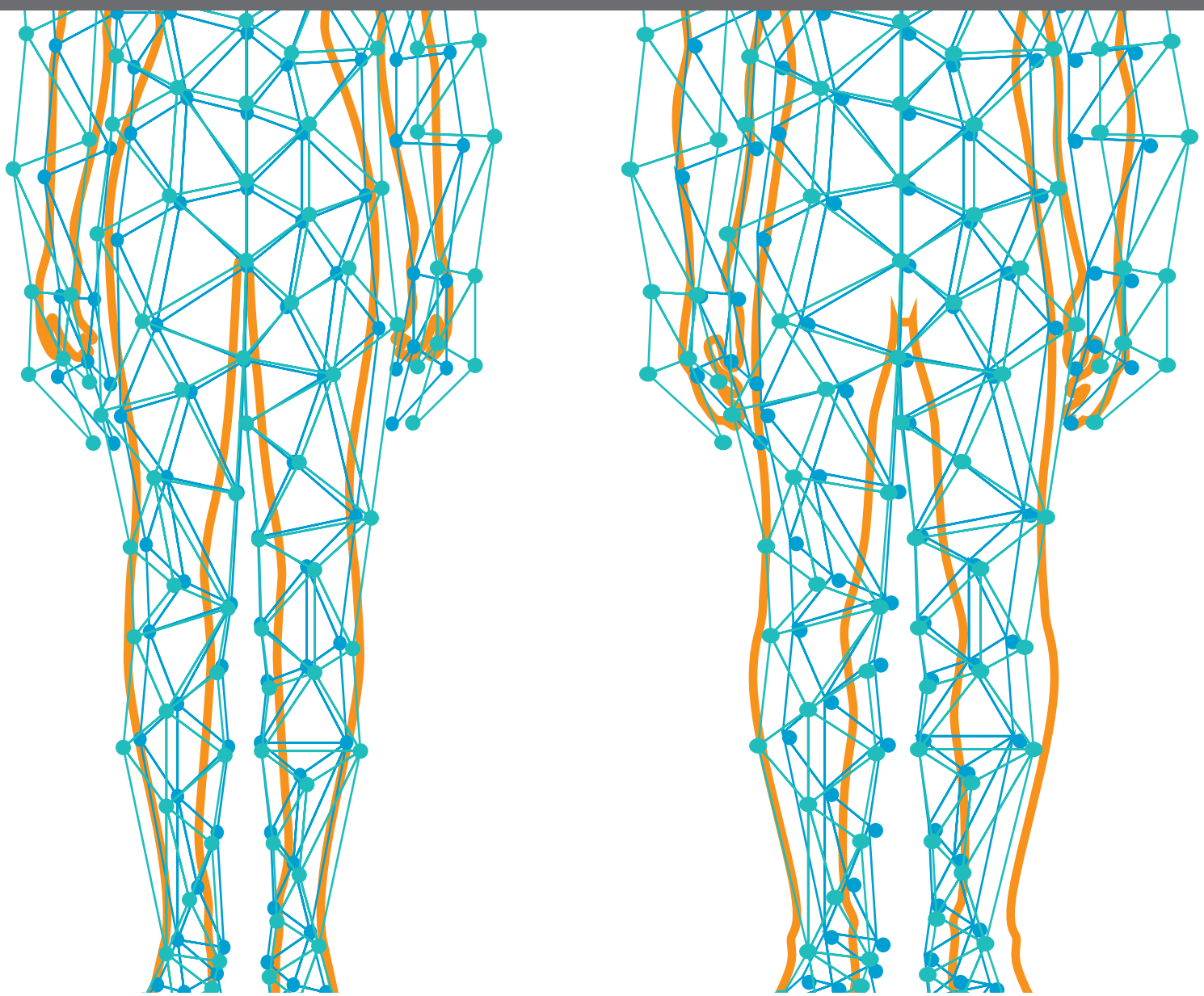
SARCOIDOSIS – THE GREAT MIMICKER

EDITED BY: Peter Korsten, Nadera J. Sweiss and Mehdi Mirsaeidi
PUBLISHED IN: Frontiers in Medicine



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88976-622-2
DOI 10.3389/978-2-88976-622-2

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

SARCOIDOSIS – THE GREAT MIMICKER

Topic Editors:
Peter Korsten, University Medical Center Göttingen, Germany
Nadera J. Sweiss, University of Illinois at Chicago, United States
Mehdi Mirsaeidi, University of Florida, United States

Citation: Korsten, P., Sweiss, N. J., Mirsaeidi, M., eds. (2022). Sarcoidosis – The Great Mimicker. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88976-622-2

Table of Contents

frontiers | Frontiers in Medicine

TYPE Editorial
PUBLISHED 02 August 2022
DOI 10.3389/fmed.2022.990714

OPEN ACCESS

APPROVED BY
João Eurico Fonseca,
University of Lisbon, Portugal

*CORRESPONDENCE
Peter Korsten
peter.korsten@med.uni-goettingen.de

SPECIALTY SECTION
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

RECEIVED 10 July 2022
ACCEPTED 18 July 2022
PUBLISHED 02 August 2022

CITATION
Korsten P, Mirsaeidi M and Sweiss NJ (2022) Editorial: Sarcoidosis—The great mimicker. *Front. Med.* 9:990714. doi: 10.3389/fmed.2022.990714

Editorial: Sarcoidosis—The great mimicker

Peter Korsten[1]*, Mehdi Mirsaeidi[2] and Nadera J. Sweiss[3]

[1]Department of Nephrology and Rheumatology, University Medical Center Göttingen, Göttingen, Germany, [2]Division of Pulmonary, Critical Care, and Sleep Disease, College of Medicine-Jacksonville, University of Florida, Jacksonville, FL, United States, [3]Division of Rheumatology, Department of Medicine, University of Illinois at Chicago, Chicago, IL, United States

KEYWORDS

sarcoidosis, chronic granulomatous disease, pulmonary sarcoidosis, hypercalcemia, case reports

Editorial on the Research Topic
Sarcoidosis—The great mimicker

Introduction

Sarcoidosis has been and remains a challenge for patients and physicians alike. Even in the year 2022, the pathophysiology of sarcoidosis is still incompletely understood. With this Research Topic entitled "Sarcoidosis—The Great Mimicker," we aimed to shed light on the various aspects of this enigmatic disease. We are thankful and indebted to all authors, reviewers, and external editors who have contributed with their research papers and time to make this a thriving collection of articles. We received many submissions indicating that sarcoidosis attracts researchers from various backgrounds and specialties. With this editorial, we will give an overview of the topics covered in the Research Topic and place them in the context of the current research landscape.

Learning from case reports

The search terms (Sarcoidosis[Title]) AND (Case Reports[Filter]) in the commonly used database Pubmed retrieved a steadily increasing number of cases over the years and a total number of 7.280 reports. Starting in 1955, only one case was reported, which increased to 276 as of 2021 (Figure 1). Thus, case reports of sarcoidosis or its mimickers are a valuable educational tool to capture the frequent and infrequent manifestations of the disease. In this regard, two case reports were accepted for this collection of articles. The first article, written by Tirelli et al. describes a case of concurrent adenocarcinoma of the lung and coexistent sarcoidosis, which is rare and therapeutically challenging. A recent systematic review identified nine case reports and highlighted that meticulous diagnostic testing is essential to differentiate metastatic disease from sarcoid or sarcoid-like lesions (1).

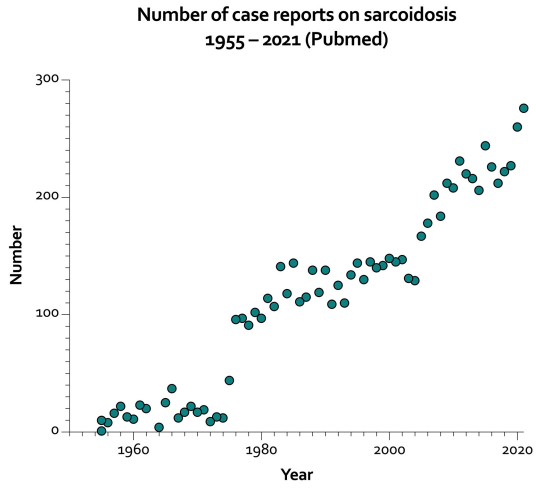


FIGURE 1
Number of case reports on sarcoidosis per year from 1955 to 2021 (https://pubmed.ncbi.nlm.nih.gov/; last accessed July 8th, 2022).

The following case report by De Cinque et al. focuses on testicular sarcoidosis, an exceedingly rare manifestation of sarcoidosis. They reviewed the literature and identified, including their case, only 20 publications where testicular sarcoidosis was the primary manifestation of sarcoidosis. Most interestingly, they described contrast-enhanced ultrasound (CEUS) features, which can be helpful in discerning sarcoidosis from other malignant testicular lesions. Specifically, sarcoid lesions are hypoechoic and show hypo-enhancement on CEUS, a clinically relevant point.

Lastly, Manansala et al. describe a case series of COVID-19 in sarcoidosis patients of African American ethnicity. They report on five cases; one ultimately expired due to a thromboembolic event. This report, published relatively early in the pandemic, was reassuring for patients with sarcoidosis, especially African American patients, who usually suffer more from sarcoidosis than other ethnicities (2, 3). However, a single-center study, which analyzed patients seen from July to December 2020, suggested that the hospitalization rates and the mortality from COVID-19 in sarcoidosis may be increased (4).

Reviews on cancer, novel treatments, pitfalls, and the microbiome

In the first of four review papers in this Research Topic, El Jammal et al. review the relationship between sarcoidosis and cancer, which complements the case report by Tirell et al. mentioned above. The authors summarize the available literature on sarcoidosis and its relationship to cancer; the main concern is the occurrence or co-occurrence of lymphomas. Further, they highlight the importance of a dedicated histopathologic analysis because some malignancies with granulomatous features may otherwise be missed. In addition, it is essential to consider infectious complications in patients with a known cancer diagnosis, especially opportunistic infections.

Next, Boleto et al. provided an overview of novel therapeutic targets for treating refractory sarcoidosis. They focused on non-tumor necrosis factor-alpha inhibitor monoclonal antibodies. Unfortunately, the available clinical trial data for many agents, including interleukin (IL)-17 and—12/23-antagonists or B-cell modulating agents, were negative. Clinical trials for anti-IL-6, anti-IL-1, and Janus kinase inhibitors are ongoing and have not been published yet. In light of the recently published American Thoracic Society/European Respiratory Society recommendations for the treatment of sarcoidosis, which confirmed the lack of robust evidence derived from high-quality clinical trials, these trial results are eagerly awaited (5). A recent, very detailed report highlighted the beneficial role of tofacitinib in sarcoidosis (6) after the first very encouraging reports for cutaneous sarcoidosis (7).

Adding to the review papers on dedicated topics concerning sarcoidosis is the article by Narula and Iannuzzi on pitfalls and challenging mimickers. Here, the authors reviewed the most common infections and non-infectious diseases across frequent (such as pulmonary sarcoidosis) and infrequent (neurosarcoidosis) manifestations. In addition, they highlight diseases and conditions that treating physicians always need to be aware of, such as drug-induced sarcoid reactions, common-variable immunodeficiency with granulomas, and differential diagnoses for hypercalcemic syndromes.

The last review of the topic by Chioma et al. described the role of the lung microbiome in interstitial lung diseases (ILD), including sarcoidosis. Among the few dedicated studies available, the evidence for alterations in the gut or lung microbiome is mixed. In sarcoidosis specifically, alterations of the microbiome in the lungs have not been firmly established.

Original papers

A total of four original papers were included in the topic. The first paper by Cacciatore et al. analyzed a French cohort regarding acute vs. chronic sarcoid arthropathy. In short, chronic arthropathy tended to be less symmetric, had more wrist involvement, and more often required second- or third-line therapies.

The subsequent exciting investigation by Cameli et al. identified serum and urinary calcium levels as a useful biomarker in sarcoidosis. The investigators compared sarcoidosis patients with idiopathic pulmonary fibrosis and hypersensitivity pneumonitis. Further, receiver operating characteristics curve analysis revealed an excellent specificity

(89.7%) of hypercalciuria for sarcoidosis vs. non-sarcoidosis ILD and a specificity of 82.5% for fibrotic sarcoidosis vs. non-sarcoidosis ILD. This paper highlights the importance of calcium and vitamin D dysregulation in sarcoidosis, which is an area deserving of further investigation.

The following paper by Nickles et al. analyzed similarities between discoid lupus erythematosus (DLE), cutaneous sarcoidosis (CS), and psoriasis by gene co-expression networks. These complex analyses revealed seven common hub genes in DLE and CS: TLR1, ITGAL, TNFRSF1B, CD86, SPI1, BTK, and IL10RA. Furthermore, these analyses also identified several disease-type specific hub genes. These results identified potential molecular targets for treating skin lesions of these diseases.

Lastly, Vagts et al. used unsupervised clustering in a cohort of sarcoidosis patients who had undergone a positron emission tomography (PET) scan as part of their workup. A detailed analysis of clinical, laboratory, and imaging features revealed three clusters (African Americans with quiescent disease, African Americans with chronically active and more frequent extrathoracic disease, and Caucasians with more acute illness). In addition, the data showed a reduction in lymphocyte counts in cluster 3 and high PET avidity in clusters 2 and 3.

These results are relevant since the current recommendations for the diagnosis and detection of sarcoidosis (8) still very much rely on the exclusion of similar disorders. With further research, one goal should be to add confidence in the diagnostic certainty when sarcoidosis is suspected.

Conclusions

Sarcoidosis remains an enigmatic and complicated disease. Within this Research Topic, experts in the field have shared their insights, experience, and research data to deepen our understanding of sarcoidosis. In our view, the agenda for future research includes but is not limited to: (1) what does assist us in making a confident diagnosis of sarcoidosis?, (2) the development of disease clustering criteria for clinical trials, (3) a definition of an applicable response index for measuring outcomes in clinical trials, (4) further explore the role of calcium/vitamin D metabolism in sarcoidosis, and (5) investigate the molecular mechanisms underlying the pathogenesis of the disease. Specialists from all fields, such as pulmonologists, rheumatologists, dermatologists, and neurologists, among many others, should embrace these challenges to improve the diagnosis and treatment of sarcoidosis patients for whom very few therapeutic options are available.

Author contributions

PK conceived the article, wrote the manuscript, and created the figure. NS and MM reviewed and edited the paper. All authors contributed to the article and approved the submitted version.

Acknowledgments

The authors acknowledge the time and effort of all reviewers and guest editors of the included articles who contributed significantly to this Research Topic.

Conflict of interest

Author PK has received honoraria or travel support from Abbvie, Amgen, AstraZeneca, Boehringer Ingelheim, Bristol-Myers-Squibb, Chugai, Galapagos Biopharma, GlaxoSmithKline, Janssen-Cilag, Lilly, Pfizer, and Sanofi-Aventis, all unrelated to this paper. Author PK received research grants from GlaxoSmithKline and Diamed Medizintechnik GmbH, all unrelated to this paper. Author MM reports research awards from Mallinckrodt, advisory membership in Mallinckrodt.

The remaining 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.

Publisher's note

References

1. Srinivasan M, Thangaraj SR, Arzoun H, Govindasamy Kulandaisamy LB, Mohammed L. The association of lung cancer and sarcoidosis: a systematic review. *Cureus.* (2022) 14:e21169. doi: 10.7759/cureus.21169

2. Hena KM. Sarcoidosis epidemiology: race matters. *Front Immunol.* (2020) 11:537382. doi: 10.3389/fimmu.2020.537382

3. Gerke AK, Judson MA, Cozier YC, Culver DA, Koth LL. Disease burden and variability in sarcoidosis. *Ann Am Thorac Soc.* (2017) 14:S421–8. doi: 10.1513/AnnalsATS.201707-564OT

4. Baughman RP, Lower EE. COVID-19 infections in sarcoidosis: a prospective single center study of 886 sarcoidosis patients. *Sarcoidosis Vasc Diffuse Lung Dis.* (2021) 38:e2021029. doi: 10.36141/svdld.v38i2.11646

5. Baughman RP, Valeyre D, Korsten P, Mathioudakis AG, Wuyts WA, Wells A, et al. ERS clinical practice guidelines on treatment of sarcoidosis. *Eur Respir J.* (2021) 2004079. doi: 10.1183/13993003.04079-2020

6. Damsky W, Wang A, Kim DJ, Young BD, Singh K, Murphy MJ, et al. Inhibition of type 1 immunity with tofacitinib is associated with marked improvement in longstanding sarcoidosis. *Nat Commun.* (2022) 13:3140. doi: 10.1038/s41467-022-30615-x

7. Damsky W, Thakral D, Emeagwali N, Galan A, King B. Tofacitinib treatment and molecular analysis of cutaneous sarcoidosis. *N Engl J Med.* (2018) 379:2540–6. doi: 10.1056/NEJMoa1805958

8. Crouser ED, Maier LA, Wilson KC, Bonham CA, Morgenthau AS, Patterson KC, et al. Diagnosis and detection of sarcoidosis. An official american thoracic society clinical practice guideline. *Am J Respir Crit Care Med.* (2020) 201:e26–51. doi: 10.1164/rccm.202002-0251ST

frontiers in Medicine

ORIGINAL RESEARCH
published: 29 October 2020
doi: 10.3389/fmed.2020.568020

Hypercalciuria in Sarcoidosis: A Specific Biomarker With Clinical Utility

Paolo Cameli[1†], Carla Caffarelli[2†], Rosa Metella Refini[1], Laura Bergantini[1], Miriana d'Alessandro[1], Martina Armati[1], Maria Dea Tomai Pitinca[2], Piersante Sestini[1], Stefano Gonnelli[2] and Elena Bargagli[1]*

[1] Respiratory Diseases Unit, Department of Medical, Surgical and Neurosciences Sciences, Siena University Hospital, Siena, Italy, [2] Internal Medicine Unit, Department of Medical, Surgical and Neurosciences Sciences, Siena University Hospital, Siena, Italy

OPEN ACCESS

Edited by:
Peter Korsten,
Nephrology and Rheumatology
University Medical Center
Göttingen, Germany

Reviewed by:
Anabela Barcelos,
New University of Lisbon, Portugal
Federica Meloni,
University of Pavia, Italy
Arthur Yee,
Hospital for Special Surgery,
United States

***Correspondence:**
Paolo Cameli
paolocameli88@gmail.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

Received: 31 May 2020
Accepted: 28 September 2020
Published: 29 October 2020

Citation:
Cameli P, Caffarelli C, Refini RM, Bergantini L, d'Alessandro M, Armati M, Tomai Pitinca MD, Sestini P, Gonnelli S and Bargagli E (2020) Hypercalciuria in Sarcoidosis: A Specific Biomarker With Clinical Utility. Front. Med. 7:568020. doi: 10.3389/fmed.2020.568020

Background: Changes in calcium metabolism are quite common in sarcoidosis: hypercalciuria is linked to a persistent clinical phenotype and more active disease. No data is yet available on the specificity of parameters of calcium metabolism as biomarkers for distinguishing different chronic interstitial lung diseases (ILD). Here we assessed calcium metabolism in an Italian population of sarcoidosis patients, which included a group with stage IV fibrotic disease, and compared the results with those of idiopathic pulmonary fibrosis (IPF) and chronic hypersensitivity pneumonitis (cHP) patients.

Population and Methods: We recruited sarcoidosis, IPF and cHP patients retrospectively. All patients were diagnosed through multidisciplinary discussion and were monitored at the Regional ILD Referral Centre in Siena. Clinical, radiological, functional, immunological and laboratory parameters were collected and entered in an electronic database for data analysis.

Results: A total of 305 patients (237 sarcoidosis, 40 IPF and 28 cHP) were enrolled. Sarcoidosis patients included a predominance of females and were significantly younger than IPF and cHP patients ($p < 0.0001$ for both). In the sarcoidosis population, 17 patients (7.2%) showed radiological evidence of lung fibrosis, according the Scadding classification; fibrotic disease was also confirmed by CT scan. Concerning calcium metabolism, sarcoidosis patients showed significantly higher serum and urinary concentrations of calcium than IPF and cHP patients ($p = 0.0004$ and $p < 0.0001$, respectively). These findings were also confirmed when comparing groups with fibrotic sarcoidosis, IPF and cHP ($p = 0.0237$ and $p = 0.0138$). According to receiver operating characteristics (ROC) curve analysis, urinary calcium showed better diagnostic accuracy than serum calcium in discriminating sarcoid and non-sarcoid lung fibrosis (AUC 0.7658 vs. 0.6205; $p = 0.0026$ vs. $p = 0.1820$).

Discussion: Our results confirmed that changes in calcium metabolism, particularly hypercalciuria, occur in a substantial percentage of patients with sarcoidosis. Higher serum and urinary concentrations of calcium were found than in IPF and cHP; the same results were observed when the comparison was limited to patients with fibrotic

sarcoidosis, supporting the hypothesis that dysregulation of calcium metabolism may be a special feature of sarcoid granulomas. Hypercalciuria distinguished fibrotic sarcoidosis from IPF and cHP, suggesting that assessment of calcium metabolism may be useful in the diagnostic pathway of ILDs.

Keywords: sarcoidosis, calcium metabolism, biomarker, interstitial lung disease, specificity

INTRODUCTION

Sarcoidosis is a systemic disease included in the wide group of interstitial lung diseases (ILDs) that commonly require a multidisciplinary approach for diagnosis and clinical management. Due to its systemic nature, sarcoidosis needs to be assessed by a holistic approach that should include all possible localizations and expressions of disease. Changes in calcium metabolism, including hypercalcemia and hypercalciuria, are quite common (1–5). Their specific assessment is recommended and was recently endorsed by American Thoracic Society guidelines (6). Since 1960's, altered calcitriol production, parathyroid hormone (PTH) activity and sensitivity to Vitamin D have been described in sarcoidosis (7). Increased calcitriol levels cause absorption of calcium in the intestine and resorption from bone. Stimulated by interferon-γ (IFN- γ), tumor necrosis factor- α (TNF-α), interleukin-1 (IL1) and−2 (IL2), macrophages from sarcoid granulomas can spontaneously release 1,25-dihydroxy vitamin D, further boosting calcium resorption from the gastrointestinal tract and bone, leading to hypercalcemia and hypercalciuria (8, 9). Since abnormal resorption is associated with bone fragility and changes in bone mineral density, calcium metabolism is an issue in sarcoidosis, especially in patients requiring prolonged steroid treatment.

Changes in calcium metabolism and subsequent renal involvement (e.g., nephrocalcinosis) have been reported to have a prevalence of 5–60% in sarcoidosis patients (6, 10, 11). It has been clearly demonstrated that hypercalciuria and hypercalcemia are reliable negative prognostic factors, being associated with a chronic-persistent disease phenotype, high angiotensin-converting enzyme (ACE) levels, old age, hypergammaglobulinemia, and extrapulmonary sarcoid localizations (particularly in the spleen, bone and kidneys) (12–14). Our research group recently demonstrated a correlation between hypercalciuria and chitotriosidase concentrations, radiological evidence of severe lung involvement, deterioration of lung function (particularly concerning lung alveolar diffusion) and hepatosplenic disease (15), confirming the potential of this non-invasive and cost-sparing biomarker in routine clinical practice.

