosis and no study has been done to assess its usefulness for diagnosis. There are not dedicated studies comparing PET/CT in sarcoidosis and other granulomatous or non-granulomatous diseases like for example lymphomas. Moreover, its use has not been recommended at diagnosis except in rare cases where a cardiac sarcoidosis is suspected (1).

3.2. More on alternative diseases: when can they mimic sarcoidosis and which tools can establish the differential diagnosis?

3.2.1. Infections

In Western European countries, Africa, and Asia, tuberculosis is the leading differential diagnosis of sarcoidosis. In North America, in contrast, nontuberculous mycobacterial infections and histoplasmosis may be more common than tuberculosis.

3.2.1.1. Tuberculosis

Tuberculosis is the main differential diagnosis in most countries. The importance of assessing the epidemiological setting is highlighted

above (section 3.1.1.) The prompt and accurate diagnosis of pulmonary tuberculosis is essential.

3.2.1.1.1. When is the differential diagnosis most difficult?

Sarcoidosis is particularly difficult to diagnose in patients with rare and atypical radiological presentations such as unilateral hilar or mediastinal lymphadenopathy, necrotic lymphadenopathy, predominantly bronchiolar micronodular infiltration or miliary, lung cavitations, or pleural effusion. These presentations should suggest tuberculosis, particularly in endemic regions (section 3.1.1.). The absence of caseating granulomas does not rule out tuberculosis (103). In all patients, including those with imaging-study findings typical for sarcoidosis, mycobacteria should be sought in biopsy specimens and, if performed, by BAL.

3.2.1.1.2. How to definitively rule out tuberculosis

Tuberculosis is easily ruled out when the following criteria are combined: country with a low incidence of tuberculosis, no contact with tuberculosis patients, radiological findings typical for sarcoidosis (section 3.1.3.), absence of clinical or imaging-study findings suggestive of tuberculosis, and negative microbiological tests on respiratory samples (sputum, BAL fluid) or tissue specimens (obtained by endobronchial ultrasound or other methods and examined using specific stains and microbiological techniques, section 3.1.5.1.).

However, in countries where tuberculosis is endemic (e.g., India), tuberculosis is more difficult to definitively rule out at presentation, as shown by a recent study of the Sarcoidosis Diagnostic Scores (7). Sarcoidosis often produces misleading constitutional symptoms such as fever, weight loss, and fatigue (104). In a study of patients with sarcoidosis in India, the lymph nodes appeared necrotic or nonhomogeneous by chest CT in 5.9% of cases, and necrotizing granulomas were found in tissue samples in 13.5% of cases (104). Stronger arguments for sarcoidosis are tuberculin anergy (71.9% of cases), typical isolated bilateral lymphadenopathy, and absence of necrosis by CT and/or histopathology. In tuberculosis-endemic countries, the proportion of presentations mimicking sarcoidosis is, as everywhere else, low but the high absolute number of tuberculosis cases increases the risk of confusion with sarcoidosis, particularly as microbiological studies for *M. tuberculosis*, including PCR tests, can produce false-negative results (105). Moreover, the tuberculin skin test and interferon-γ release assay can be negative despite confirmed tuberculosis, notably in elderly patients or when the peripheral lymphocyte count is low (106–108). In doubtful cases, the initiation of tuberculosis treatment is often wise, as the diagnosis of sarcoidosis may be confirmed only by the clinical course during tuberculosis treatment, which usually differs between tuberculosis and sarcoidosis.

Interestingly, in one study, the risk of developing sarcoidosis was significantly increased in patients with tuberculosis, particularly involving extrapulmonary sites (109). Moreover, the two diseases may co-exist at presentation. In this situation, only the accumulation of evidence over time can clarify the diagnosis.