Little data is available on bone metabolism in other diffuse ILDs, despite the fact that osteoporosis is a common comorbidity in these patients (estimated prevalence >10%) (16). Moreover, since steroid therapy is the first-line therapy in many "inflammatory" ILDs, such as chronic hypersensitivity pneumonitis (cHP), assessment of bone and calcium metabolism may be useful in the management of these patients. Interestingly, in a cross-sectional study, our research group found a increased bone fragility with higher risk of vertebral fractures, irrespective of steroid therapy, in patients with idiopathic pulmonary fibrosis (IPF), suggesting that fibrotic lung disease *per se* may influence bone status and fracture risk (17).

Sarcoidosis is generally, but unwisely, viewed as a benign disease, although 9% of patients die from respiratory failure, particularly those with stage IV disease. Fibrotic lung sarcoidosis is observed in 5–15% of patients at presentation and is associated with poorer survival (18, 19). Isolated pulmonary stage IV sarcoidosis is a diagnostic challenge: differential diagnosis with respect to cHP and pneumoconiosis can be difficult due to similar clinical, immunological and radiological features in the end stage (20).

In the present study, we focused on changes in calcium metabolism in patients with granulomatous and non-granulomatous ILD, in order to evaluate their specificity as biomarkers in sarcoidosis and the clinical utility of differentiating stage IV sarcoidosis from other ILDs.

MATERIALS AND METHODS

Study Population and Design

We recruited sarcoidosis, IPF and cHP patients retrospectively from the population monitored at the Siena Regional ILD Referral Center. Diagnosis was made according to international guidelines (6, 21); all patients underwent chest high resolution computed tomography (HRCT) for diagnostic purposes. All diagnoses were confirmed by multidisciplinary discussion: histological confirmation was available for 147 patients with sarcoidosis, six with IPF and four with cHP. For those patients in which histological sampling was not available or obtainable, diagnosis of sarcoidosis was made according to a multidisciplinary evaluation of clinical and radiological features, in order to exclude potential alternative diseases, as recently endorsed by American Thoracic Society guidelines (6). Sarcoid patients were also classified according to disease localization, as suggested by Genotype-Phenotype Relationship in Sarcoidosis project (GenPhenResA) (22). All patients underwent regular clinical evaluations at the Siena Referral Center for Osteoporosis. To be included in the study, patients had to provide serum and 24-h urine samples for assessment of calcium metabolism. Patients were specifically trained in 24-h urine collection. All patients were carefully evaluated in order to exclude potential

Abbreviations: DL_{co}, diffusing capacity of the lung for CO; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; IPF, idiopathic pulmonary fibrosis, cHP, chronic hypersensitivity pneumonitis; HRCT, high resolution computed tomography.

comorbidities that may significantly influence serum and urinary biomarkers of calcium metabolism.

Patients were excluded if they were taking calcium or vitamin D supplements or drugs for osteoporosis. Demographic, radiological, immunological and functional data was collected from the medical records and entered in an electronic database.

Serum samples were also obtained from the sarcoidosis cohort to measure the disease-specific biomarkers chitotriosidase, and ACE.

All patients gave their informed consent to the study that was approved by the local Ethic committee. The study was conducted according to Declaration of Helsinki principles.

TABLE 1 | Demographic features, functional parameters, radiological classification, and serum biomarkers' assessment of study population.

	Sarcoidosis	IPF	cHP	*p*-value
N°	236	40	28	
Male (%)	92 (38.9)	33 (82.5)	17 (60.7)	<0.0001
Age (yrs)	56.1 ± 12.1	68 ± 8.4	66.8 ± 8.7	<0.0001
Smoking history				
Former (%)	85 (36)	34 (85)	15 (53.5)	<0.0001
Never (%)	151 (63.9)	6 (15)	13 (46.4)	<0.0001
PFTs				
FVC l (%)	3.5 ± 1 (106.7 ± 17.8)	2.6 ± 0.9 (73.3 ± 27.2)	2.4 ± 0.9 (75.7 ± 17.2)	<0.0001 <0.0001
FEV1 l (%)	2.6 ± 0.9 (98.3±18.4)	2 ± 0.7 (76.4 ± 23.5)	1.9 ± 0.8 (76.2 ± 20.9)	<0.0001 <0.0001
FEV1/FVC	75.6 ± 7.4	78.8 ± 7.2	80.4 ± 10.4	0.0003*
DLCO %	80.1 ± 15.1	46.3±17.1	54.2±18.2	<0.0001
GenPhenResA phenotypes				
Abdominal (%)	17 (7.2)			
OCCC (%)	10 (4.2)			
Musculoskeletalcutaneous (%)	31 (13.1)			
Isolated pulmonary (%)	170 (72)			
Extrapulmonary (%)	8 (3.3)			
Radiological assessment¶				
CXR stage 0 (%)	99 (41.9)			
CXR stage 1 (%)	19 (8)			
CXR stage 2 (%)	51 (21.6)			
CXR stage 3 (%)	50 (21.1)			
CXR stage 4 (%)	17 (7.2)			
Sarcoidosis biomarkers				
Chitotriosidase (nmol/ml/h) [1–45 nmol/ml/h]	174.1 ± 90.6			
ACE (U/l) [30–80 U/l]	56.6 ± 22.8			

*If not otherwise reported, p-values referred to comparison between sarcoidosis and all other subgroups. ¶according to Scadding classification; *statistically significant difference between sarcoidosis and cHP patients; IPF, idiopathic pulmonary fibrosis; cHP, chronic hypersensitivity pneumonitis; PFTs, pulmonary function tests; FVC, forced volume capacity; FEV1, forced expiratory volume in the 1st s; DLCO, lung diffusion capacity for carbon monoxide; GenPhenResA, Genotype-Phenotype Relationship in Sarcoidosis; OCCC, ocular-cardiac-cutaneous-central nervous system; CXR, chest x-rays; AC, angiotensin-converting enzyme. Normal ranges of laboratory values are reported in square brackets.*

Lung Function Tests

The following lung function measurements were recorded according to American Thoracic Society/European Respiratory Society (ATS/ERS) standards (23, 24), using a Jaeger body plethysmograph with corrections for temperature and barometric pressure: forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), FEV1/FVC, total lung capacity (TLC), residual volume (RV), lung diffusion capacity for carbon monoxide (DLCO) and DLCO/VA (alveolar volume).

Chitotriosidase Assay

Human chitotriosidase activity was determined by a fluorimetric method using 22 μM 4-methylumbelliferyl β D-NNN-triacetylchitotriosidase (Sigma Chemical Co.) in citrate-phosphate buffer, pH 5.2; 100 μl substrate was incubated for 1 h at 37°C and the reaction was stopped with 1.4 ml 0.1 M glycine-NaOH buffer, pH 10.8. Fluorescence was read at 450 nm with a Perkin Elmer Victor X4 fluorimeter (excitation wavelength 365 nm). Serum chitotriosidase concentrations were expressed in mg/ml (normal values 1-44 mg/ml).

Angiotensin Converting Enzyme Assay

ACE activity was measured previously described by a colorimetric method (FAR kit, FAR srl, Verona, Italy), widely used to determine ACE activity in serum, urine and tissues, as (16). The normal range of ACE concentrations is 30–80 U/l.

Assessment of Calcium Metabolism

Serum concentrations of calcium (corrected for albumin), phosphate, total alkaline phosphatase and creatinine were

TABLE 2 | Calcium metabolism on serum and 24-h urinary sampling in sarcoidosis, IPF and cHP subgroups.

Parameters	Sarcoidosis	IPF	cHP	*p*-value
Serum				
Calcium (mg/dl) [8.5–10.5 mg/dl)	9.58 ± 0.46	9.17 ± 0.51	9.29 ± 0.33	0.0004
Phosphate (mg/dl) [2.5–4.5 mg/dl]	3.42 ± 0.5	3.35 ± 0.42	3.57 ± 0.64	0.0957
Creatinine (mg/dl) [0.5–1.2 mg/dl]	0.94 ± 0.15	0.98 ± 0.13	0.98 ± 0.08	0.1265
Creatinine clearance (ml/min) [> 80 ml/min]	87.1 ± 40.3	88.5 ± 48.3	84.7 ± 35.9	0.8278
Alkaline phosphatase (U/l) [30–120 U/l]	66.1 ± 27.5	65 ± 19.3	58.8 ± 17.7	0.7145
24 h-urine				
Calcium (mg/24 h) [50–250 mg/24 h]	176.6 ± 120.1	111.4 ± 81.7	114 ± 53.4	0.0007
Phosphate (mg/24 h) [300–800 mg/24 h]	715.3 ± 307.4	758.7 ± 312.1	655.7 ± 312.1	0.1138
Creatinine (mg/24 h) [800–1,200 mg/24 h]	1,166.8 ± 506.7	1,219.1 ± 582.8	1,194.8 ± 501.4	0.7992

IPF, idiopathic pulmonary fibrosis; cHP, chronic hypersensitivity pneumonitis. Normal ranges of values are reported in square brackets. P-value refers to sarcoidosis patients vs. all others.

measured using standard automated laboratory techniques. Urinary calcium, phosphate and creatinine were determined by a colorimetric method (Cobas C311 analyser, Roche Diagnostics, USA) in 24-h urine samples. Serum PTH was assessed by immunoradiometric assay (DiaSorin, Saluggia, Italy).

Statistical Analysis

Data was expressed as mean ± standard deviations. Study variables were tested for normal distribution. The Mann-Whitney test or *t*-test were used for group comparisons on the basis of normality of data. The Kruskall-Wallis test was used to compare more than two groups. The Spearman test was used to find correlations. The analysis was run in GraphPad version 5.0.

RESULTS

Clinical, Functional and Radiological Features

A total of 304 patients (236 sarcoidosis, 40 IPF and 28 cHP) were enrolled retrospectively in the study. Demographic, clinical, radiological and immunological data and functional parameters are reported in **Table 1**. As expected, sarcoidosis patients were younger, prevalently female and non-smokers, compared with IPF and cHP patients ($p < 0.0001$ for all comparisons). Concerning respiratory function, sarcoidosis patients showed normal lung volumes and diffusion capacity, while we observed mild restrictive impairment associated with moderate reduction in DLCO in IPF and cHP patients. Regarding radiological assessment, 17 sarcoidosis patients (7.2%) showed fibrotic lung disease, confirmed to be stage IV sarcoidosis by chest X-ray and HRCT. Despite their inclusion in stage 0 of disease according to Scadding classification, 91/99 sarcoidosis patients reported typical features of sarcoid lung involvement at HRCT; thus, only 8 patients showed (3.3%) an extrapulmonary disease phenotype, according to GenPhenResA assessment.

Assessment of Calcium Metabolism

Table 2 shows the serum and urinary parameters of calcium metabolism measured in our center. We observed significantly higher levels of urinary calcium in sarcoidosis than in IPF and cHP patients ($p = 0.0007$) (**Figure 1**). This statistical difference remained significant when the comparison was limited to fibrotic stage IV sarcoidosis patients ($p = 0.0138$). Similarly, sarcoidosis patients showed significantly higher serum concentrations of calcium (whole population $p = 0.0004$; fibrotic group $p = 0.0237$).

ROC analysis was performed in order to evaluate the accuracy of serum and urinary calcium in discriminating sarcoidosis from IPF and cHP. Of the two biomarkers, urinary calcium showed better performance (AUC 0.7368, 95% CI 0.6573–0.8164, $p < 0.0001$ vs. AUC 0.6195, 95% CI 0.5090–0.7300, $p = 0.01756$), with a sensitivity of 49.5% and a specificity of 89.7% for a cut-off value of 176.5 mg/24 h (likelihood ratio 4.67). Comparing stage IV sarcoidosis patients with IPF and cHP patients, urinary calcium was confirmed to have a moderate-to-good accuracy, significantly better than serum calcium (AUC 0.7708, 95% CI 0.6284–0.9133, $p = 0.0021$ vs. AUC 0.6205, 95% CI 0.4558–0.7853, $p = 0.1828$) (**Figure 2**).

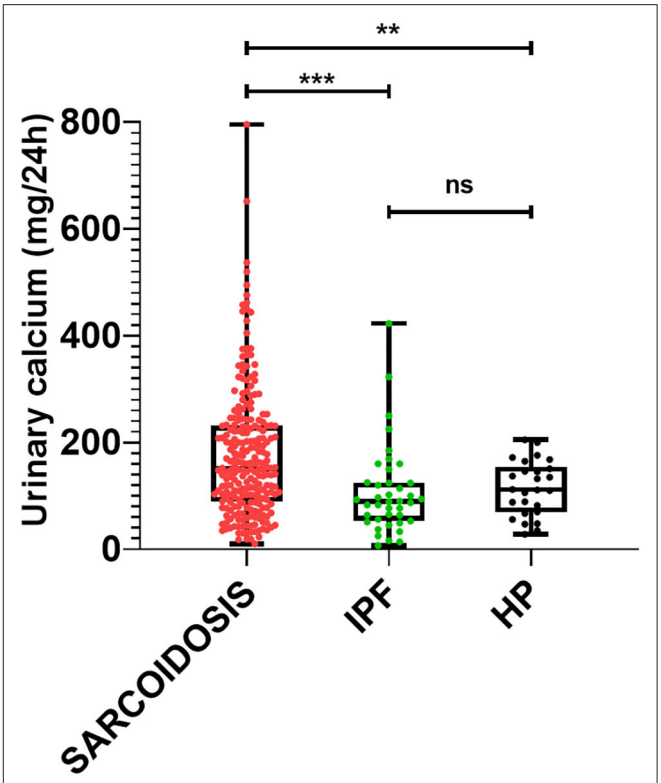


FIGURE 1 | Comparison of 24-h urinary calcium concentrations among patients with sarcoidosis, IPF and cHP. Ns: not significant; ***$p = 0.0007$; **$p = 0.003$.

No significant differences of serum and urinary parameters of calcium metabolism were found among different clinical phenotypes or radiological stages in the sarcoidosis group (**Figure 3**).

Correlations

We observed a significant direct correlation between urinary calcium and chitotriosidase activity in patients with sarcoidosis ($r = 0.2564$, $p = 0.009$), but not with ACE ($r = 0.0938$, $p = 0.1535$). Urinary calcium was also inversely correlated with DLCO ($r = -0.1428$, $p = 0.0373$) in patients with sarcoidosis, but not in IPF and cHP patients ($p = 0.3893$, and $p = 0.8091$).

DISCUSSION

The aim of the present study was to evaluate the potential of parameters of calcium metabolism in the diagnostic algorithm of diffuse ILDs. Hypercalciuria and hypercalcemia are quite common anomalies in sarcoidosis patients and need to be addressed to prevent severe complications or chronic organ failure (e.g., ventricular arrhythmia, nephrolithiasis, nephrocalcinosis, chronic renal failure). The clinical features of calcium dysregulation in sarcoidosis have been repeatedly

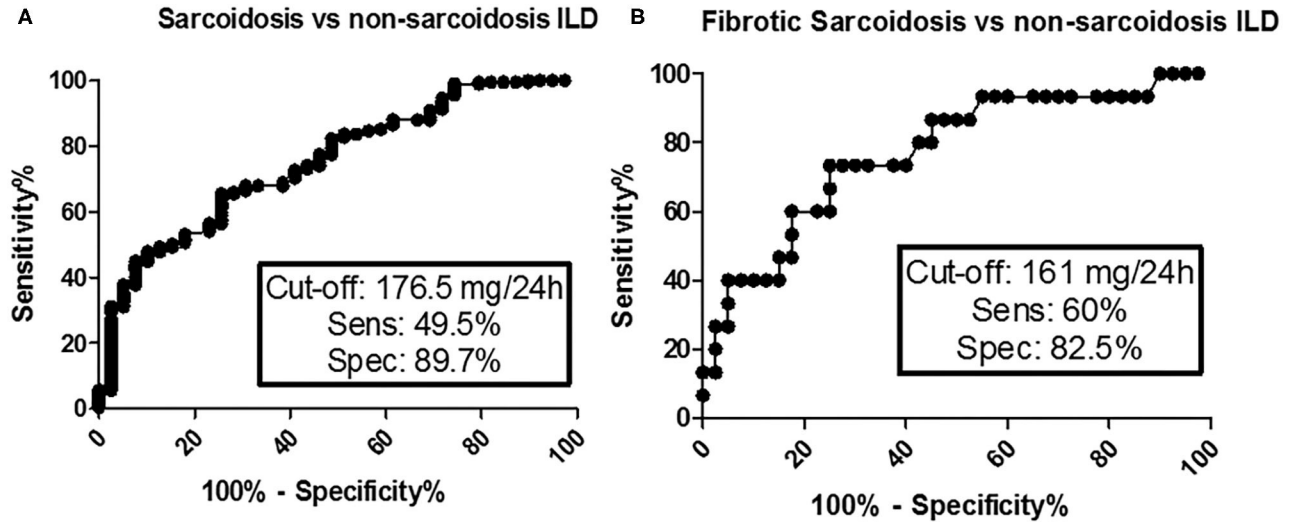


FIGURE 2 | ROC analysis for diagnostic accuracy of urinary calcium between sarcoidosis and non-sarcoidosis ILD **(A)** and between fibrotic sarcoidosis and non-sarcoidosis ILD **(B)**.

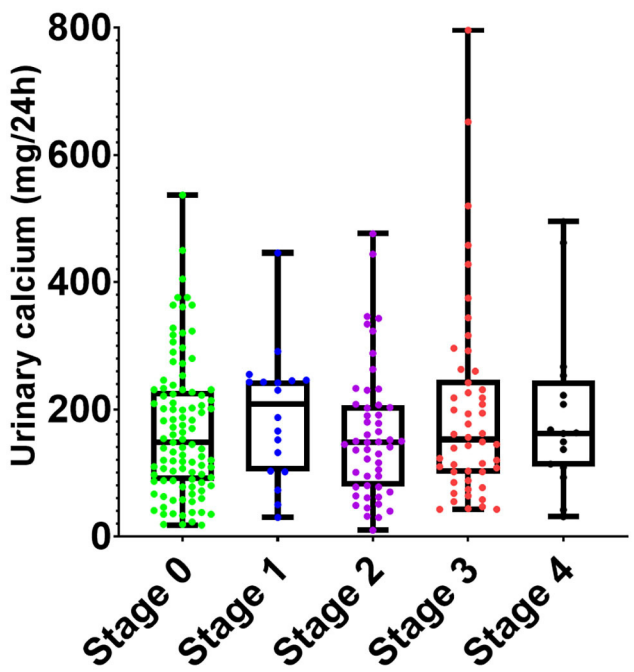


FIGURE 3 | Comparison of 24-h urinary calcium concentrations among radiological stages of sarcoidosis. No statistically significant differences of urinary calcium concentration were observed among the subgroups.

described (2, 15, 25), as well as its underlying pathophysiological processes: however, there are still many concerns about how it should be treated and monitored (26). The guidelines published recently by the ATS endorsed the utility of assessing calcium metabolism in sarcoidosis, recommending serum calcium testing at diagnosis and follow-up to screen for specific alterations (6).

Our results showed that urinary and serum calcium are both significantly higher in sarcoidosis than in IPF and cHP. Twenty-four hour urinary calcium (not serum calcium assay) showed promising accuracy in discriminating sarcoidosis from the other two ILDs. To our knowledge, the specificity of hypercalcemia or hypercalciuria in the setting of ILDs has never been researched, and no studies have ever investigated the potential of alterations in calcium metabolism as an indicator of non-sarcoid lung fibrosis. Sarcoid-related calcium alterations are possibly determined by overexpression of 1-alpha-hydroxylase and parathyroid hormone-related proteins by granulomatous macrophages (26–28), but it is not known whether this dysregulation is specific to sarcoidosis or also occurs in other granulomatous ILDs, such as cHP. Since no differences were observed between IPF and cHP patients, our results suggest that changes in calcium metabolism may be related to sarcoid granuloma activity. These assumptions are also supported by the significant correlation between urinary calcium and chitotriosidase, a macrophage-derived chitinase, specifically linked to sarcoidosis activity and severity. This correlation was previously reported by our research group and is probably determined by the aberrant activation of macrophages in sarcoid granulomas (12). Our results therefore confirm that urinary calcium is related to disease activity, although it appeared to be less sensitive than chitotriosidase, if compared with the studies available in literature (12, 29, 30). It is still unknown whether specific patterns of macrophage activation, linked to different cytokine overexpression patterns, may lead to different clinical features or disease phenotypes. However, another finding of the present study, in line with previous reports, was that urinary calcium is also inversely correlated with DLCO percentages (15). As DLCO was substantially normal in our sarcoidosis cohort, these findings suggest that urinary calcium could be useful as an early indicator of a chronic-progressive

sarcoid phenotype, leading to lung fibrosis. These results are interesting and worthy of further research in large prospective cohorts.

The accuracy of urinary calcium for differential diagnosis was also confirmed when the comparison was limited to stage IV fibrotic sarcoidosis and IPF-cHP subgroups. Fibrotic sarcoidosis may have clinical onset and progression indistinguishable from IPF and cHP, with restrictive functional impairment and similar HRCT features in many patients. Stage IV sarcoidosis, like HP, is regarded as a "great mimicker" and may therefore be a diagnostic challenge in the differential diagnosis of ILDs (20, 31). Since no biomarkers have yet been approved to distinguish these ILDs (32), changes in calcium metabolism may be suggested as a bioindicator specific to sarcoidosis among ILDs. Our findings suggest that urinary calcium assessment may be useful to discriminate end-stage sarcoidosis from other fibrotic ILDs and as a biomarker in a multidisciplinary setting. It has the advantage of being simple, non-invasive and economical.

Our study has some limitations: first, the sample size, though relevant for rare diseases such as sarcoidosis, IPF and cHP, is not sufficient to properly assess the reliability of our results, as well as the monocentric nature of the study. Second, the retrospective design is intrinsically prone to referral and reporting bias, that may significantly influence the analysis and the interpretation of data. Third, due to the lack of data contemporary to urinary sampling, we didn't include in the analysis serum 25-OH and 1, 25-OH vitamin D concentrations. Considering the prominent role of vitamin D in calcium metabolism, future and prospective studies will address the concentration of 25-OH and 1-25-OH forms to further clarify their potential value on this field.

In conclusion, in this study we found a significant increase in serum and urinary concentrations of calcium in sarcoidosis patients with respect to IPF and cHP patients. The finding sustains the specificity of changes in calcium metabolism in this granulomatous lung disease. Urinary calcium revealed good specificity for fibrotic (stage IV) sarcoidosis, suggesting that it has potential as a biomarker for the differential diagnosis of ILDs and in the estimation of sarcoid disease activity.

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 Comitato Etico Area Vasta Sud Est (C.E.A.V.S.E.). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PC, CC, RR, SG, and EB: conception, study design, interpretation of results, and writing of the manuscript. LB, MT, MA, and Md'A: data acquisition and analysis, revision of the study, and interpretation of results. PC, CC, and PS: statistical analysis and revision of the study. All authors approved the final version of the study and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Baughman RP, Teirstein AS, Judson MA, Rossman MD, Yeager H, Bresnitz EA, et al. Clinical characteristics of patients in a case control study of sarcoidosis. *Am J Respir Crit Care Med.* (2001) 164:1885–9. doi: 10.1164/ajrccm.164.10.2104046
2. Baughman R, Janovcik J, Ray M, Sweiss N, Lower E. Calcium and vitamin D metabolism in sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* (2013) 30:113–20. Available online at: https://www.mattioli1885journals.com/index.php/sarcoidosis/article/view/3022
3. RuŽa I, Lucāne Z. Serum and urinary calcium level in Latvian patients with sarcoidosis. *Reumatologia.* (2018) 56:377–81. doi: 10.5114/reum.2018.80715
4. Bickett AN, Lower EE, Baughman RP. Sarcoidosis diagnostic score: a systematic evaluation to enhance the diagnosis of sarcoidosis. *Chest.* (2018) 154:1052–60. doi: 10.1016/j.chest.2018.05.003
5. Rizzato G, Colombo P. Nephrolithiasis as a presenting feature of chronic sarcoidosis: a pro-spective study. *Sarcoidosis Vasc Diffuse Lung Dis.* (1996) 13:167–72.
6. Crouser ED, Maier LA, Wilson KC, Bonham CA, Morgenthau AS, Patterson KC, et al. Di-agnosis and detection of sarcoidosis. An official american thoracic society clinical practice guideline. *Am J Respir Crit Care Med.* (2020) 201:e26–51. doi: 10.1164/rccm.202002-0251ST
7. Mayock RL, Bertrand P, Morrison CE, Scott JH. Manifestations of sarcoidosis analysis of 145 patients, with a review of nine series selected from the literature. *Am J Med.* (1963) 35:67–89. doi: 10.1016/0002-9343(63)90165-7
8. Koeffler HP, Reichel H, Bishop JE, Norman AW. gamma-Interferon stimulates production of 1,25-dihydroxyvitamin D3 by normal human macrophages. *Biochem Biophys Res Commun.* (1985) 127:596–603. doi: 10.1016/S0006-291X(85)80202-3
9. Sexton DJ, O'Reilly MW, Geoghegan P, Kinsella S, Moran P, O'Regan A. Serum fibroblastic growth factor 23 in acute Sarcoidosis and normal kidney function. *Sarcoidosis Vasc Diffuse Lung Dis.* (2016) 33:139–42. Available online at: https://www.mattioli1885journals.com/index.php/sarcoidosis/article/view/4049
10. Sharma OP. Hypercalcemia in granulomatous disorders: a clinical review. *Curr Opin Pulm Med.* (2000) 6:442–7. doi: 10.1097/00063198-200009000-00010
11. Rottoli P, Rottoli L, Gommelli S, Zacchei F, Coviello G, Piccolo L, et al. Abnormalities in calcium metabolism in sarcoidosis. *Sarcoidosis.* (1991) 8:180–1.
12. Bennett D, Cameli P, Lanzarone N, Carobene L, Bianchi N, Fui A, et al. Chitotriosidase: a biomarker of activity and severity in patients with sarcoidosis. *Respir Res.* (2020) 21:6. doi: 10.1186/s12931-020-1303-8
13. Rizzato G. Clinical impact of bone and calcium metabolism changes in sarcoidosis. *Thorax.* (1998) 53:425–9. doi: 10.1136/thx.53.5.425
14. Doubková M, Pospíšil Z, Skričková J, Doubek M. Prognostic markers of sarcoidosis: an analysis of patients from everyday pneumological practice. *Clin Respir J.* (2015) 9:443–9. doi: 10.1111/crj.12160
15. Cameli P, Gonnelli S, Bargagli E, d'Alessandro M, Bergantini L, Favetta V, et al. The role of urinary calcium and chitotriosidase in a cohort of chronic sarcoidosis patients. *Respiration.* (2020) 99:207–12. doi: 10.1159/000505653

16. Caplan-Shaw CE, Arcasoy SM, Shane E, Lederer DJ, Wilt JS, O'Shea MK, et al. Osteopo-rosis in diffuse parenchymal lung disease. *Chest.* (2006) 129:140–6. doi: 10.1378/chest.129.1.140
17. Caffarelli C, Gonnelli S, Tomai Pitinca MD, Francolini V, Fui A, Bargagli E, et al. Idiopathic pulmonary fibrosis a rare disease with severe bone fragility. *Intern Emerg Med.* (2016) 11:1087–94. doi: 10.1007/s11739-016-1501-z
18. Valeyre D, Prasse A, Nunes H, Uzunhan Y, Brillet P-Y, Müller-Quernheim J. Sarcoidosis. *Lancet.* (2014) 383:1155–67. doi: 10.1016/S0140-6736(13)60680-7
19. Patterson KC, Strek ME. Pulmonary fibrosis in sarcoidosis. Clinical features and outcomes. *Ann Am Thorac Soc.* (2013) 10:362–70. doi: 10.1513/AnnalsATS.201303-069FR
20. Cottin V, Hirani NA, Hotchkin DL, Nambiar AM, Ogura T, Otaola M, et al. Presentation, diagnosis and clinical course of the spectrum of progressive-fibrosing interstitial lung diseases. *Eur Respir Rev.* (2018) 27:180076. doi: 10.1183/16000617.0076-2018
21. Costabel U, Hunninghake GW. ATS/ERS/WASOG statement on sarcoidosis. Sarcoidosis statement committee. American thoracic society. European respiratory society. World associa-tion for sarcoidosis and other granulomatous disorders. *Eur Respir J.* (1999) 14:735–7. doi: 10.1034/j.1399-3003.1999.14d02.x
22. Schupp JC, Freitag-Wolf S, Bargagli E, Mihailović-Vučinić V, Rottoli P, Grubanovic A, et al. Phenotypes of organ involvement in sarcoidosis. *Eur Respir J.* (2018) 51:1700991. doi: 10.1183/13993003.00991-2017
23. Miller MR, Crapo R, Hankinson J, Brusasco V, Burgos F, Casaburi R, et al. General consid-erations for lung function testing. *Eur Respir J.* (2005) 26:153–61. doi: 10.1183/09031936.05.00034505
24. Graham BL, Brusasco V, Burgos F, Cooper BG, Jensen R, Kendrick A, et al. 2017 ERS/ATS standards for single-breath carbon monoxide uptake in the lung. *Eur Respir J.* (2017) 49:1600016. doi: 10.1183/13993003.00016-2016
25. Baughman RP, Papanikolaou I. Current concepts regarding calcium metabolism and bone health in sarcoidosis. *Curr Opin Pulm Med.* (2017) 23:476–81. doi: 10.1097/MCP.0000000000000400
26. Gwadera Ł, Białas AJ, Iwański MA, Górski P, Piotrowski WJ. Sarcoidosis and calcium ho-meostasis disturbances-Do we know where we stand? *Chron Respir Dis.* (2019) 16:1479973119878713. doi: 10.1177/1479973119878713
27. Saidenberg-Kermanac'h N, Semerano L, Nunes H, Sadoun D, Guillot X, Boubaya M, et al. Bone fragility in sarcoidosis and relationships with calcium metabolism disorders: a cross sectional study on 142 patients. *Arthritis Res Ther.* (2014) 16:R78. doi: 10.1186/ar4519
28. Zeimer HJ, Greenaway TM, Slavin J, Hards DK, Zhou H, Doery JC, et al. Parathyroid-hormone-related protein in sarcoidosis. *Am J Pathol.* (1998) 152:17–21.
29. Bargagli E, Margollicci M, Perrone A, Luddi A, Perari MG, Bianchi N, et al. Chitotriosidase analysis in bronchoalveolar lavage of patients with sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* (2007) 24:59–64. doi: 10.1007/s11083-007-9059-z
30. Bargagli E, Maggiorelli C, Rottoli P. Human chitotriosidase: a potential new marker of sar-coidosis severity. *Respiration.* (2008) 76:234–8. doi: 10.1159/000134009
31. Salvatore M, Ishikawa G, Padilla M. Is it idiopathic pulmonary fibrosis or not? *J Am Board Fam Med.* (2018) 31:151–62. doi: 10.3122/jabfm.2018.01.170288
32. Raghu G, Remy-Jardin M, Myers JL, Richeldi L, Ryerson CJ, Lederer DJ, et al. Diagnosis of idiopathic pulmonary fibrosis. An official ATS/ERS/JRS/ALAT clinical practice guideline. *Am J Respir Crit Care Med.* (2018) 198:e44–68. doi: 10.1164/rccm.201807-1255ST

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.

frontiers in Medicine

CASE REPORT
published: 04 November 2020
doi: 10.3389/fmed.2020.588527

Case Series: COVID-19 in African American Patients With Sarcoidosis

Michael Manansala[1], Christian Ascoli[1], Ana Goico Alburquerque[1], David Perkins[1], Mehdi Mirsaedi[2], Patricia Finn[1] and Nadera J. Sweiss[1]*

[1] Department of Internal Medicine, University of Illinois at Chicago, Chicago, IL, United States, [2] Miller School of Medicine, University of Miami, Miami, FL, United States

Data on the clinical presentation and outcomes of sarcoidosis patients with coronavirus disease 19 (COVID-19) are scarce. In this case series, we identified 5 out of 238 sarcoidosis patients who are enrolled in an ongoing longitudinal observational study who developed COVID-19 during the study period and follow their clinical course. Four patients recovered completely, whereas one patient expired during hospital admission. Our preliminary experience suggests that African American patients with chronic sarcoidosis treated with disease-modifying anti-rheumatic drugs (DMARDs) or anti-tumor necrosis factor (TNF) therapy do not seem to be at increased risk of respiratory or life-threatening complications from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) compared with the general population, although at the present time, we advocate for maintaining a high level of vigilance and strict follow-up in this patient population.

Keywords: sarcoidosis, COVID- 19, immunosuppression, DMARDs, African American (AA), SAR-CoV-2

OPEN ACCESS

Edited by:
Xinhua Yu,
Research Center Borstel (LG), Germany

Reviewed by:
Jing Yuan,
Children's Hospital of Capital Institute of Pediatrics, China
Su-Boon Yong,
Show Chwan Memorial Hospital, Taiwan

****Correspondence:***
Michael Manansala
mmanan3@uic.edu

Specialty section:
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

Received: *29 July 2020*
Accepted: *05 October 2020*
Published: *04 November 2020*

Citation:
Manansala M, Ascoli C, Alburquerque AG, Perkins D, Mirsaedi M, Finn P and Sweiss NJ (2020) Case Series: COVID-19 in African American Patients With Sarcoidosis. Front. Med. 7:588527. doi: 10.3389/fmed.2020.588527

INTRODUCTION

The recent outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) responsible for severe acute respiratory syndrome (SARS) represents a source of concern for the management of patients with sarcoidosis (1). Within the realm of sarcoidosis, it has been shown that African Americans have 12 times the rate of age-adjusted mortality compared with similar Caucasian patients (2). This is compounded by the observation that African American patients are at disproportionately increased risk of mortality and morbidity from coronavirus disease 19 (COVID-19) (3). Data on COVID-19 in patients with sarcoidosis are scarce. Additionally, there is a concern that immunocompromised patients are at increased risk of mortality from COVID-19. Here, we report a case series describing the clinical course of five African American patients with sarcoidosis after infection with SARS-CoV-2.

METHODS

We assessed patients with sarcoidosis care established at the University of Illinois of Chicago Bernie Mac STAR Clinic who are enrolled in an ongoing longitudinal observational study for SARS-CoV-2 infection during the period of March 12 to April 30, 2020. We identified five out of 238 patients (2.1%) with confirmed SARS-CoV-2 infection by PCR and clinical symptoms consistent with COVID-19 disease. Demographic and clinical data were collected.

TABLE 1 | Clinical characteristics of five African American sarcoidosis patients with confirmed COVID-19.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age	45	62	50	48	46
Gender	M	F	M	F	M
Sarcoidosis clinical phenotype requiring treatment	Pulmonary	Advanced pulmonary	Ocular cardiac	Neurologic	Testicular
BMI	28	28	31	46	43
Comorbidities	Asthma	Pulmonary hypertension	Uncontrolled hypertension, uncontrolled diabetes	Uncontrolled hypertension	Uncontrolled hypertension
Smoking history	None	None	Active smoker	None	Active smoker
Sarcoid medications at the time of presentation	Methylprednisolone 8 mg daily	MTX 10 mg weekly, HCQ 200 mg daily, methylprednisolone 4 mg daily	None	None	Infliximab every 8 weeks, MTX 7.5 mg weekly

BMI, body mass index; MTX, methotrexate; HCQ, hydroxychloroquine.

TABLE 2 | Clinical data for five African American sarcoidosis patients with confirmed COVID-19.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
COVID-19 symptoms	Dyspnea on exertion, cough	Cough, anosmia, dysgeusia, myalgia	Cough, shortness of breath, fever, myalgias, diarrhea	Cough, fever	Diarrhea
Symptom duration prior to presentation (days)	2	7	4	7	10
Relevant laboratories prior to presentation					
WBC (3.9–12 thou/μl)	3.8	7.2	3.9	6.7	3.9
Abs lymphocytes (1.3–4.2 thou/μl)	1.7	1.7	1.3	1.9	1.8
Abs CD4 (438–1,501 cells/mm^3)	696	1,107	–	1,501	833
CRP (0–18 mg/L)	<1.0	6.9	6.8	4.3	4.9
ESR (0–10 mm/h)	8	19	16	64	21
Relevant laboratories at the time of presentation					
WBC (3.9–12 thou/μl)	4.5	–	3.1	–	2.8
Abs lymphocytes (1.3–4.2 thou/μl)	1.4	–	0.9	–	1.2
Abs CD4 (438–1,501 cells/mm^3)	–	–	152	–	420
LDH (90–180 U/L)	273	–	270	–	–
Ferritin (10–259 ng/ml)	152	–	730	–	–
CRP (0–18 mg/L)	1	–	62.5	–	10.3
ESR (0–10 mm/h)	–	–	40	–	55
COVID-19 treatment	None	HCQ, azithromycin	HCQ, azithromycin, hydrocortisone, tocilizumab	HCQ, azithromycin, prednisone	None
Chest X-ray at the initial COVID-19 evaluation	No acute cardiopulmonary process	Diffuse advanced interstitial lung disease	Bibasilar reticular infiltrates, later progressed to bilateral pulmonary opacities consistent with ARDS	Bibasilar atelectasis, bilateral pulmonary opacities	No acute cardiopulmonary process
Outcome	Discharged to home from ED, doing well at 2-week follow-up	Did not require hospitalization, complete recovery at 2-week follow-up	Death due to pulmonary embolism after prolonged hospitalization requiring ICU admission	Discharged from the ICU, doing well at 2-week follow-up	Did not require hospitalization, complete recovery at 2-week follow-up

Available relevant laboratory data, treatment, and outcome at follow-up are presented.
WBC, white blood cell; Abs, absolute; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; ED, emergency department; HCQ, hydroxychloroquine; ARDS, acute respiratory distress syndrome; ICU, intensive care unit.

CASE PRESENTATION

We identified 5 out of 238 sarcoidosis patients who were infected with SARS-Cov-2 during the study period. All patients were of African American descent. The most common presenting symptom was cough. One patient had an atypical presentation of gastrointestinal discomfort and diarrhea. Four patients recovered completely despite having comorbidities and being on chronic immunosuppression. Two of the five patients did not receive any additional treatment for COVID-19. Three of the five patients received hydroxychloroquine (HCQ) and azithromycin for treatment for COVID-19. No changes were made to the patients' current immunosuppressive regimen. They also did not experience significant relapses of sarcoidosis from the time of COVID-19 diagnosis to date. One patient died after developing a likely thromboembolic event during hospitalization in the intensive care unit (ICU). Additional clinical characteristics of these patients are summarized in **Table 1**. The clinical data, including symptoms, laboratory data, and outcomes, are included in **Table 2**.

DISCUSSION

The case fatality rate of COVID-19 is estimated to be 1–6% in the general population (4). Currently, the Center for Disease Control and Prevention (CDC) lists several risk factors for severe COVID-19, including immunocompromised status (5). However, preliminary data from an observational study of 320 Italian patients on immunosuppressive therapy for rheumatoid arthritis did not show increased risk of respiratory or life-threatening complications from SARS-CoV-2 compared with the general population (6). Indeed, it has been postulated that the pathogenesis of severe COVID-19 disease is in large part due to virally driven hyperinflammation that is perpetuated by the host immune response (7–9). Analysis from a cohort of COVID-19 cases from Wuhan, China revealed that patients requiring ICU had increased levels of pro-inflammatory cytokines (10).

Our findings do not provide any conclusions on the incidence rate of SARS-CoV-2 infection in patients with sarcoidosis, nor on the severity or overall outcome of immunocompromised sarcoidosis patients affected by COVID-19 disease. However, our preliminary experience suggests that African American patients with chronic sarcoidosis treated with disease-modifying anti-rheumatic drugs (DMARDs) or anti-tumor necrosis factor (TNF) therapy do not seem to be at increased risk of respiratory or life-threatening complications from SARS-CoV-2 compared with the general population. Better understanding of the implications of COVID-19 in patients with sarcoidosis and the effects of immunosuppressive therapies on COVID-19 infection outcome is urgently needed to guide clinicians in patient care. At the present time, we advocate for maintaining a high level of vigilance and strict follow-up in this patient population, including the exclusion of superimposed infections.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

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

NS identified patients of interest within the cohort. MMa wrote the manuscript with assistance from CA, AA, DP, PF, and MMi. All authors contributed to the article and approved the submitted version.

FUNDING

Funding for publication of this article was provided by the Bernie Mac Foundation STAR Clinic.

REFERENCES

1. Sweiss NJ, Korsten P, Syed HJ, Syed A, Baughman RP, Yee AMF, et al. When the game changes: Guidance to adjust sarcoidosis management during the COVID-19 pandemic. *Chest*. (2020) 158:892–5. doi: 10.1016/j.chest.2020.04.033
2. Mirsaeidi M, Machado RF, Schraufnagel D, Sweiss NJ, Baughman RP. Racial difference in sarcoidosis mortality in the United States. *Chest*. (2015) 147:438–49. doi: 10.1378/chest.14-1120
3. Chowkwanyun M, Reed AL. Racial health disparities and COVID-19 — caution and context. *New Engl J Med*. (2020) 383:201–20. doi: 10.1056/nejmp2012910
4. Sun P, Lu X, Xu C, Sun W, Pan B. Understanding of COVID-19 based on current evidence. *J Med Virol*. (2020) 92:548–51. doi: 10.1002/jmv.25722
5. CDC. *People who are at Higher Risk for Severe Illness*. (2020). Available online at: www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-at-higher-risk.html (accessed May 5, 2020)
6. Monti S, Balduzzi S, Delvino P, Bellis E, Quadrelli VA, Montecucco C. Clinical course of COVID-19 in a series of patients with chronic arthritis treated with immunosuppressive targeted therapies. *Ann Rheum Dis*. (2020) 79:667–8. doi: 10.1136/annrheumdis-2020-217424
7. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the 'Cytokine Storm' in COVID-19. *J Infect*. (2020) 80:607–13. doi: 10.1016/j.jinf.2020.03.037
8. Shi Y, Wang Y, Shao C, Huang J, Gan J, Huang X, et al. COVID-19 infection: the perspectives on immune responses. *Cell Death Differ*. (2020) 27:1451–4. doi: 10.1038/s41418-020-0530-3
9. Haberman R, Axelrad J, Chen A, Castillo R, Yan D, Izmirly P, et al. COVID-19 in Immune-Mediated Inflammatory Diseases — Case Series from New York. *N Engl J Med*. (2020) 383:85–8. doi: 10.1056/nejmc2009567

10. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. (2020) 395:497–506. doi: 10.1016/s0140-6736(20)30183-5

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.

frontiers in Medicine

MINI REVIEW
published: 24 November 2020
doi: 10.3389/fmed.2020.594133

Emerging Molecular Targets for the Treatment of Refractory Sarcoidosis

Gonçalo Boleto[1,2], *Matheus Vieira*[1,2], *Anne Claire Desbois*[1,2,3,4,5], *David Saadoun*[1,2,3,4,5] *and Patrice Cacoub*[1,2,3,4,5]*

[1] AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Department of Internal Medicine and Clinical Immunology, Paris, France, [2] Centre de Référence des Maladies Auto-Immunes et Systémiques Rares, Centre de Référence des Maladies Auto-Inflammatoires et de l'Amylose, Bordeaux, France, [3] Sorbonne Université, UPMC Univ Paris 06, UMR 7211, Inflammation-Immunopathology-Biotherapy Department (DHU i2B), Paris, France, [4] INSERM, UMR_S 959, Paris, France, [5] CNRS, FRE3632, Paris, France

Sarcoidosis is a multisystem granulomatous disease of unknown origin that has variable clinical course and can affect nearly any organ. It has a chronic course in about 25% of patients. Corticosteroids (CS) are the cornerstone of therapy but their long-term use is associated with cumulative toxicity. Commonly used CS-sparing agents include methotrexate, cyclophosphamide, azathioprine, and mycophenolate mofetil. Twenty to forty percentage of sarcoidosis patients are refractory to these therapies or develop severe adverse events. Therefore, additional and targeted CS-sparing agents are needed for chronic sarcoidosis. Macrophage activation, interferon response, and formation of the granuloma are mainly mediated by T helper-1 responses. Different pro-inflammatory cytokines such as interleukin (IL)-8, IL-12, IL-6, and tumor necrosis factor-alpha (TNF-α) have been shown to be highly expressed in sarcoidosis-affected tissues. As a result of increased production of these cytokines, Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling is constitutively active in sarcoidosis. Several studies of biological agents that target TNF-α have reported their efficacy and appear today as a second line option in refractory sarcoidosis. Some case series report a positive effect of tocilizumab an anti-IL-6 monoclonal antibody in this setting. More recently, JAK inhibition appears as a new promising strategy. This review highlights key advances on the management of chronic refractory sarcoidosis. Novel therapeutic strategies and treatment agents to manage the disease are described.