3.2.1.2. Nontuberculous mycobacteria

The clinical presentation and imaging-study findings closely resemble those seen in tuberculosis. Several specific features that vary across mycobacterial species deserve note. Fibrocavitary lesions in the lung apices are more common with *Mycobacterium kansasii* and *Mycobacterium malmoense*, whereas bronchiolar micronodules with a tree-in-bud appearance and bronchiectasis more often indicate *Mycobacterium avium* complex (MAC) infection. Isolated or multiple nodules can be found in *M. avium* complex and *Mycobacterium xenopi* infections.

To rule out nontuberculous mycobacterial infections, microbiological studies should be performed, as they usually allow the isolation and identification of mycobacteria. However, the diagnosis may be difficult in patients with multiple pulmonary nodules by chest CT, since investigations for mycobacteria in respiratory secretions or BAL fluid may be falsely negative. In this situation, the only definitive test is a surgical biopsy examined using specific microbiological techniques (section 3.1.5.1.).

3.2.1.3. Histoplasmosis

Histoplasmosis may be a differential diagnosis of sarcoidosis in several circumstances.

3.2.1.3.1. When is the differential diagnosis most difficult?

When the presentation is acute, imaging studies may be misleading if they show both lymphadenopathy, which may be bilateral, and diffuse lung micronodules. However, the clinical presentation more closely resembles that of community-acquired pneumonia, and the epidemiological setting is suggestive (section 3.1.1.).

In everyday practice, the differential diagnosis with sarcoidosis is most difficult in patients with (i) multiple chronic pulmonary nodules or (ii) pulmonary hypertension revealing previously unrecognized late-stage mediastinal fibrosis.

3.2.1.3.2. How to definitively rule out histoplasmosis

Histoplasmosis occurs in specific epidemiological settings (section 3.1.1.) The diagnosis relies on the identification of the causal organism by microscopic examination of sputum or tissue and by culturing or PCR testing of whole blood.

3.2.1.4. Other infections

Other bacteria that may cause symptoms and chest-imaging findings suggestive of pulmonary sarcoidosis are very rare. Q fever is usually diagnosed indirectly, by serological or PCR testing. Whipple's disease is an infection by the Gram-positive bacillus *Tropheryma whipplei*. Patients usually present with weight loss (93%), diarrhea (81%), and arthralgia (73%). Pulmonary involvement is extremely rare and produces diffuse pulmonary ground-glass opacities predominating in the lower subpleural areas, pleural involvement, and noncaseating pulmonary granulomas (70, 72, 110, 111). The diagnosis relies on the identification of *T. whipplei* in various samples, including BAL fluid, using quantitative PCR (111). The BAL fluid shows neutrophilia and eosinophilia, as opposed to lymphocytosis, and stains with periodic acid-Schiff. As indicated above (section 3.1.5.2.), syphilis is an exceedingly rare cause of lung granulomatosis.

Tests for viruses such as the varicella-zoster virus and cytomegalovirus should be selected based on the clinical history and epidemiology. Persistent diffuse lung micronodules can be found long after typical adulthood varicella. Although COVID-19 does not appear to cause granulomas, possibly coincidental sarcoidosis-like disease diagnosed shortly after COVID-19 onset has been reported in three patients (112).

Several fungal infections other than histoplasmosis can cause granulomatous lung diseases that usually mimic tuberculosis. These infections occur chiefly in immunocompromised patients, but rare cases have been reported in immunocompetent individuals. They include cryptococcosis, coccidioidomycosis, and mucormycosis (113–115).

Parasitic infections cause pulmonary granulomatous diseases only very rarely and only in endemic countries. Leishmaniosis and paragonimiasis are examples, as well as schistosomiasis due to *S. mansoni*, which can present as pulmonary arterial hypertension with liver fibrosis and portal hypertension (72, 116, 117).

3.2.2. Occupational and environmental diseases

3.2.2.1. Chronic beryllium disease

Chronic beryllium disease can develop in individuals who are exposed to beryllium, usually at the workplace, and develop sensitization to this metal (118, 119). The features of the pulmonary granulomatous disease caused by beryllium closely match those of pulmonary sarcoidosis.