Keywords: sarcoidosis, therapy, JAK inhibitors, interleukin-1, interleukin-6, granuloma

OPEN ACCESS

Edited by:
Mehdi Mirsaeidi, University of Miami, United States

Reviewed by:
Kunihiro' Yamaoka, Keio University, Japan
Luis Ferreira Moita, Gulbenkian Institute of Science (IGC), Portugal

***Correspondence:**
Patrice Cacoub
patrice.cacoub@aphp.fr

Specialty section:
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

Received: *12 August 2020*
Accepted: *02 November 2020*
Published: *24 November 2020*

Citation:
Boleto G, Vieira M, Desbois AC, Saadoun D and Cacoub P (2020) Emerging Molecular Targets for the Treatment of Refractory Sarcoidosis. Front. Med. 7:594133. doi: 10.3389/fmed.2020.594133

INTRODUCTION

Sarcoidosis is a multisystem granulomatous disease that can involve virtually any organ though the lungs and the lymphatics are the most commonly affected sites (1). The disease may remit spontaneously or upon treatment usually within the first 2–3 years after diagnosis but can have a chronic course in about 25% of cases (2). The exact cause of sarcoidosis is still not known. However, genetic susceptibility and environmental factors have been suggested as contributors of disease development (3, 4). Several studies strongly suggest that sarcoidosis might be the result of an exaggerated granulomatous reaction to a microbial-induced host response and persistent presence of antigens causing sarcoid lesions (5). Systematic treatment

is often reserved for life-threatening organ involvement [i.e., severe interstitial lung disease, central nervous system (CNS), kidneys, liver, heart] or severe disabling functional symptoms such as skin disease, arthritis and bone disease or posterior uveitis (1). Corticosteroids (CS) remain the mainstay of treatment; however, their long-term use is associated with cumulative toxicity. Alternative therapies commonly used as CS-sparing agents include hydroxychloroquine, methotrexate, azathioprine and mycophenolate mofetil (6). About 10% of patients are refractory to these first and second-line therapies or develop adverse events necessitating additional CS-sparing targeted agents (7). Despite limited data on extra pulmonary manifestations, cyclophosphamide (CYC) has been successfully used for refractory CNS and cardiac disease (8–10). However, the known toxic and carcinogenic profile of CYC limits its use especially in young patients. In this setting, biological agents that target the tumor necrosis factor (TNF) have been introduced as a third-line therapy and have proved to be effective in a proportion of patients with severe/refractory sarcoidosis (11). Despite their excellent safety profile in other rheumatic conditions, TNF-α antagonists use showed to be less well tolerated with severe infections and malignancies being more frequent during sarcoidosis treatment (12). Hence, there is currently an unmet therapeutic need for a proportion of refractory or intolerant patients to TNF antagonists. Identifying new molecular targets for the treatment of refractory sarcoidosis should be a priority. Here, we review new therapeutic approaches for the treatment of refractory sarcoidosis with targeted biologic and synthetic agents.

A summary of the current data on the potential therapeutic targets in refractory sarcoidosis is available in **Table 1**. A focus on the reported cases of targeted biologic and synthetic agents (other than TNF inhibitors) use in sarcoidosis is available in **Supplementary Table 1**. **Figure 1** illustrates the pathophysiology and potential therapeutic targets of refractory sarcoidosis.

TUMOR NECROSIS FACTOR-ALPHA INHIBITION

TNF-α plays a crucial role in the development of noncaseating granulomas in a variety of diseases. Previous studies showed that high levels of TNF-α from alveolar macrophages correlated with disease progression (13). Two randomized controlled trials have investigated infliximab, a chimeric monoclonal anti-TNF-α antibody, therapy in sarcoidosis and showed significant though modest improvement in lung function after 14 weeks of treatment (14, 15). Another randomized controlled trial showed significant improvement in extrapulmonary sarcoidosis as assessed by a the extrapulmonary physician organ severity tool (ePOST) in patients treated with infliximab (16). A large French nationwide multicentric retrospective study on 132 patients with refractory sarcoidosis showed TNF-antagonists to be efficient in about two-thirds of patients, despite higher rates of adverse events (11). Adalimumab, another monoclonal anti- TNF-α antibody, showed to be efficient in a randomized controlled clinical trial of 16 patients with skin sarcoidosis (17). These positive effects of TNF inhibition are supported by a high relapse rate after discontinuation of infliximab therapy in 47 patients with severe sarcoidosis (18). Despite the lack of high quality evidence, TNF inhibition can currently be considered as standard-of-care in severe cases of refractory sarcoidosis.

INTERLEUKIN-6 BLOCKING

A subset of $CD4^+$ effector T cell population called T helper 17 (Th17), which express a transcription factor known as retinoic acid-related orphan receptor (ROR)γι, has been described in sarcoid lesions (19). Active sarcoidosis patients have an increased Th17/T regulator (Treg) ratio in the peripheral blood and bronchoalveolar lavage fluid (BALF) that is reversed by immunosuppressive therapy (20). Interleukin-6 (IL-6) is a key pleiotropic cytokine. It induces the development of Th17 cells from naïve T cells together with transforming growth fractor β (TGF-β) and it inhibits anti-inflammatory Treg cells (21). Previous reports showed increased IL-6 levels in the BALF of sarcoidosis patients. Significant correlations were found between IL-6 levels and $CD4^+/CD8^+$ ratio, IL-8 and BALF neutrophil percentage (22–24). Another study reported increased IL-6 levels in the cerebrospinal fluid (CSF) of patients with neurosarcoidosis as compared to patients with multiple sclerosis or other inflammatory disorders. CSF concentration of IL-6 $>$ 50 pg/mL was associated with a higher risk of relapse or progression of neurosarcoidosis (25). Genetic variations in the genes encoding IL-6 were preferentially upregulated in patients with severe and progressive sarcoidosis thus giving further evidence of the pathogenic role of this proinflammatory cytokine (26, 27). IL-6 is a potent up regulator of serum amyloid A protein (SAA), an acute phase reactant which has demonstrated a potential key role in the pathogenesis of sarcoidosis (28).

Currently, there are two humanized IL-6 receptor monoclonal antibodies [tocilizumab (TCZ) and sarilumab (SAR)] that have been approved for the treatment of different inflammatory rheumatic conditions. Despite robust evidence on the role of IL-6 in the pathogenesis of sarcoidosis, data on the use of anti-IL-6 agents in sarcoidosis are scarce. Previous case reports showed clinical improvement under TCZ in patients with chronic sarcoidosis associated with Castleman's disease (29) and Still's disease (30). In a case series of four patients with refractory chronic sarcoidosis the authors reported dramatic responses to TCZ with improved symptoms and organ function allowing steroid tapering (31). In a model of experimental uveitis, Yoshimura et al. investigated the role of IL-6 in the formation of refractory ocular inflammation (32). The authors showed that IL-6-deficient mice had reduced Th17 responses and ameliorated ocular inflammation. Using the same model of experimental uveitis, systemic administration of an anti-IL-6 receptor antibody also ameliorated ocular inflammation by suppressing Th17 responses. TCZ has shown a positive signal in the management of patients with ocular involvement (refractory uveitis, cystoid macular oedema) refractory to conventional immunosuppressive drugs (33, 34). Of note, despite these encouraging data, cases of paradoxal new-onset sarcoidosis during TCZ therapy must also be acknowledge. To our knowledge, four reports [three patients

TABLE 1 | Current data on the potential therapeutic targets in refractory sarcoidosis.

Target	Agents	Evidence	Primary endpoint	Ongoing clinical trials
IL-6	Tocilizumab Sarilumab	Case-reports		Phase II (Sarilumab) (NCT04008069)
IL-1	Anakinra Canakinumab	Basic studies		Phase II (Canakinumab) for pulmonary sarcoidosis (NCT02888080) Phase II (Anakinra) for cardiac sarcoidosis (NCT04017936)
IL-17	Secukinumab Ixekizumab Brodalumab	Case-reports Phase II RCT (Secukinumab for non-infectious uveitis)	NA (only 4 patients)	None
IL-12/23	Ustekinumab (IL-12/23) Guselkumab (IL-23) Risankizumab (IL-23)	Phase II RCT (Ustekinumab for pulmonary and skin sarcoidosis)	Negative	None
CTLA4	Abatacept	Basic studies		STAR trial (NCT00739960) (the study has been terminated prematurely due to funding constraints)
B-cell	Rituximab Belimumab	Case-reports Open-label phase I/II trial (Rituximab for pulmonary sarcoidosis)	Negative	None
JAK	Ruxolitinib Tofacitinib Baracitinib Upadacitinib Filgotinib	Case-reports		Open-label trial (Tofacitinib) for pulmonary sarcoidosis (NCT03793439) Open-label trial (Tofacitinib) for cutaneous sarcoidosis (NCT03910543)
PDEA4	Apremilast	Open-label trial (cutaneous sarcoidosis)	Positive	Phase II/III open-label trial for cutaneous sarcoidosis (NCT00794274)

IL, interleukin; RCT, randomized-clinical trial; NA, not evaluated; CTLA4, cytotoxic T-lymphocyte antigen 4; JAK, janus kinase; PDEA4, phosphodiesterase type 4.

with rheumatoid arthritis (RA) (35–37) and one with giant cell arteritis (38)] have described cases of cutaneous and mediastinal sarcoidosis triggered by TCZ therapy. The exact mechanism for these paradoxical reactions is not known, but data on murine models of granulomatosis suggest that anti-IL-6 treatment might enhance TNF-α production contributing to granuloma formation (39, 40). Currently there is an ongoing clinical trial comparing the effectiveness and the safety of SAR in patients with glucocorticoid-dependent sarcoidosis (NCT04008069).

INTERLEUKIN-1 BLOCKING

Interleukin-1 (IL-1) is a cytokine with potent pro-inflammatory properties that has been shown to be implicated in the pathogenesis of sarcoidosis (41). An imbalance between the levels of IL-1β and IL-1 receptor antagonist has been observed in the BALF of patients with pulmonary sarcoidosis (42, 43). Anakinra is a recombinant human IL-1 receptor antagonist that was firstly approved for the treatment of RA. It is currently used mostly for the management autoinflammatory conditions and difficult-to-treat gout (44, 45). We were unable to identify case reports or trials of anakinra use in the treatment of sarcoidosis. However, two cases of anakinra-induced sarcoidosis have been reported (46, 47). A phase 2 randomized controlled trial assessing the efficacy and safety of canakinumab, another IL-1 antagonist, in patients with pulmonary sarcoidosis (NCT02888080) has completed the recruitment process. The Interleukin-1 Blockade for Treatment of Cardiac Sarcoidosis (MAGiC-ART) trial (NCT04017936) is another ongoing phase 2 randomized-controlled trial evaluating anakinra for the treatment of cardiac sarcoidosis.

INTERLEUKIN-17 BLOCKING

Recent investigations indicate that Th17 cells are key players in all stages of granuloma formation and are upregulated in patients with active sarcoidosis (20, 48). The hallmark of the Th17 pathway is the production of IL-17. This proinflammatory cytokine with pleiotropic properties has been shown to be involved in the pathogenesis of various granulomatous diseases (49). Ten Berge et al. showed enhanced IL-17 expression in granulomas as well as increased numbers of IL-17 memory Th cells in the circulation and BALF of newly diagnosed sarcoidosis patients (50). Ostadkarampour et al. demonstrated the presence of T cells producing IL-17 in response to a mycobacterial antigen in patients with pulmonary sarcoidosis. The authors also observed higher levels of IL-17 and IL-17 producing cells in patients with Löfgren's syndrome suggesting a potential biomarker for the prognosis of sarcoidosis (51). Increased IL-17 responses might be related to aberrant metabolic pathways as shown by the abundant expression of hypoxia inducible factor (HIF) isoforms in granulomas and their association with Glut1 protein levels and enhanced IL-17 production. Downregulation of HIF in sarcoidosis peripheral blood mononuclear cells lead to a decrease in IL-17 production (52). An elevated expression of IL-17 receptor C on $CD8^+$ T cells in peripheral blood was found in patients with ocular sarcoidosis (53).

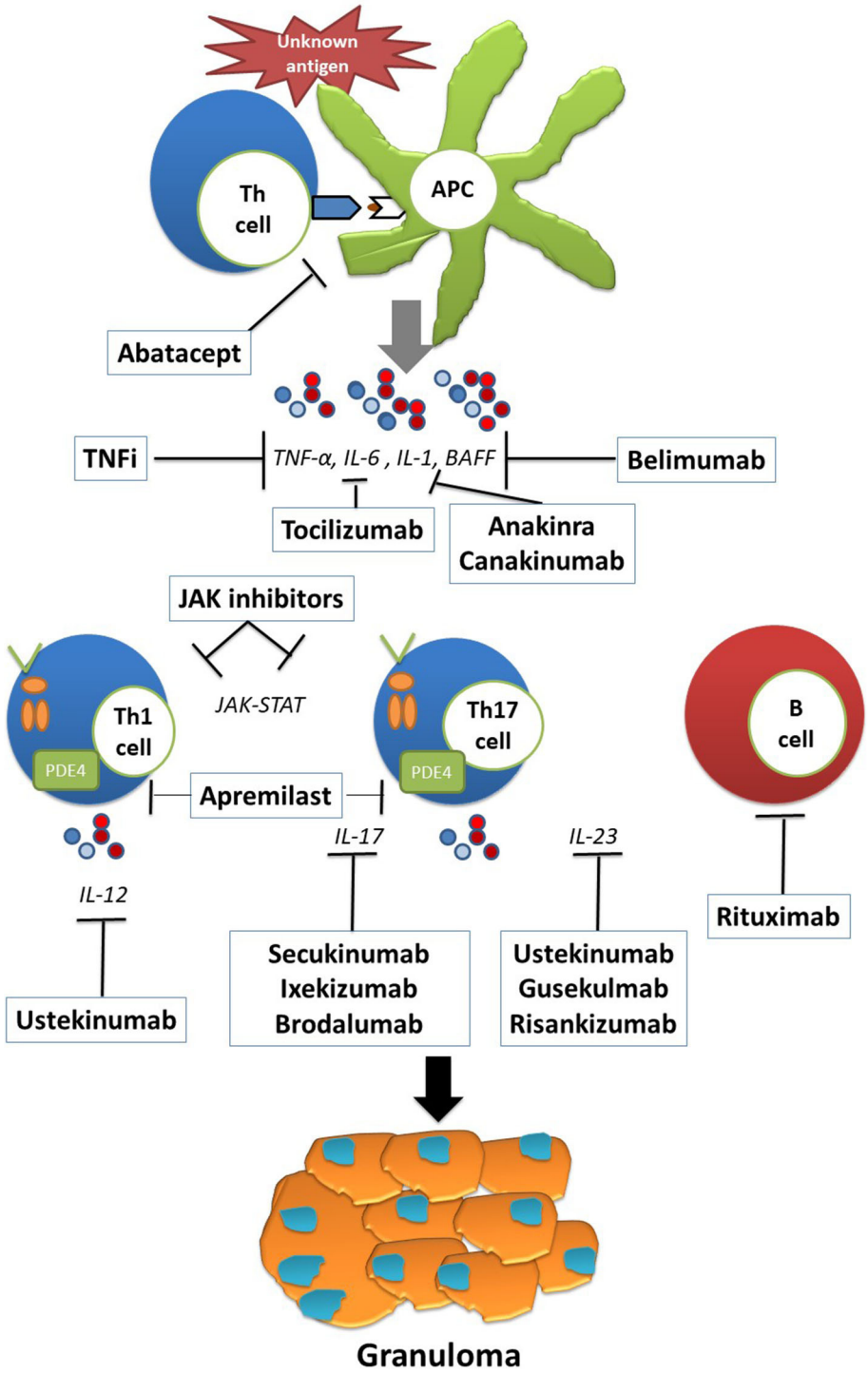


FIGURE 1 | The pathophysiology and potential therapeutic targets of refractory sarcoidosis. Exposure to an unknown antigen leads to the activation and proliferation of T cells through antigen presenting cells (APCs). The release of proinflammatory cytokines such as IL-6, IL-1, and BAFF skews the immune response to Th1 and Th17 responses as well as B cell activation and proliferation. Persistent antigen presentation leads to granuloma formation and to the development of sarcoid lesions. Th, T-helper cell; APC, antigen presenting cell; IL, interleukin; BAFF, B lymphocyte stimulator; JAK, janus kinase.

Currently, three human monoclonal antibodies that target IL-17 have been approved for the treatment of psoriasis, psoriatic arthritis and axial spondyloarthritis (secukinumab, ixekizumab and brodalumab). In Crohn's disease, which is another disorder characterized by granuloma formation, IL-17 inhibition can exacerbate the inflammatory disease. In sarcoidosis patients, the

role of IL-17 inhibition is less clear (54). Most of the available evidence on IL-17 blockade in patients with sarcoidosis comes from case reports. Previous reports suggest a favorable effect of secukinumab in two cases of sarcoidosis associated with TNF antagonists (55, 56). Conversely, our review of the literature identified two cases of sarcoidosis that either developed or worsened in patients taking secukinumab and ixekizumab (57, 58). However, a previous multicentre, randomized, double blind, phase II trial, assessing the efficacy and safety of secukinumab in non-infectious uveitis did not identify any safety concerns in the included patients with sarcoidosis ($n = 4$) (59). Further trials assessing the efficacy and safety of IL-17 blockade in sarcoidosis are warranted.

INTERLEUKIN-12 AND INTERLEUKIN-23 BLOCKING

Sarcoid granuloma formation is characterized by an influx of Th1 cells which spontaneously express IL-2 receptors and release interferon γ (IFN-γ), TNF-α, and IL-2 (5). IL-12 through its receptor (IL-12R) expressed on Th1 cells is one of the most important cytokines for inducing T cell response toward Th1 differentiation (60). Multiple studies have reported overexpression of IL-12 by activated alveolar macrophages in patients with active pulmonary sarcoidosis (61–65). IL-23 is a member of the IL-12 cytokine family which promotes Th17 responses through its receptor (IL-23R) (66). Transcriptomic analyses demonstrated upregulation of IL-23 in sarcoid lesions (67). IL-23R polymorphisms have also been shown to be implicated in the pathogenesis of sarcoidosis (68).

Ustekinumab is a monoclonal antibody that binds to the shared p40 unity of human IL-12 and IL-23, thus blocking Th1 and Th17 responses, respectively. It is approved for the treatment of psoriasis, psoriatic arthritis, Crohn's disease and ulcerative colitis (69). In a phase II, multicentre, randomized, double-blind, placebo-controlled trial (70), Judson et al. evaluated the safety and efficacy of ustekinumab in patients with chronic pulmonary and/or cutaneous sarcoidosis (70). Patients received ustekinumab 180 mg subcutaneously at week 0 and 90 mg at week 8, 16, and 24. Despite an excellent safety profile, ustekinumab failed to achieve the primary efficacy endpoint [change from baseline at week 16 in % predicted forced vital capacity (FVC%)] and there were no significant improvements in the major secondary endpoints. Guselkumab and risankizumab are specific anti-IL-23 monoclonal antibodies recently approved for the treatment of moderate-to-severe psoriasis. However, no data is currently available in patients with sarcoidosis. Similarly to IL-17 antagonists, several cases of induced or worsened sarcoidosis with ustekinumab or guselkumab have been reported in the literature (71–74).

CYTOTOXIC T-LYMPHOCYTE ANTIGEN 4 BLOCKADE

Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is a costimulatory molecule that is an important regulator of T cell activation and proliferation. CTLA-4 polymorphisms were shown to significantly influence phenotypes of sarcoidosis (75). Abatacept is a fusion protein of the extracellular domain of the CTLA-4 linked to a modified Fc of human immunoglobulin 1 (IgG1) inhibiting the activation of T cell responses (76). It is currently approved for the treatment of RA. To the best of our knowledge, there are no published studies or case reports concerning abatacept in sarcoidosis. We identified a prospective open-label trial (STAR trial NCT00739960) evaluating abatacept in refractory sarcoidosis. Unfortunately, this study has been terminated prematurely due to funding constraints.

B-CELL INHIBITION

Innate and T cell immunity play major roles in the pathogenesis of sarcoidosis. Several studies suggest a potential involvement of B cell immune responses in this disease (5, 77). Hypergammaglobulinemia and B cell accumulation in sarcoid lesions are frequently observed (78, 79). Saussine et al. showed increased levels of B-cell-activating factor (BAFF), also called BlyS (B lymphocyte stimulator), which is a cytokine involved in the survival and maturation of B cells in patients with active sarcoidosis (80). Rituximab (RTX) is an anti-CD20 monoclonal chimeric antibody that selectively depletes $CD20^+$ B cell population. RTX was first approved for the treatment of non-Hodgkin B-cell lymphoma and later for rheumatoid arthritis and anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis. Despite several case reports of the effectiveness of RTX in patients with refractory sarcoidosis (81–90) data from clinical trials are scarce. In a prospective, open-label phase I/II trial, the authors assessed the effect of RTX in 10 patients with symptomatic moderate-to-severe pulmonary sarcoidosis refractory to corticosteroids plus one or more corticosteroid-sparing agents, including methotrexate and azathioprine (91). The authors observed very modest and inconsistent improvements in FVC% and 6-min walk test (6MWD) with only two patients having >10% improvement of FCV% and three patients having >50 m improvement in 6MWD by week 52. The prednisone doses were not changed during the study. RTX was generally well tolerated with only one patient hospitalized for pneumonia. Two patients died of respiratory failure linked to sarcoidosis progression. Belimumab, a human monoclonal antibody that inhibits BAFF/BlyS, is approved for the treatment of systemic lupus erythematosus and may represent a potential drug candidate for the management of refractory sarcoidosis. However, our literature review did not identify reports or ongoing trials concerning belimumab in sarcoidosis.

JANUS KINASE INHIBITION

The activation of macrophages in sarcoid lesions is thought to be driven by Th1 immune responses and mediated by several cytokines including interferon-γ (IFN-γ) (92, 93). IFN-γ activates the Janus Kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway which is involved in upregulating a set of genes involved in the inflammatory response (94). Transcriptomic analysis showed that JAK-STAT pathway

activation signatures, especially STAT1 pathway, is activated in granulomatous diseases (95–98).