3.2.2.1.1. When is the differential diagnosis most difficult?

The circumstances of the diagnosis vary widely. Chronic beryllium disease is readily diagnosed when the manifestations are detected during routine workplace monitoring of workers exposed to beryllium. However, individuals may be unaware of the exposure. Unless a systematic and detailed occupational history is obtained, sarcoidosis is often erroneously diagnosed at first, given the similarities in radiological, serum angiotensin-converting enzyme assay, BAL, and histopathology findings (120, 121). Moreover, the tuberculin test is often negative in chronic beryllium disease. Interestingly, gene expression patterns in peripheral-blood mononuclear cells were not different between patients with chronic beryllium disease and sarcoidosis (122). The only reported differences are rare extrapulmonary manifestations and rare voluminous hilar and mediastinal lymphadenopathy in chronic beryllium disease (118). In one study, among 84 patients with potential beryllium exposure and suspected or diagnosed sarcoidosis, 34 were diagnosed as having chronic beryllium disease instead, based on a positive beryllium lymphocyte proliferation test (120). Of these 34 patients, 28 had first been diagnosed with sarcoidosis, the median time between the two diagnoses being 3 [0.25–18] years.

3.2.2.1.2. How to definitively rule out chronic beryllium disease

Differentiating chronic beryllium disease from sarcoidosis relies chiefly on two investigations: first, a systematic and detailed occupational history including questions about possible occupational beryllium exposure should be obtained in every patient with suspected pulmonary sarcoidosis and, second, a beryllium hypersensitivity test is mandatory in all patients with a history of beryllium exposure, even in the distant past.

Occupational beryllium exposure may occur during primary beryllium and beryllium-alloy production; in dental laboratories; in nuclear power plants; during nuclear weapon manufacturing; and during aerospace or ceramic manufacturing (119). Beryllium exposure has also been reported in security guards, accountants, exposed workers' spouses, and people living near beryllium-production facilities. Exposure may be demonstrated unexpectedly, as shown recently in workers who inhaled concrete dusts containing high beryllium concentrations (123).

Beryllium hypersensitivity can be demonstrated by performing a beryllium lymphocyte proliferation test. The result is abnormal when the lymphocyte count is greater than 15%. Two or more abnormal results on blood, one abnormal and one borderline result on blood, or one positive result on BAL fluid confirms beryllium hypersensitivity (119). In clinical practice, the absence of an occupational history of beryllium exposure combined with two negative beryllium lymphocyte proliferation tests on blood rule out chronic beryllium disease.

HLA-DPB1 alleles encoding a glutamic acid residue at position 69 of the β-chain (Glu69) are associated with an increased risk of both beryllium hypersensitivity and chronic beryllium disease, with odds ratios greater than 10 (118). However, the Glu69 variant can be found in healthy individuals and is absent in 25% of patients with chronic beryllium disease. Thus, HLA-DPB1 testing has no role as a diagnostic investigation for chronic beryllium disease.

3.2.2.2. Non-beryllium-metal-induced granulomatosis

Positive lymphocyte transformation tests to metals other than beryllium have been reported in patients with sarcoidosis-like presentations and workplace exposures to metals (e.g., during jewelry manufacturing or welding). The metals/minerals were aluminum, nickel, titanium, chromium, palladium, silica, mercury, and zirconium. Thus, some patients given a diagnosis of sarcoidosis may have metal-induced sarcoidosis-like granulomatous disease (124, 125).

3.2.2.3. Hypersensitivity pneumonitis

Hypersensitivity pneumonitis, notably in its nonfibrotic form, is caused by exposure to airborne environmental or occupational antigens and can share features with sarcoidosis. However, differentiation from sarcoidosis is generally easy, as shown by a study of the Sarcoidosis Diagnostic Scores (7). The onset may be acute, subacute, or insidious, and recurrent episodes may develop. Crackles are common and squawks may be heard. Although many patients have constitutional symptoms including weight loss, the disease is limited to the lungs, without extrapulmonary organ involvement. At least 40% of patients have either exposure to or serum IgG against an environmental or occupational inciting agent (e.g., avian antigens, bacteria, mycobacteria, fungi, isocyanates). The imaging findings typically differ from those in sarcoidosis (section 3.1.3.) (76, 126). Although BAL fluid lymphocytosis occurs in both hypersensitivity pneumonitis and sarcoidosis, a relative lymphocyte count above 50% has been reported in half the patients with the former versus almost none with the latter (80). In BAL fluid, the total cell count, percentages of neutrophils and eosinophils, and percentage of mast cells — this last having the greatest discriminating power — tend to be higher in hypersensitivity pneumonitis than in sarcoidosis, whereas the CD4/CD8 T-cell ratio tends to be above normal in sarcoidosis and below normal in hypersensitivity pneumonitis (80, 127). Combining the history with blood-test, imaging-study, and BAL findings usually suffices to differentiate hypersensitivity pneumonitis from sarcoidosis. The granulomas found in transbronchial lung-biopsy specimens in hypersensitivity pneumonitis are typically small and poorly formed and tend to predominate in the peribronchiolar interstitium (section 3.1.3.). This finding combined with those of the above-listed investigations is highly discriminating.

3.2.2.4. Hot-tub lung

Hot-tub lung is an infrequent interstitial lung disease secondary to the inhalation of aerosolized hot-tub water containing nontuberculous mycobacteria (*M. avium or Mycobacterium phocaicum*) (128). Chest CT findings may closely resemble those in hypersensitivity pneumonitis, with diffuse ground-glass opacities and mosaic attenuation. However, the lung biopsy shows different features consisting in nonnecrotizing and mostly well-formed granulomas in a bronchocentric distribution. Differentiation from sarcoidosis is easy even in the absence of a lung biopsy, based on (i) use of a hot tub, (ii) the very different CT images, and (iii) the recovery of nontuberculous mycobacteria from respiratory specimens.

3.2.2.5. Granulomatous talcosis

Pulmonary granulomatous talcosis can produce radiological abnormalities resembling those seen in sarcoidosis (section 3.1.3.) (39). A history of inhaled or intravenous drug abuse suggests the diagnosis. Confirmation is provided by lung biopsy, which shows small diffuse granulomas at the level of the terminal or respiratory bronchioles, with birefringent bodies within giant cells by polarized light microscopy (129). These lesions are more similar to foreign-body granulomas than to sarcoid granulomas.

3.2.3. Drugs

A recent WHO pharmacovigilance review reported 2,425 cases of drug-induced sarcoidosis and strong associations with TNF-a antagonists, interferon, pegylated interferon, and immune checkpoint inhibitors (19, 130, 131).