There are currently four JAK inhibitors available for clinical use: ruxolitinib for the treatment of myeloproliferative disorders; tofacitinib for rheumatoid arthritis (RA), psoriatic arthritis and ulcerative colitis; and baricitinib, upadacitinib and filgotinib for RA. Our literature review identified 7 reports concerning JAK inhibitors use (ruxolitinib $n = 3$; tofacitinib $n = 3$; baricitinib $n = 1$) in cases of refractory sarcoidosis. Ruxolitinib, a JAK 1 and 2 inhibitor, improved multivisceral involvement in three patients with sarcoidosis among whom two were treated for concomitant polycythemia vera (99–101). Damsky et al. reported dramatic positive effects of tofacitinib, a JAK 1 and 3 inhibitor, in a patient with severe cutaneous sarcoidosis refractory to topical and systemic corticosteroids and multiple corticosteroid-sparing agents including minocycline, hydroxychloroquine, methotrexate, tacrolimus and adalimumab. During treatment, the authors observed a downregulation of JAK/STAT signature on skin samples (102). The positive effect of tofacitinib was also observed in four patients with refractory cutaneous sarcoidosis ($n = 3$) and granuloma annular ($n = 1$) (103). Tofacitinib resulted in a mean improvement in clinical activity and histologic resolution was documented in all patients. The same group reported another case of severe multiorgan sarcoidosis involving the lungs, lymph nodes, bones, and skin, not controlled with prednisone, mycophenolate sodium, methotrexate, infliximab, rituximab and intravenous immunoglobulins (104). In this patient, tofacitinib treatment resulted in clinical remission of cutaneous lesions as well as resolution of positron emission tomography hypermetabolized lesions in internal organs after 6 months. Scheinberg et al. reported the case of a 35-year-old female patient with fever, arthralgia and hilar and cervical adenopathy revealing sarcoidosis initially treated with prednisone (105). Due to persistent daily low grade fever and arthralgia she was started on baricitinib, a selective JAK 1 and 2 inhibitor, with complete resolution of clinical symptoms and lymph node disease after week 12. Despite these encouraging data, the safety profile of JAK inhibitors in sarcoidosis remains to be confirmed. There are currently two ongoing open-label trials evaluating tofacitinib 5 mg twice daily as a corticosteroid-sparing agent in pulmonary sarcoidosis (NCT03793439) and in the treatment of cutaneous sarcoidosis and granuloma annular (NCT03910543).

PHOSPHODIESTERASE TYPE 4 INHIBITION

Inhibition of PDEA4 prevents cyclic AMP (cAMP) being hydrolysed to AMP resulting in increased intracellular levels of cAMP and inhibiting the expression of proinflammatory cytokines (106). Apremilast is an orally administered phosphodiesterase type 4 (PDEA4) inhibitor approved for the treatment of psoriasis and psoriatic arthritis. Baughman et al. reported the effects of apremilast in 15 patients with active cutaneous sarcoidosis (107). The authors observed significant improvements in skin scores in 14 patients treated with apremilast 20 mg twice daily. Three patients developed worsening of cutaneous sarcoid lesions within 3 months after discontinuation of apremilast. A phase 2 and 3 open-label trial evaluating the efficacy and safety of apremilast in chronic cutaneous sarcoidosis (NCT00794274) has been completed; however the results are not yet available.

CONCLUSION

Refractory sarcoidosis, broadly defined as failure to attain clinical remission after appropriate treatment with corticosteroids and conventional immunosuppressant, is associated with increased morbidity and mortality. At present, TNF antagonists can be considered standard-of-care therapy in severe cases of refractory sarcoidosis. However, treatment options in patients with multidrug-refractory sarcoidosis failing initial anti-TNF therapy is a challenging issue in clinical practice. In the clinical practice, assessing adherence, ruling out differential diagnosis, checking anti-TNF blood concentrations and testing for anti-drug antibodies, should be done before classifying patients as refractory and intensifying treatment. In this setting, new potential useful agents including IL-6, IL-17, and JAK inhibitors with evidence consisting largely of observation or uncontrolled studies are the most promising ones. Data from ongoing prospective clinical trials should give further information on clinical effects of these agents in refractory sarcoidosis.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and approved the final version to be submitted for publication. PC had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.594133/full#supplementary-material

REFERENCES

1. Valeyre D, Prasse A, Nunes H, Uzunhan Y, Brillet P-Y, Müller-Quernheim J. Sarcoidosis. *Lancet Lond Engl.* (2014) 383:1155–67. doi: 10.1016/S0140-6736(13)60680-7
2. Pereira CAC, Dornfeld MC, Baughman R, Judson MA. Clinical phenotypes in sarcoidosis. *Curr Opin Pulm Med.* (2014) 20:496–502. doi: 10.1097/MCP.0000000000000077
3. Newman LS, Rose CS, Bresnitz EA, Rossman MD, Barnard J, Frederick M, et al. A case control etiologic study of sarcoidosis: environmental and

occupational risk factors. *Am J Respir Crit Care Med.* (2004) 170:1324–30. doi: 10.1164/rccm.200402-249OC
4. Iannuzzi MC. Genetics of sarcoidosis. *Semin Respir Crit Care Med.* (2007) 28:15–21. doi: 10.1055/s-2007-970330
5. Zissel G, Prasse A, Müller-Quernheim J. Immunologic response of sarcoidosis. *Semin Respir Crit Care Med.* (2010) 31:390–403. doi: 10.1055/s-0030-1262208
6. Baughman RP, Nunes H, Sweiss NJ, Lower EE. Established and experimental medical therapy of pulmonary sarcoidosis. *Eur Respir J.* (2013) 41:1424–38. doi: 10.1183/09031936.00060612
7. Baughman RP, Culver DA, Judson MA. A concise review of pulmonary sarcoidosis. *Am J Respir Crit Care Med.* (2011) 183:573–81. doi: 10.1164/rccm.201006-0865CI
8. Doty JD, Mazur JE, Judson MA. Treatment of corticosteroid-resistant neurosarcoidosis with a short-course cyclophosphamide regimen. *Chest.* (2003) 124:2023–6. doi: 10.1378/chest.124.5.2023
9. Chapelon-Abric C, Sene D, Saadoun D, Cluzel P, Vignaux O, Costedoat-Chalumeau N, et al. Cardiac sarcoidosis: diagnosis, therapeutic management and prognostic factors. *Arch Cardiovasc Dis.* (2017) 110:456–65. doi: 10.1016/j.acvd.2016.12.014
10. Joubert B, Chapelon-Abric C, Biard L, Saadoun D, Demeret S, Dormont D, et al. Association of prognostic factors and immunosuppressive treatment with long-term outcomes in neurosarcoidosis. *JAMA Neurol.* (2017) 74:1336–44. doi: 10.1001/jamaneurol.2017.2492
11. Jamilloux Y, Cohen-Aubart F, Chapelon-Abric C, Maucort-Boulch D, Marquet A, Pérard L, et al. Efficacy and safety of tumor necrosis factor antagonists in refractory sarcoidosis: a multicenter study of 132 patients. *Semin Arthritis Rheum.* (2017) 47:288–94. doi: 10.1016/j.semarthrit.2017.03.005
12. Maneiro JR, Salgado E, Gomez-Reino JJ, Carmona L, BIOBADASER Study Group. Efficacy and safety of TNF antagonists in sarcoidosis: data from the Spanish registry of biologics BIOBADASER and a systematic review. *Semin Arthritis Rheum.* (2012) 42:89–103. doi: 10.1016/j.semarthrit.2011.12.006
13. Marques LJ, Zheng L, Poulakis N, Guzman J, Costabel U. Pentoxifylline inhibits TNF-alpha production from human alveolar macrophages. *Am J Respir Crit Care Med.* (1999) 159:508–11. doi: 10.1164/ajrccm.159.2.9804085
14. Baughman RP, Drent M, Kavuru M, Judson MA, Costabel U, du Bois R, et al. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. *Am J Respir Crit Care Med.* (2006) 174:795–802. doi: 10.1164/rccm.200603-402OC
15. Rossman MD, Newman LS, Baughman RP, Teirstein A, Weinberger SE, Miller W, et al. A double-blinded, randomized, placebo-controlled trial of infliximab in subjects with active pulmonary sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* (2006) 23:201–8. Available online at: https://pubmed.ncbi.nlm.nih.gov/18038919/
16. Judson MA, Baughman RP, Costabel U, Flavin S, Lo KH, Kavuru MS, et al. Efficacy of infliximab in extrapulmonary sarcoidosis: results from a randomised trial. *Eur Respir J.* (2008) 31:1189–96. doi: 10.1183/09031936.00051907
17. Pariser RJ, Paul J, Hirano S, Torosky C, Smith M. A double-blind, randomized, placebo-controlled trial of adalimumab in the treatment of cutaneous sarcoidosis. *J Am Acad Dermatol.* (2013) 68:765–73. doi: 10.1016/j.jaad.2012.10.056
18. Vorselaars ADM, Verwoerd A, van Moorsel CHM, Keijsers RGM, Rijkers GT, Grutters JC. Prediction of relapse after discontinuation of infliximab therapy in severe sarcoidosis. *Eur Respir J.* (2013) 43:602–9. doi: 10.1183/09031936.00055213
19. Facco M, Cabrelle A, Teramo A, Olivieri V, Gnoato M, Teolato S, et al. Sarcoidosis is a Th1/Th17 multisystem disorder. *Thorax.* (2011) 66:144–50. doi: 10.1136/thx.2010.140319
20. Huang H, Lu Z, Jiang C, Liu J, Wang Y, Xu Z. Imbalance between Th17 and regulatory T-Cells in sarcoidosis. *Int J Mol Sci.* (2013) 14:21463–73. doi: 10.3390/ijms141121463
21. Kimura A, Kishimoto T. IL-6: regulator of Treg/Th17 balance. *Eur J Immunol.* (2010) 40:1830–5. doi: 10.1002/eji.201040391
22. Sahashi K, Ina Y, Takada K, Sato T, Yamamoto M, Morishita M. Significance of interleukin 6 in patients with sarcoidosis. *Chest.* (1994) 106:156–60. doi: 10.1378/chest.106.1.156
23. Girgis RE, Basha MA, Maliarik M, Popovich J, Iannuzzi MC. Cytokines in the bronchoalveolar lavage fluid of patients with active pulmonary sarcoidosis. *Am J Respir Crit Care Med.* (1995) 152:71–5. doi: 10.1164/ajrccm.152.1.7599865
24. Takizawa H, Satoh M, Okazaki H, Matsuzaki G, Suzuki N, Ishii A, et al. Increased IL-6 and IL-8 in bronchoalveolar lavage fluids (BALF) from patients with sarcoidosis: correlation with the clinical parameters. *Clin Exp Immunol.* (1997) 107:175–81. doi: 10.1046/j.1365-2249.1997.d01-905.x
25. Chazal T, Costopoulos M, Maillart E, Fleury C, Psimaras D, Legendre P, et al. The cerebrospinal fluid CD4/CD8 ratio and interleukin-6 and−10 levels in neurosarcoidosis: a multicenter, pragmatic, comparative study. *Eur J Neurol.* (2019) 26:1274–80. doi: 10.1111/ene.13975
26. Bihl MP, Laule-Kilian K, Bubendorf L, Rutherford RM, Baty F, Kehren J, et al. Progressive pulmonary sarcoidosis–a fibroproliferative process potentially triggered by EGR-1 and IL-6. *Sarcoidosis Vasc Diffuse Lung Dis.* (2006) 23:38–50. doi: 10.1183/09031936.00051907
27. Grutters JC, Sato H, Pantelidis P, Ruven HJT, McGrath DS, Wells AU, et al. Analysis of IL6 and IL1A gene polymorphisms in UK and Dutch patients with sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* (2003) 20:20–7.
28. Chen ES, Song Z, Willett MH, Heine S, Yung RC, Liu MC, et al. Serum amyloid a regulates granulomatous inflammation in sarcoidosis through toll-like receptor-2. *Am J Respir Crit Care Med.* (2010) 181:360–73. doi: 10.1164/rccm.200905-0696OC
29. Awano N, Inomata M, Kondoh K, Satake K, Kamiya H, Moriya A, et al. Mixed-type multicentric Castleman's disease developing during a 17-year follow-up of sarcoidosis. *Intern Med Tokyo Jpn.* (2012) 51:3061–6. doi: 10.2169/internalmedicine.51.8120
30. Semiz H, Kobak S. Coexistence of sarcoidosis and adult onset Still disease. *Reumatol Clin.* (2019) 15:e18–20. doi: 10.1016/j.reuma.2017.04.004
31. Sharp M, Donnelly SC, Moller DR. Tocilizumab in sarcoidosis patients failing steroid sparing therapies and anti-TNF agents. *Respir Med X.* (2019) 1:100004. doi: 10.1016/j.yrmex.2019.100004
32. Yoshimura T, Sonoda K-H, Ohguro N, Ohsugi Y, Ishibashi T, Cua DJ, et al. Involvement of Th17 cells and the effect of anti-IL-6 therapy in autoimmune uveitis. *Rheumatol Oxf Engl.* (2009) 48:347–54. doi: 10.1093/rheumatology/ken489
33. Silpa-Archa S, Oray M, Preble JM, Foster CS. Outcome of tocilizumab treatment in refractory ocular inflammatory diseases. *Acta Ophthalmol.* (2016) 94:e400–6. doi: 10.1111/aos.13015
34. Vegas-Revenga N, Calvo-Río V, Mesquida M, Adán A, Hernández MV, Beltrán E, et al. Anti-IL6-receptor tocilizumab in refractory and noninfectious uveitic cystoid macular edema: multicenter study of 25 patients. *Am J Ophthalmol.* (2019) 200:85–94. doi: 10.1016/j.ajo.2018.12.019
35. Nutz A, Pernet C, Combe B, Cohen JD. Sarcoidosis induced by tocilizumab: a paradoxical event? *J Rheumatol.* (2013) 40:1773–4. doi: 10.3899/jrheum.130278
36. Bustamente L, Buscot M, Marquette CH, Roux C. Sarcoidosis and tocilizumab: is there a link? *Clin Exp Rheumatol.* (2017) 35:716.
37. Shono Y, Kamata M, Takeoka S, Ikawa T, Tateishi M, Fukaya S, et al. Cutaneous sarcoidosis in a patient with rheumatoid arthritis receiving tocilizumab. *J Dermatol.* (2018) 45:e217–8. doi: 10.1111/1346-8138.14268
38. Del Giorno R, Iodice A, Mangas C, Gabutti L. New-onset cutaneous sarcoidosis under tocilizumab treatment for giant cell arteritis: a quasi-paradoxical adverse drug reaction. Case report and literature review. *Ther Adv Musculoskelet Dis.* (2019) 11:1759720X19841796. doi: 10.1177/1759720X19841796
39. Yimin null, Kohanawa M. A regulatory effect of the balance between TNF-alpha and IL-6 in the granulomatous and inflammatory response to *Rhodococcus aurantiacus* infection in mice. *J Immunol.* (2006) 177:642–50. doi: 10.4049/jimmunol.177.1.642
40. Yimin null, Kohanawa M, Minagawa T. Up-regulation of granulomatous inflammation in interleukin-6 knockout mice infected with *Rhodococcus aurantiacus*. *Immunology.* (2003) 110:501–6. doi: 10.1111/j.1365-2567.2003.01762.x
41. Rolfe MW, Standiford TJ, Kunkel SL, Burdick MD, Gilbert AR, Lynch JP, et al. Interleukin-1 receptor antagonist expression in sarcoidosis. *Am Rev Respir Dis.* (1993) 148:1378–84. doi: 10.1164/ajrccm/148.5.1378

42. Mikuniya T, Nagai S, Shimoji T, Takeuchi M, Morita K, Mio T, et al. Quantitative evaluation of the IL-1 beta and IL-1 receptor antagonist obtained from BALF macrophages in patients with interstitial lung diseases. *Sarcoidosis Vasc Diffuse Lung Dis.* (1997) 14:39–45.
43. Mikuniya T, Nagai S, Takeuchi M, Mio T, Hoshino Y, Miki H, et al. Significance of the interleukin-1 receptor antagonist/interleukin-1 beta ratio as a prognostic factor in patients with pulmonary sarcoidosis. *Respir Int Rev Thorac Dis.* (2000) 67:389–96. doi: 10.1159/000029536
44. Cvetkovic RS, Keating G. Anakinra. *BioDrugs.* (2002) 16:303–11. doi: 10.2165/00063030-200216040-00005
45. Kalliolias GD, Liossis S-NC. The future of the IL-1 receptor antagonist anakinra: from rheumatoid arthritis to adult-onset Still's disease and systemic-onset juvenile idiopathic arthritis. *Expert Opin Investig Drugs.* (2008) 17:349–59. doi: 10.1517/13543784.17.3.349
46. Sacre K, Pasqualoni E, Descamps V, Choudat L, Debray M-P, Papo T. Sarcoid-like granulomatosis in a patient treated by interleukin-1 receptor antagonist for TNF-receptor-associated periodic syndrome. *Rheumatol Oxf Engl.* (2013) 52:1338–40. doi: 10.1093/rheumatology/kes377
47. Friedman BE, English JC. Drug-induced sarcoidosis in a patient treated with an interleukin-1 receptor antagonist for hidradenitis suppurativa. *JAAD Case Rep.* (2018) 4:543–5. doi: 10.1016/j.jdcr.2018.03.007
48. Crouser ED. Role of imbalance between Th17 and regulatory T-cells in sarcoidosis. *Curr Opin Pulm Med.* (2018) 24:521–6. doi: 10.1097/MCP.0000000000000498
49. Curtis MM, Way SS. Interleukin-17 in host defence against bacterial, mycobacterial and fungal pathogens. *Immunology.* (2009) 126:177–85. doi: 10.1111/j.1365-2567.2008.03017.x
50. Ten Berge B, Paats MS, Bergen IM, van den Blink B, Hoogsteden HC, Lambrecht BN, et al. Increased IL-17A expression in granulomas and in circulating memory T cells in sarcoidosis. *Rheumatol Oxf Engl.* (2012) 51:37–46. doi: 10.1093/rheumatology/ker316
51. Ostadkarampour M, Eklund A, Moller D, Glader P, Olgart Höglund C, Lindén A, et al. Higher levels of interleukin IL-17 and antigen-specific IL-17 responses in pulmonary sarcoidosis patients with Löfgren's syndrome. *Clin Exp Immunol.* (2014) 178:342–52. doi: 10.1111/cei.12403
52. Talreja J, Talwar H, Bauerfeld C, Grossman LI, Zhang K, Tranchida P, et al. HIF-1α regulates IL-1β and IL-17 in sarcoidosis. *eLife.* (2019) 8:e44519. doi: 10.7554/eLife.44519
53. Wu W, Jin M, Wang Y, Liu B, Shen D, Chen P, et al. Overexpression of IL-17RC associated with ocular sarcoidosis. *J Transl Med.* (2014) 12:152. doi: 10.1186/1479-5876-12-152
54. Orrell KA, Murphrey M, Kelm RC, Lee HH, Pease DR, Laumann AE, et al. Inflammatory bowel disease events after exposure to interleukin 17 inhibitors secukinumab and ixekizumab: postmarketing analysis from the RADAR ("research on adverse drug events and reports") program. *J Am Acad Dermatol.* (2018) 79:777–8. doi: 10.1016/j.jaad.2018.06.024
55. Toussirot E, Bernard C, Bossert M. Safety of the use of anti-IL17A treatment in a patient with certolizumab-induced sarcoidosis. *Clin Exp Rheumatol.* (2019) 37:344–5.
56. Eichhoff G. Management with secukinumab of tumour necrosis factor inhibitor-induced pulmonary sarcoidosis-like reaction in a patient with psoriasis. *Clin Exp Dermatol.* (2020) 45:455–6. doi: 10.1111/ced.14101
57. Nyckowski T, Ceilley R, Wilson J. Sarcoidosis developing during secukinumab therapy: case report. *SKIN J Cutan Med.* (2017) 1:95–9. doi: 10.25251/skin.1.2.7
58. Sambharia M, Magge T, Ramakrishna S. Worsening of pulmonary sarcoidosis induced by ixekizumab: a rare paradox. *Chest.* (2018) 154:898A. doi: 10.1016/j.chest.2018.08.808
59. Letko E, Yeh S, Foster CS, Pleyer U, Brigell M, Grosskreutz CL, et al. Efficacy and safety of intravenous secukinumab in noninfectious uveitis requiring steroid-sparing immunosuppressive therapy. *Ophthalmology.* (2015) 122:939–48. doi: 10.1016/j.ophtha.2014.12.033
60. Trinchieri G. Interleukin-12 and its role in the generation of TH1 cells. *Immunol Today.* (1993) 14:335–8. doi: 10.1016/0167-5699(93)90230-I
61. Mroz RM, Korniluk M, Stasiak-Barmuta A, Chyczewska E. Increased levels of interleukin-12 and interleukin-18 in bronchoalveolar lavage fluid of patients with pulmonary sarcoidosis. *J Physiol Pharmacol.* (2008) 59 (Suppl. 6):507–13.
62. Taha RA, Minshall EM, Olivenstein R, Ihaku D, Wallaert B, Tsicopoulos A, et al. Increased expression of IL-12 receptor mRNA in active pulmonary tuberculosis and sarcoidosis. *Am J Respir Crit Care Med.* (1999) 160:1119–23. doi: 10.1164/ajrccm.160.4.9807120
63. Shigehara K, Shijubo N, Ohmichi M, Kon S, Shibuya Y, Takahashi R, et al. Enhanced mRNA expression of Th1 cytokines and IL-12 in active pulmonary sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* (2000) 17:151–7.
64. Shigehara K, Shijubo N, Ohmichi M, Takahashi R, Kon S, Okamura H, et al. IL-12 and IL-18 are increased and stimulate IFN-gamma production in sarcoid lungs. *J Immunol.* (2001) 166:642–9. doi: 10.4049/jimmunol.166.1.642
65. Shigehara K, Shijubo N, Ohmichi M, Kamiguchi K, Takahashi R, Morita-Ichimura S, et al. Increased circulating interleukin-12 (IL-12) p40 in pulmonary sarcoidosis. *Clin Exp Immunol.* (2003) 132:152–7. doi: 10.1046/j.1365-2249.2003.02105.x
66. Parham C, Chirica M, Timans J, Vaisberg E, Travis M, Cheung J, et al. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. *J Immunol.* (2002) 168:5699–708. doi: 10.4049/jimmunol.168.11.5699
67. Judson MA, Marchell RM, Mascelli M, Piantone A, Barnathan ES, Petty KJ, et al. Molecular profiling and gene expression analysis in cutaneous sarcoidosis: the role of interleukin-12, interleukin-23, and the T-helper 17 pathway. *J Am Acad Dermatol.* (2012) 66:901–10.e1–2. doi: 10.1016/j.jaad.2011.06.017
68. Kim HS, Choi D, Lim LL, Allada G, Smith JR, Austin CR, et al. Association of interleukin 23 receptor gene with sarcoidosis. *Dis Markers.* (2011) 31:17–24. doi: 10.1155/2011/185106
69. Sands BE, Sandborn WJ, Panaccione R, O'Brien CD, Zhang H, Johanns J, et al. Ustekinumab as induction and maintenance therapy for ulcerative colitis. *N Engl J Med.* (2019) 382:91. doi: 10.1056/NEJMoa1900750
70. Judson MA, Baughman RP, Costabel U, Drent M, Gibson KF, Raghu G, et al. Safety and efficacy of ustekinumab or golimumab in patients with chronic sarcoidosis. *Eur Respir J.* (2014) 44:1296–307. doi: 10.1183/09031936.00000914
71. Powell JB, Matthews P, Rattehalli R, Woodhead F, Perkins P, Powell G, et al. Acute systemic sarcoidosis complicating ustekinumab therapy for chronic plaque psoriasis. *Br J Dermatol.* (2015) 172:834–6. doi: 10.1111/bjd.13365
72. Gad MM, Bazarbashi N, Kaur M, Gupta A. Sarcoid- like phenomenon - ustekinumab induced granulomatous reaction mimicking diffuse metastatic disease: a case report and review of the literature. *J Med Case Rep.* (2019) 13:257. doi: 10.1186/s13256-019-2137-1
73. Kobak S, Semiz H. Ustekinumab-induced sarcoidosis in a patient with psoriatic arthritis. *Curr Drug Saf.* (2020) 15:163–6. doi: 10.2174/1574886315666200316113312
74. Thomas AS, Rosenbaum JT. Poor control of sarcoidosis-related panuveitis with an antibody to IL-23. *Ocul Immunol Inflamm.* (2020) 28:491–3. doi: 10.1080/09273948.2019.1569245
75. Hattori N, Niimi T, Sato S, Achiwa H, Maeda H, Oguri T, et al. Cytotoxic T-lymphocyte antigen 4 gene polymorphisms in sarcoidosis patients. *Sarcoidosis Vasc Diffuse Lung Dis.* (2005) 22:27–32.
76. Dubois EA, Cohen AF. Abatacept. *Br J Clin Pharmacol.* (2009) 68:480–1. doi: 10.1111/j.1365-2125.2009.03502.x
77. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. *N Engl J Med.* (2007) 357:2153–65. doi: 10.1056/NEJMra071714
78. Lee N-S, Barber L, Akula SM, Sigounas G, Kataria YP, Arce S. Disturbed homeostasis and multiple signaling defects in the peripheral blood B-cell compartment of patients with severe chronic sarcoidosis. *Clin Vaccine Immunol CVI.* (2011) 18:1306–16. doi: 10.1128/CVI.05118-11
79. Fazel SB, Howie SE, Krajewski AS, Lamb D. B lymphocyte accumulations in human pulmonary sarcoidosis. *Thorax.* (1992) 47:964–7. doi: 10.1136/thx.47.11.964
80. Saussine A, Tazi A, Feuillet S, Rybojad M, Juillard C, Bergeron A, et al. Active chronic sarcoidosis is characterized by increased transitional blood B cells, increased IL-10-producing regulatory B cells and high BAFF levels. *PLoS ONE.* (2012) 7:e43588. doi: 10.1371/journal.pone.0043588
81. Beccastrini E, Vannozzi L, Bacherini D, Squatrito D, Emmi L. Successful treatment of ocular sarcoidosis with rituximab. *Ocul Immunol Inflamm.* (2013) 21:244–6. doi: 10.3109/09273948.2012.762982