3.2.3.1. TNF-a antagonist-induced granulomatous diseases

Despite data supporting the use of TNF-a antagonists to treat sarcoidosis, these drugs have been reported to induce sarcoid-like reactions (18, 132). Etanercept was most often involved, but cases were also seen with adalimumab, infliximab, golimumab, and biosimilars. The reason for TNF-a antagonist therapy was usually rheumatoid arthritis, although some patients had ankylosing arthritis, Crohn's disease, or even sarcoidosis (133–136). TNF-a antagonist-induced granulomatous disease is rare; thus, only four (0.57%) cases were identified among 697 patients with rheumatoid arthritis (133). The mean duration of TNF-a antagonist exposure has varied widely, from 7 to 123 months in rheumatoid arthritis and 1 to 180 months in Crohn's disease. Diffuse lung infiltration with or without hilar lymphadenopathy was common. Peripheral lymphadenopathy, uveitis, and involvement of the spleen, muscle, parotid glands, and spinal cord have also been reported. Laboratory test abnormalities included hypercalcemia, which was severe in some patients, and elevated serum angiotensin-converting enzyme levels. Diagnostic challenges arise due to the low incidence of TNF-a antagonist-induced granulomatosis, wide variability in exposure duration at symptom onset, and possible presence of symptoms due to the underlying disease (e.g., rheumatoid arthritis or Crohn's disease) (137, 138). The prompt fading of the manifestations after withdrawal of the suspected drug strongly supports TNF-a antagonist-induced granulomatous disease. In patients with sarcoidosis, worsening of the disease during TNF-a antagonist therapy is difficult to interpret, particularly given the paucity of relevant information in the literature. A relapse after an initial response to TNF-a antagonist therapy may indicate either drug-induced granulomatous disease or the development of antidrug antibodies, as reported with infliximab or adalimumab in patients with rheumatoid arthritis (139, 140). Antidrug antibody assays are very useful in this situation.

3.2.3.2. Sarcoid-like reaction to anticancer drugs

Some of the most recent anticancer drugs including immunotherapeutic agents and targeted therapies have been incriminated in the development of granulomas. The recent WHO pharmacovigilance report recognizes PD-1, CTLA4 (103 cases), and BRAF and MEK inhibitors (37 cases) as causes of sarcoid-like granulomatosis (130).

3.2.3.2.1. Immune checkpoint inhibitors

Pulmonary granulomatosis was first described with CTLA-4 inhibitors then with PD-1 and PD-L1 inhibitors. In several patients, the CTLA-4 inhibitors ipilimumab and tremelimumab seem to have caused cutaneous, lymph-node, and lung involvement with noncaseating granulomas, as well as lymphocytic alveolitis (141–144). Time to symptom onset was usually three to 6 months. The PD-1 inhibitor nivolumab has been associated with granulomatosis of the lungs and of other organs including the eyes (145). This appears to be a class effect, as granuloma formation has been reported with the PD1 inhibitors pembrolizumab (146) and nivolumab (147) and with the PD-L1 inhibitors atezolizumab (148), durvalumab (149), and avelumab (150). The time to onset in patients given both CTLA-4 and PD-1 inhibitor therapy may be shorter, as with all toxicities from immunotherapies (151). Retrospective data suggest that a sarcoid-like reaction may be associated with a better prognosis. Of 434 retrospectively reviewed patients with adverse effects of checkpoint inhibitor therapy, 28 had sarcoid-like reactions, which were asymptomatic in half the cases and often manifested only as mild dyspnea or a cough with no serious lung impairments in the other cases. The most common primary cancer was melanoma and most patients with taking both CTLA-4 and PD-1 inhibitors. Compared to the 406 patients with other adverse events, those with sarcoid-like reactions had better overall survival (hazard ratio, 0.232; 95% confidence interval, 0.086–0.630; $p = 0.002$) in this retrospective study (152).

Importantly, patients with sarcoid-like reactions are at risk of being misdiagnosed either with cancer progression in the absence of histological studies or with an infection precipitated by the treatment-induced immunosuppression (combined chemotherapy and immunotherapy). Sarcoid-like reactions can mimic cancer progression. Of 45 patients given adjuvant combined nivolumab-ipilimumab therapy for melanoma, 10 (22%) were diagnosed with sarcoid-like reactions manifesting most often as hilar and mediastinal lymphadenopathy and in some patients as skin and bone involvement (153). An unresolved issue is whether the type of primary cancer and/or disease stage affect the incidence of sarcoid-like reactions (153). Findings that strongly support a need for histological studies include atypical changes in the radiological abnormalities, a dissociated radiological response, and a marked discrepancy between the radiological and clinical signs.