82. Cinetto F, Compagno N, Scarpa R, Malipiero G, Agostini C. Rituximab in refractory sarcoidosis: a single centre experience. *Clin Mol Allergy.* (2015) 13:19. doi: 10.1186/s12948-015-0025-9
83. Gottenberg JE, Guillevin L, Lambotte O, Combe B, Allanore Y, Cantagrel A, et al. Tolerance and short term efficacy of rituximab in 43 patients with systemic autoimmune diseases. *Ann Rheum Dis.* (2005) 64:913–20. doi: 10.1136/ard.2004.029694
84. Zella S, Kneiphof J, Haghikia A, Gold R, Woitalla D, Thöne J. Successful therapy with rituximab in three patients with probable neurosarcoidosis. *Ther Adv Neurol Disord.* (2018) 11: 1756286418805732. doi: 10.1177/1756286418805732
85. Bomprezzi R, Pati S, Chansakul C, Vollmer T. A case of neurosarcoidosis successfully treated with rituximab. *Neurology.* (2010) 75:568–70. doi: 10.1212/WNL.0b013e3181ec7ff9
86. Sawaya R, Radwan W. Sarcoidosis associated with neuromyelitis optica. *J Clin Neurosci.* (2013) 20:1156–8. doi: 10.1016/j.jocn.2012.09.030
87. Lower EE, Baughman RP, Kaufman AH. Rituximab for refractory granulomatous eye disease. *Clin Ophthalmol.* (2012) 6:1613–8. doi: 10.2147/OPTH.S35521
88. Dalia T, Liu D, Fraga GR, Springer J. A rare case of sarcoidosis presenting with cutaneous medium-vessel granulomatous vasculitis treated with rituximab. *J Clin Rheumatol.* (2018) 5.
89. Earle B, Wolf DS, Ramsay ES. Novel use of rituximab in treatment of refractory neurosarcoidosis in an 11-year-old girl. *J Clin Rheumatol.* (2019) 25:e101–3. doi: 10.1097/RHU.0000000000000900
90. Krause ML, Cooper LT, Chareonthaitawee P, Amin S. Successful use of rituximab in refractory cardiac sarcoidosis. *Rheumatol Oxf Engl.* (2016) 55:189–91. doi: 10.1093/rheumatology/kev309
91. Sweiss NJ, Lower EE, Mirsaeidi M, Dudek S, Garcia JGN, Perkins D, et al. Rituximab in the treatment of refractory pulmonary sarcoidosis. *Eur Respir J.* (2014) 43:1525–8. doi: 10.1183/09031936.00224513
92. Broos CE, Hendriks RW, Kool M. T-cell immunology in sarcoidosis: disruption of a delicate balance between helper and regulatory T-cells. *Curr Opin Pulm Med.* (2016) 22:476–83. doi: 10.1097/MCP.0000000000000303
93. Ramstein J, Broos CE, Simpson LJ, Ansel KM, Sun SA, Ho ME, et al. IFN-γ-Producing T-Helper 17.1 cells are increased in sarcoidosis and are more prevalent than T-helper type 1 cells. *Am J Respir Crit Care Med.* (2016) 193:1281–91. doi: 10.1164/rccm.201507-1499OC
94. Rosenbaum JT, Pasadhika S, Crouser ED, Choi D, Harrington CA, Lewis JA, et al. Hypothesis: sarcoidosis is a STAT1-mediated disease. *Clin Immunol.* (2009) 132:174–83. doi: 10.1016/j.clim.2009.04.010
95. Zhou T, Casanova N, Pouladi N, Wang T, Lussier Y, Knox KS, et al. Identification of Jak-STAT signaling involvement in sarcoidosis severity via a novel microRNA-regulated peripheral blood mononuclear cell gene signature. *Sci Rep.* (2017) 7:4237. doi: 10.1038/s41598-017-04109-6
96. Zhou T, Zhang W, Sweiss NJ, Chen ES, Moller DR, Knox KS, et al. Peripheral blood gene expression as a novel genomic biomarker in complicated sarcoidosis. *PLoS ONE.* (2012) 7:e44818. doi: 10.1371/journal.pone.0044818
97. Li H, Zhao X, Wang J, Zong M, Yang H. Bioinformatics analysis of gene expression profile data to screen key genes involved in pulmonary sarcoidosis. *Gene.* (2017) 596:98–104. doi: 10.1016/j.gene.2016.09.037
98. Rosenbaum JT, Hessellund A, Phan I, Planck SR, Wilson DJ. The expression of STAT-1 and phosphorylated STAT-1 in conjunctival granulomas. *Ocul Immunol Inflamm.* (2010) 18:261–4. doi: 10.3109/09273941003797934
99. Rotenberg C, Besnard V, Brillet PY, Giraudier S, Nunes H, Valeyre D. Dramatic response of refractory sarcoidosis under ruxolitinib in a patient with associated JAK2-mutated polycythemia. *Eur Respir J.* (2018) 52:1801482. doi: 10.1183/13993003.01482-2018
100. Levraut M, Martis N, Viau P, Suarez F, Queyrel V. Refractory sarcoidosis-like systemic granulomatosis responding to ruxolitinib. *Ann Rheum Dis.* (2019) 78:1606–7. doi: 10.1136/annrheumdis-2019-215387
101. Wei JJ, Kallenbach LR, Kreider M, Leung TH, Rosenbach M. Resolution of cutaneous sarcoidosis after Janus kinase inhibitor therapy for concomitant polycythemia vera. *JAAD Case Rep.* (2019) 5:360–1. doi: 10.1016/j.jdcr.2019.02.006
102. Damsky W, Thakral D, Emeagwali N, Galan A, King B. Tofacitinib treatment and molecular analysis of cutaneous sarcoidosis. *N Engl J Med.* (2018) 379:2540–6. doi: 10.1056/NEJMoa1805958
103. Damsky W, Thakral D, McGeary MK, Leventhal J, Galan A, King B. Janus kinase inhibition induces disease remission in cutaneous sarcoidosis and granuloma annulare. *J Am Acad Dermatol.* (2020) 82:612–21. doi: 10.1016/j.jaad.2019.05.098
104. Damsky W, Young BD, Sloan B, Miller EJ, Obando JA, King B. Treatment of multiorgan sarcoidosis with tofacitinib. *ACR Open Rheumatol.* (2020) 2:106–9. doi: 10.1002/acr2.11112
105. Scheinberg M, Maluf F, Wagner J. Steroid-resistant sarcoidosis treated with baricitinib. *Ann Rheum Dis.* (2020) 79:1259–60. doi: 10.1136/annrheumdis-2020-217271
106. Keating GM. Apremilast: a review in psoriasis and psoriatic arthritis. *Drugs.* (2017) 77:459–72. doi: 10.1007/s40265-017-0709-1
107. Baughman RP, Judson MA, Ingledue R, Craft NL, Lower EE. Efficacy and safety of apremilast in chronic cutaneous sarcoidosis. *Arch Dermatol.* (2012) 148:262–4. doi: 10.1001/archdermatol.2011.301

Conflict of Interest: PC has received consulting and lecturing fees from Abbvie, Alnylam, Bristol-Myers Squibb, Gilead, Glaxo Smith Kline, Innotech, Janssen, Servier and Vifor. DS has received consulting and lecturing fees from from Medimmune, Abbvie, Bristol Meyer Squibb, Celgene, Sanofi, Roche, Servier, Gilead, AstraZeneca and Glaxo Smith Kline.

The remaining 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.

frontiers in Medicine

MINI REVIEW
published: 24 November 2020
doi: 10.3389/fmed.2020.594118

Sarcoidosis and Cancer: A Complex Relationship

*Thomas El Jammal[1], Michel Pavic[2], Mathieu Gerfaud-Valentin[1], Yvan Jamilloux[1,3] and Pascal Sève[1,4,5]**

[1] Internal Medicine Department, La Croix-Rousse Hospital, Lyon, France, [2] Medicine Department, Sherbrooke University, Sherbrooke, QC, Canada, [3] INSERM U1111, Center International de Recherche en Infectiologie/International Research Center in Infectiology (CIRI), University Claude-Bernard Lyon 1, Villeurbanne, France, [4] Pôle IMER, Hospices Civils de Lyon, Lyon, France, [5] HESPER EA 7425, Lyon University, University Claude-Bernard Lyon 1, Lyon, France

Sarcoidosis is a systemic disease of unknown etiology, characterized by the presence of non-caseating granulomas in various organs, mainly the lungs, and the lymphatic system. Since the individualization of sarcoidosis-lymphoma association by Brincker et al., the relationship between sarcoidosis or granulomatous syndromes and malignancies has been clarified through observational studies worldwide. Two recent meta-analyses showed an increased risk of neoplasia in sarcoidosis. The granulomatosis can also reveal malignancy, either solid or hematological, defining paraneoplastic sarcoidosis. Recent cancer immunotherapies, including immune checkpoint inhibitors (targeting PD-1, PD-L1, or CTLA-4) and BRAF or MEK inhibitors were also reported as possible inducers of sarcoidosis-like reactions. Sarcoidosis and neoplasia, especially lymphoma, can show overlapping presentations, thus making the diagnosis and treatment harder to deal with. There are currently no formal recommendations to guide the differential diagnosis workup between the evolution of lymphoma or a solid cancer and a granulomatous reaction associated with neoplasia. Thus, in atypical presentations (e.g., deeply impaired condition, compressive lymphadenopathy, atypical localization, unexplained worsening lymphadenopathy, or splenomegaly), and treatment-resistant disease, targeted biopsies on suspect localizations with histological examination could help the clinician to differentiate neoplasia from sarcoidosis. Pathological diagnosis could sometimes be challenging since very few tumor cells may be surrounded by massive granulomatous reaction. The sensitization of currently available diagnostic tools should improve the diagnostic accuracy, such as the use of more "cancer-specific" radioactive tracers coupled with Positron Emission Tomography scan.

Keywords: sarcoidosis, granulomatosis, neoplasia, lymphoma, checkpoint inhibitor

OPEN ACCESS

Edited by:
Mehdi Mirsaeidi, University of Miami, United States

Reviewed by:
Gonçalo Boleto, Hôpitaux Universitaires Pitié Salpêtrière, France
Mukunthan Murthi, John H. Stroger, Jr. Hospital of Cook County, United States
Viktor Korendovych, University Medical Center Göttingen, Germany

***Correspondence:**
Pascal Sève
pascal.seve@chu-lyon.fr

Specialty section:
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

Received: *12 August 2020*
Accepted: *30 October 2020*
Published: *24 November 2020*

Citation:
El Jammal T, Pavic M, Gerfaud-Valentin M, Jamilloux Y and Sève P (2020) Sarcoidosis and Cancer: A Complex Relationship. Front. Med. 7:594118. doi: 10.3389/fmed.2020.594118

INTRODUCTION

Sarcoidosis is a systemic disease of unknown etiology characterized by multiple granuloma formation in various sites, especially in the lungs, lymph nodes, liver, eyes, and skin (1). Although its etiology is still unknown; sarcoidosis is thought to be the consequence of an exaggerated immune response to an environmental trigger in a genetically predisposed patient. Mortality in sarcoidosis is mainly represented by respiratory failure due to pulmonary fibrosis, central nervous system involvement, and cardiac damage (2, 3). In a French epidemiological study of 2417 patients, the

cause of death was linked to neoplasms in 1.8% of the patients. Most of the time, the cause was a solid neoplasm (1.4%) while hematological malignancies represented 0.4% of the deaths in this series (4).

The occurrence of sarcoidosis or sarcoidosis-like reaction (SLR) in cancer patients has been known for several years, either through case reports or larger series (5–8). A localized granulomatous reaction can be found in draining lymph nodes of a solid tumor or in distant sites (mostly spleen, liver, or bone marrow) (6). The granulomatous reactions can also be found at the primary site of the tumor itself. Brincker reported that 3–7% of primary tumor sites may present with epithelioid cell granulomas (9). An SLR can also be found in the case of opportunistic infections in such a population (e.g., cryptococcosis, atypical mycobacteria, tuberculosis, nocardiosis, actinomycosis, …), in case of disseminated *Bacille de Calmette de Guérin* (BCG) infection after instillation of BCG therapy for bladder cancer, or in case of cancer-specific treatments [anti-programmed death-(ligand)1 (PD-(L)1)/anti-cytotoxic T-lymphocyte antigen 4 (CTLA4)/anti-MAP/ERK kinase (MEK)/anti-B-Raf proto-oncogene (BRAF)] (10). Moreover, typical sarcoidosis can occur in solid or hematological malignancies before, during, or after the onset of the disease. In those situations, the diagnosis may be challenging and requires a careful diagnostic workup. Herein, we summarize the specifics for sarcoidosis or SLR mimicking cancer, especially regarding positive and differential diagnosis of sarcoidosis or cancer in this particular association. We provide a brief literature review performed through the PubMed platform (https://www.pubmed.ncbi.nlm.nih.gov) using the keywords "sarcoidosis," "cancer," "lymphoma," "sarcoidosis-lymphoma syndrome", "sarcoid-like reaction" and "drug-induced sarcoidosis" that allowed us to find most of the references used to build this article.

INTERACTIONS BETWEEN SARCOIDOSIS AND CANCER

Cancer Risk in Sarcoidosis Patients

In previous series and cohorts, patients with sarcoidosis were found to have a higher risk of cancer compared to the general population, especially lymphoma (**Table 1**). Brincker et al. first reported an increased risk of cancer in sarcoidosis patients (7). Indeed, in this series, the risk of lymphoma was 11 times higher in the sarcoidosis group, compared to the expected risk of lymphoma in the general population. The risk of developing lung cancer was 3 times higher than the one expected in the general population. In the following years, several epidemiological studies analyzed the risk of cancer in sarcoidosis patients, with contradictory data according to the different types of cancer (lymphoma, testicular cancer, digestive cancers, breast cancer, etc.) (5, 8, 13, 17, 21, 23). Of note, Ungprasert et al. reported no increased risk of malignancy in a cohort study of patients with sarcoidosis compared with non-sarcoidosis patients but an increased risk of hematological malignancies in patients with sarcoidosis and extra thoracic involvement compared with those without extra thoracic involvement (26). An increased risk of cancer was noted (e.g., by 30–40%), especially skin cancers, hematological malignancies and leukemias. Despite conflicting data, the overall cancer risk in sarcoidosis patients is clearly higher than in the general population. Indeed, in two recent meta-analyses, the relative risk of developing cancer in patients with sarcoidosis was near 1.19–1.21 [with significant results in both studies ($p < 0.05$)] and the risk of developing hematological malignancies was even higher [RR = 1.92, 95% CI (1.41–2.62)] (27, 28). Lymphomas and particularly Hodgkin lymphomas (HL) were significantly more incident in sarcoidosis [RR = 2.91, 95% CI (1.21–6.98)] (28). There was also an increased incidence of skin cancers [RR = 2.00, 95% CI (1.69–2.36)] and especially non-melanoma skin cancers [RR = 2.29, 95% CI (1.88–2.78)]. These meta-analyses reported no increase in the risk of developing lung cancer. This interesting result is probably explained by a lower prevalence of smokers among sarcoidosis patients compared to the general population (29). Worth noting, there was no specific subgroup analysis of patients with sarcoidosis-related pulmonary fibrosis.

In a study about mortality in sarcoidosis patients in France, Jamilloux et al. reported that non-Hodgkin lymphoma was the most frequently declared cause of death in women when sarcoidosis was not the underlying cause of death, especially after the age of 50 (4). In another recent review of 115 cases, thyroid and breast cancers were the most frequently reported solid neoplasms (30).

Sarcoidosis and Sarcoid-Like Reactions in the Course of Cancer

Sarcoidosis and SLR can occur before, during or after cancer (31). While sarcoidosis is a well-defined condition, SLR is usually defined as non-caseating granulomatous reaction occurring under various conditions, which do not meet the diagnostic criteria for sarcoidosis (32). Many alternative diagnoses mimicking sarcoidosis can also be encountered in a neoplastic context (**Table 2**). In a series of 29 sarcoidosis patients with pre-existing cancer, Arish et al. described clinical and radiological features of granulomatosis (55). Histological features were not described in this article. Breast cancer and lymphoma were the most commonly observed malignancies. Sarcoidosis was frequently diagnosed at an early stage, possibly due to a more systematic follow-up with computed tomography (CT) and positron emission tomography in cancer patients. Radiological features were similar to those seen in classical sarcoidosis (mediastinal and hilar lymphadenopathy). Most patients were asymptomatic at the sarcoidosis diagnosis. The patients had bronchoalveolar fluid (BALF) lymphocytosis and granuloma on endobronchial biopsies and parenchymal biopsies, suggesting a pattern of systemic immune response rather than a local granulomatous response to neoplastic cells. In 43% of patients, the diagnosis of sarcoidosis was made more than 5 years after the diagnosis of cancer. De Charry et al. described the characteristics of granulomatosis occurring in the context of lymphoma (56). In this study, the patients developed granulomatosis at a median age of 60 while typical sarcoidosis usually occurs before 50 with a peak of incidence between 20 and 39 (1). Sarcoidosis

TABLE 1 | Risk of malignancy in sarcoidosis patients: cohort and case control studies.

References	Population	Relative risk of neoplasia
Anderson and Engels (11)	418 patients with HCL (case control, United States)	Patients with HCL were more likely to have a sarcoidosis antecedent (OR = 9.6 95% CI = 2.4–39.5)
Askling et al. (5)	474 sarcoidosis patients (cohort study in Sweden)	No increased risk of lymphoma, increased risk of melanoma (SIR = 1.6 95% CI = 1.0–2.3), and non-melanoma skin cancer (SIR = 2.8 95% CI = 2.0–3.8)
Blank et al. (12)	435 sarcoidosis patients (cohort)	Possible association (incidence 14%)
Boffetta et al. (13)	5,768 sarcoidosis patients (case-control study in USA)	No increased risk of malignancy (globally)
Brincker (14)	17 patients (case series) in Denmark	Increased risk of lymphoma
Brincker (15)	131 patients (case review)	Increased risk of lymphoma
Hemminki et al. (16)	5,149 sarcoidosis patients (cohort in Sweden)—female cancers	No global increased risk of malignancy
Ji et al. (17)	10,037 sarcoidosis patients (cohort study in Germany)	Increased risk of malignancy (SIR = 1.40) and cancer diagnosed later than 1 year of follow-up (SIR = 1.18). Increased risk of non-melanoma skin cancer, kidney and non-thyroid endocrine tumors, non-Hodgkin lymphoma, and leukemia
Kataoka et al. (18)	148 sarcoidosis patients (cohort)	Increased risk of leukemia, thyroid, and larynx cancer
Kristinsson et al. (19)	16 sarcoidosis patients in HL patients (case control study in Sweden)	Increased risk of having a sarcoidosis in patients with HL (OR = 3.7 95% CI = 1.9–7.4)
Landgren et al. (20)	7,476 patients (case control study in Sweden and Denmark)	Increased risk of lymphoma (OR = 14.1 95% CI = 5.4–36.8)
Le Jeune et al. (21)	1,153 sarcoidosis (case control study in UK)	Increased risk of malignancy (RR = 1.65 95% CI = 1.22–2.24) and especially skin cancer (RR = 1.86 95% CI = 1.11–3.11)
Mellemkjaer et al. (22)	50 sarcoidosis patients (case control study in Denmark)	Increased risk of NHL (OR = 1.9 95% CI = 1.3–2.7)
Rømer et al. (8)	555 sarcoidosis patients (cohort in Denmark)	No increased risk of malignancy (O/E ratio = 1.16 95% CI = 0.75–1.79)
Seersholm et al. (23)	254 sarcoidosis patients (cohort in Denmark)	No increased risk of malignancy (SIR = 1.4 95% CI = 0.99–2.0)
Smedby et al. (24)	3,055 patients with NHL Sweden and Denmark (case control)	No global increased risk of lymphoma in sarcoidosis patients
Søgaard et al. (25)	12,890 sarcoidosis patients (cohort study in Denmark)	Global increased risk of malignancy (SIR = 1.3 95% CI = 1.3–1.4), increased risk of lung cancer, tonsil cancer, and lymphoma. Of note, lung cancer risk seems to be more important in the first 3 months and then substantially decrease
Ungprasert et al. (26)	345 sarcoidosis patients (case control study in the USA)	No global increased risk of malignancy, but higher risk of hematological malignancies in patients with sarcoidosis and extra thoracic involvement (HR = 1.87 95% CI = 1.09–3.22)

CI, confidence interval; HCL, hairy cell leukemia; HR, hazard ratio; NHL, non-Hodgkin lymphoma; O/E, observed/expected; OR, odd ratio; RR, rate ratio; SIR, standardized incidence ratio; UK, United Kingdom; USA, United States of America.

explained 4 of the 25 patients' granulomatous manifestations in this study. Other etiologies were hematological malignancies ($n = 11$), tuberculosis ($n = 3$), allergy ($n = 1$), disseminated annular granuloma ($n = 1$), atypical inflammatory bowel disease ($n = 1$), and undetermined granulomatosis ($n = 4$). Likewise, London et al. described a series of 39 patients with sarcoidosis occurring in the setting of lymphoma (57). The median age at the onset of sarcoidosis was 49 years. Most patients had a history of high stage lymphoma (Ann Harbor III or IV) (74%). Most patients developed sarcoidosis after terminating lymphoma chemotherapy and all except two were considered in complete remission. In another series, Herron et al. reported that almost 60% of sarcoidosis cases occurring after a cancer were diagnosed within 1 year of cancer diagnosis (58).

During the course of cancer, an epithelioid granuloma can be found in regional lymph nodes or in distant metastases (6). Some authors have suggested that the presence of a cancer-associated SLR could be a marker of good prognosis, indicating a strong immune response to tumor cells (59–62). This type of reaction is mainly seen in lymphoma and testicular cancer (6, 63, 64). Other authors have provided conflicting data regarding other types of cancers, especially non-small lung carcinoma in which the presence of granulomas was not associated with better prognosis (65, 66).

Some cancer treatments can also induce granuloma formation. The description of various side effects, including sarcoidosis, has come with the recent advent of immune checkpoint inhibitors (ICI), as well as BRAF/MEK inhibitors. SLR were described either with ICI [anti-PD1: pembrolizumab (67), nivolumab (68); anti-PD-L1: atezolizumab (69), durvalumab (70), avelumab (71); anti-CTLA4: ipilimumab (72)] or with BRAF/MEK inhibitors [vemurafenib (73), dabrafenib (74) sometimes in combination with trametinib or cobimetinib (75)]. A review of the WHO pharmacovigilance database including 2425 drug-induced sarcoidosis was conducted in 2019. In this study, strong associations were found between SLR and several drugs including, pembrolizumab, nivolumab, ipilimumab ($n = 103$) along with dabrafenib, vemurafenib, trametinib, and cobimetinib ($n = 37$) (76). SLR disappeared with drug discontinuation in 17.7% of the cases. In a few

TABLE 2 | Non-exhaustive list of differential diagnosis of granulomatosis in pre-existing cancer patients.

Type of granulomatosis	Etiology	Risk factors
Opportunistic infectious granulomatosis (bacteria)	Tuberculosis	Immunosuppression [e.g., chemotherapy, hematological malignancy, …; (33)]
	Atypical mycobacteria	Immunosuppression [e.g., chemotherapy, hematological malignancies, …; (34)]
	Disseminated BCG infection (superficial bladder cancer)	BCG therapy for bladder cancer treatment could lead to disseminated BCG infection (35)
	Nocardiosis	Immunosuppression [e.g., hematological malignancies, diabetes, …; (36, 37)]
	Actinomycosis	Immunosuppression (38)
Opportunistic infectious granulomatosis (fungi)	Cryptococcosis	Immunosuppression [e.g., diabetes, cirrhosis, CD4 lymphopenia, stem cell transplant, chemotherapy, …; (39)]
	Candida spp.	Immunosuppression (chemotherapy) (40)
	Aspergillosis	Immunosuppression (41)
	Pneumocystosis	Immunosuppression [e.g., solid or hematological malignancies; (42)]
Opportunistic infectious granulomatosis (parasites)	Disseminated strongyloidosis	Immunosuppression [e.g., hematological malignancies; (43)]
	Toxoplasmosis	Immunosuppression [e.g., solid or hematological malignancies; (44)]
Opportunistic infectious granulomatosis (viruses)	HCV ++	HCV associated liver cancer or HCV associated lymphoma (45)
	HBV +/–	HBV associated liver cancer/hepatic granuloma (46)
	EBV	Lethal midline granuloma (47), lymphomatoid granulomatosis (48)
	CMV	Doughnut granuloma (immunosuppression) (49)
Drug-induced granulomatosis	ICI (nivolumab, pembrolizumab, cemiplimab, avelumab, durvalumab, ipilimumab)	Treatment of lung cancer, pharyngolaryngeal cancer, melanoma, renal cancer (10)
	BRAF/MEK inhibitors (dabrafenib, vemurafenib, trametinib, cobimetinib)	Treatment of lung cancer, melanoma (10)
	IFN-α	Formerly used in melanoma, kidney cancer, lymphoma (50)
	BCG therapy	Used in bladder cancer (35)
Histologic granuloma associated to neoplasia	Lymphoma (Hodgkin and non-Hodgkin lymphoma)	Proper to neoplasia (6)
	Solid neoplasia (testicular cancer, melanoma, breast cancer)	Proper to neoplasia (32)
	Immune restauration	Following aplasia (immune reconstitution leads to granuloma associated with T cell infiltrate) (51)
	Donor-acquired sarcoidosis	Following HSCT (52)
	Lymphomatoid granulomatosis	Proper to neoplasia (48)
Primary immunodeficiencies associated with neoplasia (especially lymphoma)	Common variable immunodeficiency, GATA2 mutations (Mono MAC syndrome)	Primary immunodeficiency related granulomatosis (53, 54)

BCG, Bacille de Calmette et Guérin; CMV, cytomegalovirus; EBV, Epstein Barr Virus; GATA2, GATA binding protein 2; HBV, hepatitis B virus; HCV, hepatitis C virus; HSCT, hematopoietic stem cell transplant; ICI, immune checkpoint inhibitors; IFN-α, interferon alpha; MAC, Mycobacterium avium complex.

patients, drug reintroduction triggered SLR recurrence. Stronger associations were found with other drugs such as tumor necrosis factor alpha inhibitors (TNFi) and especially soluble TNF receptor etanercept, interferon and PEG-interferon. These SLR can also mimic cancer progression or metastases. In a series of 45 patients treated with ICI, 10 developed SLR and 2 developed mediastinohilar lymphadenopathy misinterpreted as metastatic progression (77). Sarcoidosis and SLR have to be considered as differential diagnosis in patients with such treatments and biopsies have to be performed since no radiological nor biological marker is sufficiently specific to assess sarcoidosis diagnosis especially when presentation is suspicious [e.g., asymmetric lymph node enlargement; (78)]. The Society for Immunotherapy of Cancer Toxicity Management Working Group has made recommendations regarding immunotherapy-related SLR (79, 80). Corticosteroids (CS) have similar indications to those of "idiopathic" sarcoidosis. Pulmonary function testing and chest CT have to be performed in order to assess sarcoidosis severity. In case of DLCO decrease >20%, total lung capacity >10% or forced vital capacity >15%, persistent sarcoidosis-related symptoms, radiographic progression, or involvement of critical extrapulmonary organ systems or sarcoidosis related hypercalcemia, it is recommended to hold the treatment with ICI and add CS at 1 mg/kg/day dosage. CS tapering and withdrawal will depend on the clinical response.

Sarcoidosis has also been reported in hematopoietic stem cell transplant recipients. It was described either in allogenic (81–85) or autologous bone marrow transplantation (86, 87). In these cases, sarcoidosis was described in various organs such as lymph nodes (85), liver (88), lung, or lymph nodes (82). In some cases, a specific condition called "donor-acquired sarcoidosis" was described (52, 82, 84, 85). This condition refers to the occurrence of sarcoidosis in a solid organ or allogenic bone marrow transplant recipient when sarcoidosis is previously known in the donor.

In the past decade, TNFi were found to be an efficient way to treat sarcoidosis patients (89–92). The immunosuppressants can be linked to a theoretical increased risk of malignancy. However, in 2012, Maneiro et al. reported an incidence rate of cancer of 1 per 100 patients-year in sarcoidosis patients treated with TNFi (93). In a recent review, Adler et al. reported data from randomized and non-randomized clinical trials of TNFi in sarcoidosis patients. The malignancies occurred in <1% of the patients (94). In comparison with other inflammatory diseases, TNFi does not seem to increase the risk of cancer in sarcoidosis (95).

The Sarcoidosis-Lymphoma Syndrome

Sarcoidosis-lymphoma association was first described by Brincker in a series of 46 patients (14). In this series, sarcoidosis-lymphoma syndrome was defined as a condition in which sarcoidosis occurred several years before the diagnosis of lymphoma. Most frequently the diagnosis of sarcoidosis was made after 40 years old and the most frequent type of lymphoma was HL. On the contrary, Papanikolaou and Sharma have found that NHL were the most common lymphomas. Interestingly, the development of new lymphadenopathy or new splenic involvement were the main symptoms revealing lymphoma in this series. These patients were on average 10 years older at the sarcoidosis diagnosis compared to unselected patients in most series (96, 97). Compared to the general population, sarcoidosis patients had a 5.5-fold higher risk of developing lymphoma (14). In a recent monocentric study of patients with sarcoidosis-lymphoma syndrome compared to unselected sarcoidosis patients, significant differences between initial or follow-up patients' characteristics have been evidenced especially regarding angiotensin-converting enzyme (ACE) blood levels that have proved to be higher in sarcoidosis-lymphoma syndrome, while the sarcoidosis alone group was more likely to have lung involvement, a restrictive ventilatory defect and a higher relapse rate (98).

Most of the time, lymphoma occurs 2–8 years after the sarcoidosis diagnosis, preferentially in patients with a chronic course of the disease (14). CD4/CD8 lymphocyte ratio in BALF is also higher in patients with sarcoidosis-lymphoma syndrome compared to unselected patients (98). For example, B-cell activating factor (BAFF) levels are elevated in patients with sarcoidosis and are correlated with ACE levels (99). Elevation of pro-proliferative cytokines such as BAFF for B lymphocytes could be a possible explanation for the emergence of clonal proliferation in sarcoidosis patients in comparison with other autoimmune diseases (100).

WHEN SHOULD WE LOOK FOR NEOPLASIA IN PATIENTS WITH SARCOIDOSIS?

Sarcoidosis diagnosis requires three major conditions: (1) a compatible clinical/radiological presentation, (2) evidence of granulomas on a biopsy sample, and (3) exclusion of differential diagnoses (101).

Although rarely observed, physicians should be aware that sarcoidosis can present itself as a pseudo tumoral condition such as miliary nodules, peritoneal involvement, and symptomatic osteolytic or osteoblastic lesions (102–104).

Other red flags should alert the clinician about the atypical nature of sarcoidosis or the possibility of underlying neoplasia [e.g., impaired general condition, compressive phenomena, hemoptysis, refractory disease; (31, 32, 105)]. Atypical radiological manifestations should also be considered. For example, unilateral, compressive or necrotizing lymph nodes are not usually seen during the course of sarcoidosis. Isolated mediastinal lymphadenopathy without hilar lymph node enlargement, non-lymphatic diffuse lung micronodules, cavitary mass on chest X-ray should also be considered as suspicious for a differential diagnosis of sarcoidosis (106, 107). Broadly speaking, these atypical presentations should encourage the clinician to pay attention to other causes of granulomatosis, including lymphoma, infectious granulomatosis (tuberculosis, leprosy, syphilis, brucellosis, Q fever, Whipple's disease), common variable immunodeficiency, and drug-induced sarcoidosis (108).

In a patient with previously known sarcoidosis, the occurrence of atypical manifestations (e.g., peritoneal or gut involvement) or new organ involvement, and refractory disease which is defined as a disease in which a 2nd line treatment is not sufficient to achieve satisfying disease control or satisfying CS tapering, must lead to histological confirmation to rule out opportunistic infection and lymphoma, especially (31, 102, 108).

Recently, the American thoracic society (ATS) provided new guidelines concerning sarcoidosis diagnosis (32). In a large review of 16 studies enrolling a total of 556 patients with suspected stage I sarcoidosis, 85% of sampling procedures with histological examination confirmed the diagnosis of sarcoidosis. In 11% of the cases, histology was inconclusive, and in 2% of the cases, a differential diagnosis was made. Among differential diagnoses, 25% were lymphoma. On the basis of this work, ATS reminds that the diagnosis of sarcoidosis does not only rely on histological findings but also on compatible presentation and exclusion of differential diagnoses.

As noted above, sarcoidosis patients have a possibly increased risk of malignancy, either solid or hematological. The increase of the risk of developing solid neoplasia in the course of sarcoidosis seems to be less important than the risk of developing hematological malignancies such as lymphoma (7, 19, 20). Again, this emphasizes the attention the clinician should pay to any atypical symptom or presentation in a sarcoidosis patient since delayed diagnosis of cancer may impact the patient's prognosis.

HOW TO DIAGNOSE NEOPLASIA IN PATIENTS WITH SARCOIDOSIS?

A histological examination is warranted to accurately diagnose a patient with sarcoidosis or sarcoid-like reaction to neoplasia. The neoplastic cells can be found on histological examination within a granulomatous reaction. In case of atypical sarcoidosis, lymphocytes phenotyping should be performed in order to rule out clonality. Full examination of included samples (which increases sensibility of histological examination) and complementary immunohistochemical staining could also be helpful. A specific subtype of HL, the necrotic granuloma-like HL, as well as some T-cell lymphoma (NHL) could be misdiagnosed as non-neoplastic granuloma, such as sarcoidosis, because of an important tumor-related sarcoid reaction and only careful histologic examination can help to rectify the diagnosis (109–111). Among the neoplasia which can mimic sarcoidosis, special attention should be paid to lymphomatoid granulomatosis (LYG). LYG is a lymphoproliferative disorder associated with Epstein Barr virus (EBV). The aggressive behavior of the tumor is represented by its metastatic potential. The classic histological pattern of LYG is a coexistence of granulomatous inflammation made of large atypical EBV-positive B cells, T cells, necrosis, and lymphocytic vasculitis (112). Lung localization and skin involvement may mimic sarcoidosis. Almost 100% of patients present with pulmonary involvement consisting most of the time in pulmonary nodules. Skin nodules are also part of the clinical presentation. They take the form of subcutaneous nodules, most of the time erythematous and painful (113). Histological diagnosis is difficult if the pathologist is not aware of the suspected diagnosis of LYG or unfamiliar with this condition. Classical histological results consist of a mononucleated infiltrate with large and small lymphocytes invading vascular walls and a variable amount of atypical CD20+ B cells among numerous small CD3+ T cells.

Although some imaging results may point to a diagnosis of neoplasia [e.g., asymmetric lymphadenopathy, hypermetabolism of extrathoracic lymph nodes; (78)], 18-fluorodeoxyglucose (18-FDG) uptake on PET-CT is unable to differentiate malignant from non-malignant hypermetabolism. Currently, 18-FDG PET-CT may be used to identify the best biopsy sites, which may result in the observation of tumor cells or specific granuloma characteristics which suggest other causes of granulomatosis [e.g., loosely organized collections of phagocytes or multinucleated giant cells, extensive or dirty necrosis, or palisading granulomas; (114, 115)]. Other radiotracers or techniques used with PET-CT may be interesting in differentiating tumoral hypermetabolism from non-malignant hypermetabolism. Dual time point 18-FDG PET-CT with delayed acquisition sequences and 18F-3′-Fluoro-3′-deoxythymidine (18F-FLT) PET-CT could help in distinguishing malignant from non-malignant lesions but few studies are available and their roles in improving the diagnostic performances of PET-CT remain to be precised (116, 117).

Magnetic resonance imaging (MRI) changes could also be helpful in distinguishing sarcoidosis lesions from tumoral localizations. Although conventional MRI is insufficient to distinguish malignant bone lesions from bone sarcoidosis (118), Conte et al. reported one case where the differential diagnosis between sarcoidosis and metastasis was made using whole body diffusion MRI (119). In this patient, the hypersignal on diffusion sequences contrasted with a decreased signal in apparent diffusion coefficient sequences that was considered to be too low to be compatible with neoplastic origin.

Finally, specific biomarkers have been proposed to ease the diagnosis, especially in germ-cell tumors. In such cases, the elevation of serum levels of α-fetoprotein, human chorionic gonadotropin and lactate dehydrogenase may help guide the diagnosis (32). No suggestion has been made regarding other types of cancer, probably due to the lack of specificity of tumor markers in these settings.

CONCLUSION

Granulomatosis and cancer can coexist in various clinical situations that the clinician should be aware of. The risk of developing solid neoplasia or hematological malignancies, especially lymphomas, is increased in sarcoidosis patients. Sarcoidosis-lymphoma syndrome has to be considered in patients with previously known sarcoidosis and unexplained recurrence of deep or peripheral lymph nodes enlargement. Any atypical and unexplained symptom mimicking a sarcoidosis flare should encourage the clinician to be careful to differential diagnosis.

The granulomatous reactions are not uncommon in the course of solid neoplasia and hematological malignancies. Recent therapeutic advances in cancer treatment, especially the emergence of immunotherapy with ICI, have reminded the possibility of drug induced SLR as it was previously known with older therapies (e.g., interferon). CS may help control ICI-induced SLR without holding cancer treatments.

Differentiating sarcoidosis from cancer-associated granulomatosis is difficult. Atypical presentation of sarcoidosis (atypical organ involvement or refractory disease) may alert the clinician. There is currently no alternative to the histological examination to differentiate sarcoidosis from neoplasia. New radiotracers (18F-FLT) and new acquisition techniques (dual time point PET CT) are promising but currently not available in routine care.

A careful and rigorous diagnosis process is required when encountering granulomatosis, on the one hand, because of the increased risk of neoplasia in sarcoidosis patients and, on the other hand, because of the sarcoidosis-like presentation of neoplasia. Discussing with the pathologist in order to sensitize the diagnosis (full examination of included samples, complementary immunohistochemical staining, search for clonality) is fundamental.

The sarcoidosis patients are also susceptible to present neoplasia as the general population. Diagnosis can be difficult maybe due to a greater propension to present a granulomatous reaction compared to the general population. Here again, it is

essential to share and to discuss clinical presentation to ease the pathologist's work.

AUTHOR CONTRIBUTIONS

TE contributed to bibliography, most of writing, and reviewing of the manuscript. PS, YJ, MG-V, and MP contributed to reviewing and writing the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was funded by the Association pour les Soins, la Formation et la Recherche en Médecine Interne.