3.2.3.2.2. Targeted therapies

Other anticancer treatments that can induce sarcoid-like reactions include BRAF and MEK inhibitors. Cases have been reported with the BRAF inhibitor vemurafenib used alone (154) or with the BRAF inhibitor dabrafenib combined with a MEK inhibitor (trametinib or cobimetinib) (155, 156). Bilateral hilar lymphadenopathy, uveitis, and

cutaneous involvement have been reported (154). These manifestations were usually not severe, and the cutaneous and ophthalmological abnormalities resolved promptly with topical treatment despite continuation of the anticancer drugs. In one patient, however, acute kidney injury required discontinuation of the targeted treatment and the administration of corticosteroid therapy (155).

3.2.3.2.3. Intravesical BCG therapy for bladder cancer

Intravesical BCG therapy for bladder cancer can cause systemic granulomatosis (157, 158). Granulomatous pneumonitis, sometimes with systemic involvement, has been reported in 0.7%–0.9% of patients (159, 160). The pathogenesis of BCG-related granulomatous pneumonitis is debated but may involve a hypersensitivity reaction to disseminated BCG (158).

The chest radiograph may be normal or show a miliary pattern mimicking disseminated tuberculosis, a fungal infection, or hematogenous metastases (158).

3.2.3.3. Interferon-induced pulmonary granulomatous disease

New treatments for hepatitis have largely superseded interferon-a, except for patients with hepatitis delta. Interferon-induced pulmonary granulomatous disease has therefore become rare.

3.2.3.4. Highly active antiretroviral therapy (HAART) for HIV infection

A sarcoidosis-like disease can develop in HIV-positive patients who have responded to antiretroviral therapy by a rise in CD4 T-cell counts and a fall in viral loads. In 10 retrospectively identified HIV-positive patients with a newly diagnosed, sarcoidosis-like disease, the chest CT findings resembled those produced by sarcoidosis in HIV-negative patients: they consisted of lymphadenopathy, nodules, thickened interlobular septa, focal consolidation, reticular opacities, ground-glass opacities, and cyst-like or other cavities (161). In HIV-positive patients receiving HAART, sarcoidosis-like manifestations may indicate true sarcoidosis related to restoration of the immune system.

3.2.3.5. Other drug-induced pulmonary granulomatous diseases

The monoclonal antibodies rituximab, omalizumab, ustekinumab, vedolizumab, and natalizumab are suspected causes of pulmonary granulomatosis (130, 162, 163). Mesalamine has been suggested to induce pulmonary granuloma formation when used to treat Crohn's disease.

3.2.4. Immune deficiencies

3.2.4.1. Pulmonary granulomatosis-associated common variable immunodeficiency

This disease often results in recurrent bacterial respiratory infections (65% of cases), bronchiectasis, and autoimmune manifestations (40% of cases) and may co-exist with lymphoproliferative disorders (21). However, the disease may remain asymptomatic until sarcoid-like abnormalities develop. Findings may include mediastinal lymphadenopathy and bronchial or parenchymal granulomas (21). The pulmonary granulomatous lesions may be isolated or co-exist with organizing pneumonia, lymphoid interstitial pneumonitis, follicular bronchiolitis, or lymphoid hyperplasia. The term "granulomatous-lymphocytic interstitial lung disease" has been used to designate this condition but remains controversial (21, 164). Serum protein electrophoresis, which must be part of the diagnostic workup for sarcoidosis, shows hypogammaglobulinemia, thereby establishing the diagnosis (1). Importantly, the chest CT findings differ substantially from those seen in sarcoidosis (section 3.1.3.).

3.2.4.2. Pulmonary manifestations in adults with chronic granulomatous disease

Chronic granulomatous disease is a rare inherited primary immunodeficiency caused by a mutation in the NADPH oxidase gene. The respiratory manifestations are major complications. The diagnosis is usually made in early childhood upon the evaluation of recurrent infections. The pulmonary infections are often chronic and may be asymptomatic, notably when caused by *Aspergillus fumigatus*. A biopsy of persistent pulmonary nodules or consolidations is often required and frequently shows noncaseating granulomas. Radiological presentations mimicking sarcoidosis are very rare (165, 166). The repeated infections starting at a very young age combined with the radiological presentation usually make sarcoidosis very improbable.

3.2.5. Genetic diseases

Blau syndrome is an autosomal dominant disorder due to *NOD2* mutations, of which over 15 have been identified (167, 168). Familial cases are present in 40% of patients. The onset is usually at 3–4 years of age (15). Most patients have the typical triad of skin involvement, symmetrical polyarthritis, and uveitis. Lung involvement has been reported in a single patient, who had ground-glass opacities in the middle and lower lobes (169). The diagnosis is based on the presentation and results of genetic testing for *NOD2* mutations. Thus, Blau syndrome is easily differentiated from sarcoidosis (170).

3.2.6. Vasculitides and autoimmune diseases

3.2.6.1. Granulomatosis with polyangiitis

This disease typically manifests as necrotizing granulomatous inflammation of the ears, nose, and upper and lower respiratory tracts and as necrotizing vasculitis involving the small- to medium-sized vessels, often with glomerulonephritis (171).

The differential diagnosis with sarcoidosis is rarely difficult unless the lung is the only site involved, with condensations or noncavitated nodular lesions. However, differences with sarcoidosis include the absence of co-existing perilymphatic micronodular lesions and of lymphadenopathy (46). A positive assay for antineutrophil cytoplasmic antibodies directed to proteinase 3 (PR3-ANCA) is highly specific but only 60% sensitive. In doubtful cases, a lung biopsy, performed surgically to ensure the collection of sufficient material, may be indicated. The typical histological pattern is a triad of vasculitis, necrosis, and granulomatous inflammation, which may co-exist with organizing pneumonia or alveolar hemorrhage.

3.2.6.2. Eosinophilic granulomatosis with polyangiitis

Lung granulomas can develop in eosinophilic granulomatosis with polyangiitis, producing a radiological presentation very similar to that seen in granulomatosis with polyangiitis. However, differentiation with sarcoidosis is readily achieved based on the history of asthma (often severe), marked blood eosinophilia, serum C-reactive protein elevation, and positive assays for perinuclear antineutrophil cytoplasmic antibodies.

3.2.6.3. Necrotizing sarcoid granulomatosis

"Necrotizing sarcoid granulomatosis" is still a provisional diagnostic term, given the uncertainty about whether it represents necrotizing angiitis with a sarcoid reaction or true sarcoidosis with a distinctive pathological pattern (172). The epidemiology and presentation are very similar to those of sarcoidosis. However, thoracic lymphadenopathy occurs in only 33% of patients. The only clear difference lies in the pathological findings. However, a possible source of bias is that the lung specimens are often obtained surgically and are therefore larger than for sarcoidosis. The prevailing view at present is that necrotizing sarcoid granulomatosis is a form of sarcoidosis (172).

3.2.6.4. Interstitial lung disease associated with Sjögren's syndrome

Interstitial lung disease, sometimes with granulomatous pulmonary lesions, may develop in patients with sicca due to Sjögren's syndrome. Very infrequently, this condition may require differentiation from sarcoidosis with pulmonary fibrosis producing linear opacities extending from the hilum to the subpleural area (173). However, the pathological features of granulomatous lesions in Sjögren's syndrome differ markedly from those of sarcoid granulomas (section 3.1.4.).

3.2.7. Crohn's disease

Noninfectious pulmonary involvement has rarely been studied in patients with Crohn's disease. The first diagnosis to consider is an adverse drug reaction, for instance to mesalamine or a TNF-a antagonist (174). In practice, Crohn's disease is never *per se* the cause of pulmonary granulomatosis. At the trachea, in contrast, macroscopic abnormalities can be visible by endoscopy, a finding never observed in sarcoidosis (175). Crohn's disease and sarcoidosis may co-exist (176).

3.2.8. Granulomatosis associated with lymphoma and solid malignancies

Non-caseating granulomas have been reported in 0.7–13% of patients with malignancies (177) including lymphomas; testicular, breast, lung, and head-and-neck cancer; and melanoma (177, 178). Sarcoidosis-like granulomas were identified in 14% of patients with Hodgkin's lymphoma and 7% with non-Hodgkin's lymphoma (179, 180). The granulomas may develop within the tumor itself or in the regional draining lymph nodes (e.g., mediastinal nodes in lung cancer). Distant lymph nodes may be involved, and lung nodules may develop remotely from primary skin melanoma (178, 179). The granulomas may be identified only many years after the diagnosis of cancer. The main diagnostic challenge is differentiation from metastases. A granulomatous reaction to cancer is readily distinguished from sarcoidosis when it is present only in the neighborhood of the malignancy. Investigations for granulomas at other sites must therefore be performed. Sometimes, a typical sarcoidosis can be evidenced upon review of earlier investigations. In challenging cases, only the collection of further data during follow-up can establish the diagnosis.

The risk of lymphoma is 2-fold to 11-fold higher in patients with vs. without sarcoidosis (179, 181). Thus, manifestations that develop during the course of sarcoidosis may be due to lymphoma. On the other hand, clinical events in patients with lymphoma may be due to another disease. One study identified 14 new and 25 previously reported patients in whom sarcoidosis developed after a diagnosis of Hodgkin's or non-Hodgkin's lymphoma (182).

Lymphomatoid granulomatosis is a rare B-cell lymphoproliferative disease associated with Epstein–Barr virus infection. Multiple lung nodules predominating in the middle to lower lung fields may develop. The diagnosis is confirmed by the identification of atypical B cells positive for Epstein–Barr virus-encoded small non-polyadenylated RNA 1 and 2 (EBER). These cells may be sparse and associated with nonnecrotizing granulomatous inflammation (183).

4. Conclusion

Alternative diagnoses must be ruled out before a diagnosis of sarcoidosis can be given. To this end, a rigorous diagnostic strategy must be applied. First, epidemiological factors must be clarified, including local infectious diseases, regions of travel, occupational and environmental exposures, drug abuse, exposure to medications, and family history. A detailed medical history and thorough physical examination are also crucial, as they may, for instance, suggest an immunodeficiency or identify extrapulmonary abnormalities that may be characteristic of specific diseases. Chest CT is very helpful, particularly when read by a highly experienced radiologist who can characterize the findings as typical or atypical for sarcoidosis and for the alternative diagnoses. All other investigations are guided by this information. A study reported nearly three decades ago used Bayes' theorem to diagnose chronic interstitial lung diseases based only on clinical and radiological findings (184). High confidence (greater than 95% probability) in a final correct diagnosis of sarcoidosis was obtained in 80% and 78% of patients in the training and validation sets, respectively. The improvements in CT acquisition and interpretation achieved since this study was done would probably result in even better performance. Another important message is the need to obtain typical granulomas for confirming diagnosis in most of the patients to avoid misdiagnosis with some alternative diseases shown to have a confusing presentation, particularly lymphoproliferative disorders. Eventually, using scores like the Sarcoidosis Diagnosis Scores Clinical and Biopsy can be very helpful to assess sarcoidosis diagnosis before and after granuloma evidence.

Author contributions

DV and FJ conceptualized the review. DV prepared the first draft of the manuscript with MB (for imaging), J-FB (for pathology), EC (for microbiology), and BD (for drug-induced granulomatosis). MB provided the radiological figures and legends. J-FB provided the histological table and figures and legends. DV, MB, J-FB, EC, BD, CR, IB, AM, HN, J-MN, and FJ critically revised and edited the manuscript and agreed to the submitted version. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that this review was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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