REFERENCES

1. Valeyre D, Prasse A, Nunes H, Uzunhan Y, Brillet P-Y, Müller-Quernheim J. Sarcoidosis. *Lancet.* (2014) 383:1155–67. doi: 10.1016/S0140-6736(13)60680-7
2. Nardi A, Brillet P-Y, Letoumelin P, Girard F, Brauner M, Uzunhan Y, et al. Stage IV sarcoidosis: comparison of survival with the general population and causes of death. *Eur Respir J.* (2011) 38:1368–73. doi: 10.1183/09031936.00187410
3. Swigris JJ, Olson AL, Huie TJ, Fernandez-Perez ER, Solomon J, Sprunger D, et al. Sarcoidosis-related Mortality in the United States from 1988 to 2007. *Am J Respir Crit Care Med.* (2011) 183:1524–30. doi: 10.1164/rccm.201010-1679OC
4. Jamilloux Y, Maucort-Boulch D, Kerever S, Gerfaud-Valentin M, Broussolle C, Eb M, et al. Sarcoidosis-related mortality in France: a multiple-cause-of-death analysis. *Eur Respir J.* (2016) 48:1700–9. doi: 10.1183/13993003.00457-2016
5. Askling J, Grunewald J, Eklund A, Hillerdal G, Ekbom A. Increased risk for cancer following sarcoidosis. *Am J Respir Crit Care Med.* (1999) 160:1668–72. doi: 10.1164/ajrccm.160.5.9904045
6. Brincker H. Sarcoid reactions and sarcoidosis in Hodgkin's disease and other malignant lymphomata. *Br J Cancer.* (1972) 26:120–8. doi: 10.1038/bjc.1972.18
7. Brincker H, Wilbek E. The incidence of malignant tumours in patients with respiratory sarcoidosis. *Br J Cancer.* (1974) 29:247–51. doi: 10.1038/bjc.1974.64
8. Rømer FK, Hommelgaard P, Schou G. Sarcoidosis and cancer revisited: a long-term follow-up study of 555 Danish sarcoidosis patients. *Eur Respir J.* (1998) 12:906–12. doi: 10.1183/09031936.98.12040906
9. Brincker H. Sarcoid reactions in malignant tumours. *Cancer Treat Rev.* (1986) 13:147–56. doi: 10.1016/0305-7372(86)90002-2
10. Rubio-Rivas M, Moreira C, Marcoval J. Sarcoidosis related to checkpoint and BRAF/MEK inhibitors in melanoma. *Autoimm Rev.* (2020) 19:102587. doi: 10.1016/j.autrev.2020.102587
11. Anderson LA, Engels EA. Autoimmune conditions and hairy cell leukemia: an exploratory case-control study. *J Hematol Oncol.* (2010) 3:35. doi: 10.1186/1756-8722-3-35
12. Blank N, Lorenz H-M, Ho AD, Witzens-Harig M. Sarcoidosis and the occurrence of malignant diseases. *Rheumatol Int.* (2014) 34:1433–1439. doi: 10.1007/s00296-014-2983-5
13. Boffetta P, Rabkin CS, Gridley G. A cohort study of cancer among sarcoidosis patients. *Int J Cancer.* (2009) 124:2697–700. doi: 10.1002/ijc.24261
14. Brincker H. The sarcoidosis-lymphoma syndrome. *Br J Cancer.* (1986) 54:467–73. doi: 10.1038/bjc.1986.199
15. Brincker H. Coexistence of sarcoidosis and malignant disease: causality or coincidence? *Sarcoidosis.* (1989) 6:31–43.
16. Hemminki K, Liu X, Ji J, Försti A, Sundquist J, Sundquist K. Effect of autoimmune diseases on risk and survival in female cancers. *Gynecol Oncol.* (2012) 127:180–5. doi: 10.1016/j.ygyno.2012.07.100
17. Ji J, Shu X, Li X, Sundquist K, Sundquist J, Hemminki K. Cancer risk in hospitalized sarcoidosis patients: a follow-up study in Sweden. *Ann Oncol.* (2009) 20:1121–6. doi: 10.1093/annonc/mdn767
18. Kataoka M, Nakata Y, Hioka T, Hosoya S, Shiomi K, Nishizaki H, et al. [Malignancies in patients with sarcoidosis]. *Nihon Kyobu Shikkan Gakkai Zasshi.* (1992) 30:598–603.
19. Kristinsson S, Landgren O, Sjöberg J, Turesson I, Björkholm M, Goldin L. Autoimmunity and risk for Hodgkin's lymphoma by subtype. *Haematologica.* (2009) 94:1468–9. doi: 10.3324/haematol.2009.008094
20. Landgren O, Engels EA, Pfeiffer RM, Gridley G, Mellemkjaer L, Olsen JH, et al. Autoimmunity and susceptibility to Hodgkin lymphoma: a population-based case–control study in Scandinavia. *JNCI.* (2006) 98:1321–30. doi: 10.1093/jnci/djj361
21. Le Jeune I, Gribbin J, West J, Smith C, Cullinan P, Hubbard R. The incidence of cancer in patients with idiopathic pulmonary fibrosis and sarcoidosis in the UK. *Respir Med.* (2007) 101:2534–40. doi: 10.1016/j.rmed.2007.07.012
22. Mellemkjaer L, Pfeiffer RM, Engels EA, Gridley G, Wheeler W, Hemminki K, et al. Autoimmune disease in individuals and close family members and susceptibility to non-Hodgkin's lymphoma. *Arthritis Rheum.* (2008) 58:657–66. doi: 10.1002/art.23267
23. Seersholm N, Vestbo J, Viskum K. Risk of malignant neoplasms in patients with pulmonary sarcoidosis. *Thorax.* (1997) 52:892–94.
24. Smedby KE, Hjalgrim H, Askling J, Chang ET, Gregersen H, Porwit-MacDonald A, et al. Autoimmune and chronic inflammatory disorders and risk of non-Hodgkin lymphoma by subtype. *JNCI.* (2006) 98:51–60. doi: 10.1093/jnci/djj004
25. Søgaard KK, Sværke C, Thomsen RW, Nørgaard M. Sarcoidosis and subsequent cancer risk: a Danish nationwide cohort study. *Eur Respir J.* (2015) 45:269–72. doi: 10.1183/09031936.00084414
26. Ungprasert P, Crowson CS, Matteson EL. Risk of malignancy among patients with sarcoidosis: a population-based cohort study: sarcoidosis and risk of malignancy. *Arthr Care Res.* (2017) 69:46–50. doi: 10.1002/acr.22941
27. Ungprasert P, Srivali N, Wijarnpreecha K, Thongprayoon C, Cheungpasitporn W, Knight EL. Is the incidence of malignancy increased in patients with sarcoidosis? A systematic review and meta-analysis: malignancy and sarcoidosis. *Respirology.* (2014) 19:993–8. doi: 10.1111/resp.12369
28. Bonifazi M, Bravi F, Gasparini S, La Vecchia C, Gabrielli A, Wells AU, et al. Sarcoidosis and cancer risk. *Chest.* (2015) 147:778–91. doi: 10.1378/chest.14-1475
29. Valeyre D, Soler P, Clerici C, Pré J, Battesti JP, Georges R, et al. Smoking and pulmonary sarcoidosis: effect of cigarette smoking on prevalence, clinical manifestations, alveolitis, and evolution of the disease. *Thorax.* (1988) 43:516–24.
30. Spiekermann C, Kuhlencord M, Huss S, Rudack C, Weiss D. Coexistence of sarcoidosis and metastatic lesions: a diagnostic and therapeutic dilemma. *Oncol Lett.* (2017) 14:7643–52. doi: 10.3892/ol.2017.7247
31. Seve P, Jamilloux Y, Gerfaud-Valentin M, El-Jammal T, Pavic M. Faut-il rechercher un cancer après la découverte d'une granulomatose inexpliquée? *La Revue de Médecine Interne.* (2019) 40:487–90. doi: 10.1016/j.revmed.2019.05.006
32. Crouser ED, Maier LA, Wilson KC, Bonham CA, Morgenthau AS, Patterson KC, et al. Diagnosis and Detection of Sarcoidosis. An Official American Thoracic Society Clinical Practice Guideline. *Am J Respir Crit Care Med.* (2020) 201:e26–51. doi: 10.1164/rccm.202002-0251ST
33. Simonsen DF, Farkas DK, Horsburgh CR, Thomsen RW, Sørensen HT. Increased risk of active tuberculosis after cancer diagnosis. *J Infect.* (2017) 74:590–8. doi: 10.1016/j.jinf.2017.03.012
34. Reilly AF, McGowan KL. Atypical mycobacterial infections in children with cancer. *Pediatr Blood Cancer.* (2004) 43:698–702. doi: 10.1002/pbc.20061
35. Pérez-Jacoiste Asín MA, Fernández-Ruiz M, López-Medrano F, Lumbreras C, Tejido Á, San Juan R, et al. Bacillus Calmette-Guérin (BCG) infection following intravesical BCG administration as adjunctive therapy for bladder cancer: incidence, risk factors, and outcome in a single-institution series and review of the literature. *Medicine.* (2014) 93:236–54. doi: 10.1097/MD.0000000000000119

36. Apisarnthanarak A, Razavi B, Bailey T. Disseminated *Nocardia asteroides* presenting as pulmonary non-caseating granulomas in a patient with waldenstrom macroglobulinemia. *Infection.* (2002) 30:38–40. doi: 10.1007/s15010-001-2048-z
37. Singh A, Chhina D, Soni R, Kakkar C, Sidhu U. Clinical spectrum and outcome of pulmonary nocardiosis: 5-year experience. *Lung India.* (2016) 33:398. doi: 10.4103/0970-2113.184873
38. Sei H, Nouta J, Miyaji S, Hato N. Post-transplantation laryngeal actinomycosis. *Auris Nasus Larynx.* (2019) 46:917–920. doi: 10.1016/j.anl.2018.12.006
39. Nematollahi S, Dioverti-Prono V. Cryptococcal infection in haematologic malignancies and haematopoietic stem cell transplantation. *Mycoses.* (2020) 63:1033–46. doi: 10.1111/myc.13153
40. Misme-Aucouturier B, Albassier M, Alvarez-Rueda N, Le Pape P. Specific human and candida cellular interactions lead to controlled or persistent infection outcomes during granuloma-like formation. *Infect Immun.* (2017) 85:e00807-16: doi: 10.1128/IAI.00807-16
41. Dreizen S, McCredie KB. Orofacial aspergillosis in acute leukemia. (1985) 59:499–504.
42. Hartel PH, Shilo K, Klassen-Fischer M, Neafie RC, Franks TJ. Granulomatous reaction to *Pneumocystis jirovecii:* clinicopathologic review of 20 cases. *Am J Surg Pathol.* (2010) 34:730–4. doi: 10.1097/PAS.0b013e3181d9f16a
43. Peters L, McCarthy AE, Faught C. Secondary *Strongyloides stercoralis* prophylaxis in patients with human T-cell lymphotropic virus type 1 infection: report of two cases. *Int J Infect Dis.* (2009) 13:e501–3. doi: 10.1016/j.ijid.2009.02.009
44. Israelski DM, Remington JS. Toxoplasmosis in patients with cancer. *Clin Infect Dis.* (1993) 17:S423–35. doi: 10.1093/clinids/17.Supplement_2.S423
45. Gaya DR. Hepatic granulomas: a 10 year single centre experience. *J Clin Pathol.* (2003) 56:850–3. doi: 10.1136/jcp.56.11.850
46. Tahan V, Ozaras R, Lacevic N, Ozden E, Yemisen M, Ozdogan O, et al. Prevalence of hepatic granulomas in chronic hepatitis B. *Dig Dis Sci.* (2004) 49:1575–7. doi: 10.1023/B:DDAS.0000043366.18578.15
47. Harabuchi Y, Yamanaka N, Kataura A, Imai S, Kinoshita T, Osato T. Epstein-Barr virus in nasal T-cell lymphomas in patients with lethal midline granuloma. *Lancet.* (1990) 335:128–30. doi: 10.1016/0140-6736(90)90002-M
48. Alexandra G, Claudia G. Lymphomatoid granulomatosis mimicking cancer and sarcoidosis. *Ann Hematol.* (2019) 98:1309–11. doi: 10.1007/s00277-018-3505-4
49. Dejhansathit S, Miller AM, Suvannasankha A. Multiple 'doughnut' granulomas in a liver transplant patient with CMV reactivation. *BMJ Case Rep.* (2018) 11:e227252. doi: 10.1136/bcr-2018-227252
50. Otte H-G, Hartig C, Stadler R. Sarkoidose bei Interferon-alpha-Therapie. *Der Hautarzt.* (1997) 48:482–7. doi: 10.1007/s001050050614
51. Miceli MH, Maertens J, Buvé K, Grazziutti M, Woods G, Rahman M, et al. Immune reconstitution inflammatory syndrome in cancer patients with pulmonary aspergillosis recovering from neutropenia: Proof of principle, description, and clinical and research implications. *Cancer.* (2007) 110:112–20. doi: 10.1002/cncr.22738
52. Padilla ML, Schilero GJ, Teirstein AS. Donor-acquired sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* (2002) 19:18–24.
53. Bouvry D, Mouthon L, Brillet P-Y, Kambouchner M, Ducroix J-P, Cottin V, et al. Granulomatosis-associated common variable immunodeficiency disorder: a case-control study versus sarcoidosis. *Eur Respir J.* (2013) 41:115–22. doi: 10.1183/09031936.00189011
54. Overbeek MJ, van de Loosdrecht A, Vonk-Noordegraaf A. Granulomatous lung disease in a patient with a family history of hematological disorders. *Sarcoidosis Vasc Diffuse Lung Dis.* (2014) 31:350–3.
55. Arish N, Kuint R, Sapir E, Levy L, Abutbul A, Fridlender Z, et al. Characteristics of sarcoidosis in patients with previous malignancy: causality or coincidence? *Respiration.* (2017) 93:247–52. doi: 10.1159/000455877
56. de Charry F, Sadoune K, Sebban C, Rey P, de Parisot A, Nicolas-Virelizier E, et al. Association lymphome et granulomatose : à propos d'une série de cas. *La Revue de Médecine Interne.* (2016) 37:453–9. doi: 10.1016/j.revmed.2015.10.344
57. London J, Grados A, Fermé C, Charmillon A, Maurier F, Deau B, et al. Sarcoidosis occurring after lymphoma: report of 14 patients and review of the literature. *Medicine.* (2014) 93:e121. doi: 10.1097/MD.0000000000000121
58. Herron M, Chong SG, Gleeson L, Nicholson S, Fahy RJ. Paraneoplastic sarcoidosis: a review. *QJM.* (2020) 113:17–9. doi: 10.1093/qjmed/hcz207
59. O'Connell MJ. Epithelioid granulomas in Hodgkin disease. A favorable prognostic sign? *JAMA.* (1975) 233:886–9. doi: 10.1001/jama.233.8.886
60. Sacks EL, Donaldson SS, Gordon J, Dorfman RF. Epithelioid granulomas associated with Hodgkin's disease. Clinical correlations in 55 previously untreated patients. *Cancer.* (1978) 41:562–7.
61. Takeuchi H, Suchi T, Suzuki R, Sato T. Histological study of immune parameters of regional lymph nodes of gastric cancer patients. *Gan.* (1982) 73:420–8.
62. Steinfort DP, Irving LB. Sarcoidal reactions in regional lymph nodes of patients with non-small cell lung cancer: incidence and implications for minimally invasive staging with endobronchial ultrasound. *Lung Cancer.* (2009) 66:305–8. doi: 10.1016/j.lungcan.2009.03.001
63. Kaikani W, Boyle H, Chatte G, de la Roche E, Errihani H, Droz J-P, et al. Sarcoid-like granulomatosis and testicular germ cell tumor: the 'great imitator.' *Oncology.* (2011) 81:319–24. doi: 10.1159/000334239
64. Rayson D, Burch PA, Richardson RL. Sarcoidosis and testicular carcinoma. *Cancer.* (1998) 83:337–43
65. Kamiyoshihara M, Hirai T, Kawashima O, Ishikawa S, Morishita Y. Sarcoid reactions in primary pulmonary carcinoma: report of seven cases. *Oncol Rep.* (1998) 5:177–80.
66. Tomimaru Y, Higashiyama M, Okami J, Oda K, Takami K, Kodama K, et al. Surgical results of lung cancer with sarcoid reaction in regional lymph nodes. *Jpn J Clin Oncol.* (2007) 37:90–5. doi: 10.1093/jjco/hyl141
67. Cotliar J, Querfeld C, Boswell WJ, Raja N, Raz D, Chen R. Pembrolizumab-associated sarcoidosis. *JAAD Case Rep.* (2016) 2:290–3. doi: 10.1016/j.jdcr.2016.06.004
68. Danlos F-X, Pagès C, Baroudjian B, Vercellino L, Battistella M, Mimoun M, et al. Nivolumab-induced sarcoid-like granulomatous reaction in a patient with advanced melanoma. *Chest.* (2016) 149:e133–6. doi: 10.1016/j.chest.2015.10.082
69. Mitchell MA, Hogan K, Amjadi K. Atezolizumab-induced sarcoid-like granulomatous reaction in a patient with urothelial cell carcinoma. *Immunotherapy.* (2018) 10:1189–92. doi: 10.2217/imt-2018-0035
70. Rousseau PM, Raimbourg J, Robert M, Dansette D, Dréno B, Peuvrel L, Supported by GESTIM Nantes group of cutaneous adverse events induced by cancer treatments. First case of cutaneous sarcoidosis within tattoos under durvalumab. *Int J Dermatol.* (2019) 58:e168–70. doi: 10.1111/ijd.14484
71. Tun Min S, Nordman IIC, Tran HA. Hypercalcaemia due to sarcoidosis during treatment with avelumab for metastatic Merkel cell carcinoma. *Case Rep Oncol.* (2019) 12:639–43. doi: 10.1159/000502285
72. Nandavaram S, Nadkarni A. Ipilimumab-induced sarcoidosis and thyroiditis. *Am J Ther.* (2018) 25:e379–80. doi: 10.1097/MJT.0000000000000545
73. Lheure C, Kramkimel N, Franck N, Laurent-Roussel S, Carlotti A, Queant A, et al. Sarcoidosis in patients treated with vemurafenib for metastatic melanoma: a paradoxical autoimmune activation. *Dermatology.* (2015) 231:378–84. doi: 10.1159/000439400
74. Jansen YJ, Janssens P, Hoorens A, Schreuer MS, Seremet T, Wilgenhof S, et al. Granulomatous nephritis and dermatitis in a patient with BRAF V600E mutant metastatic melanoma treated with dabrafenib and trametinib: *Melanoma Res.* (2015) 25:550–554. doi: 10.1097/CMR.0000000000000186
75. Assan F, Schlemmer F, Assie J-B, Mahevas M, Sustronck P, Ortonne N, et al. Atypical systemic sarcoid-like granulomatosis in two patients treated with BRAF and MEK inhibitors. *Eur J Dermatol.* (2019) 29:556–7. doi: 10.1684/ejd.2019.3640
76. Cohen Aubart F, Lhote R, Amoura A, Valeyre D, Haroche J, Amoura Z, et al. Drug-induced sarcoidosis: an overview of the WHO pharmacovigilance database. *J Int Med.* (2019) 288: 356–62. doi: 10.1111/joim.12991
77. Chorti E, Kanaki T, Zimmer L, Hadaschik E, Ugurel S, Gratsias E, et al. Drug-induced sarcoidosis-like reaction in adjuvant immunotherapy: increased rate and mimicker of metastasis. *Eur J Cancer.* (2020) 131:18–26. doi: 10.1016/j.ejca.2020.02.024

78. Koo HJ, Kim MY, Shin SY, Shin S, Kim S-S, Lee SW, et al. Evaluation of mediastinal lymph nodes in sarcoidosis, sarcoid reaction, and malignant lymph nodes using CT and FDG-PET/CT: *Medicine.* (2015) 94:e1095. doi: 10.1097/MD.0000000000001095
79. Brahmer JR, Lacchetti C, Schneider BJ, Atkins MB, Brassil KJ, Caterino JM, et al. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American Society of Clinical Oncology Clinical Practice Guideline. *JCO.* (2018) 36:1714–68. doi: 10.1200/JCO.2017.77.6385
80. Puzanov I, Diab A, Abdallah K, Bingham CO, Brogdon C, Dadu R, et al. Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. *J Immunother Cancer.* (2017) 5:95. doi: 10.1186/s40425-017-0300-z
81. Heyll A, Meckenstock G, Schneider W. Possible transmission of sarcoidosis via allogenic bone marrow transplantation. *Bone Marrow Transpl.* (1994) 14:161–4.
82. Morita R, Hashino S, Kubota K, Onozawa M, Kahata K, Kondo T, et al. Donor cell-derived sarcoidosis after allogenic BMT. *Bone Marrow Transpl.* (2009) 43:507–8. doi: 10.1038/bmt.2008.340
83. Bhagat R, Rizzieri DA, Vredenburgh JJ, Chao NJ, Folz RJ. Pulmonary sarcoidosis following stem cell transplantation. *Chest.* (2004) 126:642–4. doi: 10.1378/chest.126.2.642
84. Kushima H, Ishii H, Ikewaki J, Takano K, Ogata M, Kadota J. Sarcoidosis in donor-derived tissues after haematopoietic stem cell transplantation. *Eur Respir J.* (2013) 41:1452–3. doi: 10.1183/09031936.00136112
85. Schattenberg AVMB, Baynes C, van Dijk MCRF, Koster A, van Cleef PHJ, Preijers FWMB, et al. A mediastinal mass after donor lymphocyte infusion for relapse of chronic myeloid leukemia after allogeneic stem cell transplantation. *Leukem Lymphoma.* (2006) 47:1188–90. doi: 10.1080/10428190500519410
86. Marchal A, Charlotte F, Maksud P, Haroche J, Lifferman F, Miyara M, et al. Sarcoidosis flare after autologous stem cell transplantation: an immune paradox? *Rev Med Interne.* (2017) 38:619–22. doi: 10.1016/j.revmed.2017.01.001
87. Teo M, McCarthy JE, Brady AP, Curran DR, Power DG. A case of sarcoidosis in a patient with testicular cancer post stem cell transplant. *Acta Oncologica.* (2013) 52:869–70. doi: 10.3109/0284186X.2012.689854
88. Gooneratne L, Lim ZY, Vivier A du, Salisbury JR, Knisely AS, Ho AYL, et al. Sarcoidosis as an unusual cause of hepatic dysfunction following reduced intensity conditioned allogeneic stem cell transplantation. *Bone Marrow Transplant.* (2007) 39:511–2. doi: 10.1038/sj.bmt.1705606
89. Jamilloux Y, Cohen-Aubart F, Chapelon-Abric C, Maucort-Boulch D, Marquet A, Pérard L, et al. Efficacy and safety of tumor necrosis factor antagonists in refractory sarcoidosis: a multicenter study of 132 patients. *Sem Arthr Rheum.* (2017) 47:288–94. doi: 10.1016/j.semarthrit.2017.03.005
90. Barba T, Marquet A, Bouvry D, Cohen-Aubart F, Ruivard M, Debarbieux S, et al. Efficacy and safety of infliximab therapy in refractory upper respiratory tract sarcoidosis: experience from the STAT registry. *Sarcoidosis Vasc Diffuse Lung Dis.* (2018) 34:343–51. doi: 10.36141/svdld.v34i4.5817
91. Marquet A, Chapelon-Abric C, Maucort-Boulch D, Cohen-Aubart F, Pérard L, Bouillet L, et al. Efficacy and safety of TNF antagonists in ocular sarcoidosis: data from the French registry STAT. *Sarcoidosis Vasc Diffuse Lung Dis.* (2017) 34:74–80. doi: 10.36141/svdld.v34i1.5368
92. Schimmelpennink MC, Vorselaars ADM, van Beek FT, Crommelin HA, Deneer VHM, Keijsers RGM, et al. Efficacy and safety of infliximab biosimilar Inflectra ® in severe sarcoidosis. *Respir Med.* (2018) 138:S7–13. doi: 10.1016/j.rmed.2018.02.009
93. Maneiro JR, Salgado E, Gomez-Reino JJ, Carmona L. Efficacy and safety of TNF antagonists in sarcoidosis: data from the Spanish registry of biologics BIOBADASER and a systematic review. *Sem Arthr Rheum.* (2012) 42:89–103. doi: 10.1016/j.semarthrit.2011.12.006
94. Adler BL, Wang CJ, Bui T-L, Schilperoort HM, Armstrong AW. Anti-tumor necrosis factor agents in sarcoidosis: a systematic review of efficacy and safety. *Sem Arthr Rheum.* (2019) 48:1093–104. doi: 10.1016/j.semarthrit.2018.10.005
95. Chen Y, Friedman M, Liu G, Deodhar A, Chu C-Q. Do tumor necrosis factor inhibitors increase cancer risk in patients with chronic immune-mediated inflammatory disorders? *Cytokine.* (2018) 101:78–88. doi: 10.1016/j.cyto.2016.09.013
96. Chalayer É, Bachy E, Occelli P, Weiler L, Faurie P, Ghesquieres H, et al. Sarcoidosis and lymphoma: a comparative study. *QJM.* (2015) 108:871–8. doi: 10.1093/qjmed/hcv039
97. Papanikolaou IC, Sharma OP. The relationship between sarcoidosis and lymphoma. *Eur Respir J.* (2010) 36:1207–19. doi: 10.1183/09031936.00043010
98. Cerri S, Fontana M, Balduzzi S, Potenza L, Clini E, Luppi F. Clinical differences in sarcoidosis patients with and without lymphoma: a single-centre retrospective cohort analysis. *Eur Respir J.* (2019) 54:1802470. doi: 10.1183/13993003.02470-2018
99. Ando M, Goto A, Takeno Y, Yamasue M, Komiya K, Umeki K, et al. Significant elevation of the levels of B-cell activating factor (BAFF) in patients with sarcoidosis. *Clin Rheumatol.* (2018) 37:2833–8. doi: 10.1007/s10067-018-4183-2
100. Mariette X. How does BAFF activate B cells in patients with autoimmune diseases? *Arthritis Res Ther.* (2012) 14:106. doi: 10.1186/ar3729
101. Jeny F, Bernaudin J-F, Cohen Aubart F, Brillet P-Y, Bouvry D, Nunes H, et al. Diagnosis issues in sarcoidosis. *Respir Med Res.* (2020) 77:37–45. doi: 10.1016/j.resmer.2019.09.002
102. Warshauer DM, Lee JKT. Imaging manifestations of abdominal sarcoidosis. *Am J Roentgenol.* (2004) 182:15–28. doi: 10.2214/ajr.182.1.1820015
103. Salahuddin M, Karanth S, Ocazionez D, Estrada-Y-Martin RM, Cherian SV. Clinical characteristics and etiologies of miliary nodules in the US: a single-center study. *Am J Med.* (2019) 132:767–9. doi: 10.1016/j.amjmed.2018.12.030
104. Brandy-García AM, Cabezas-Rodriguez I, Caminal-Montero L, Suarez-Cuervo C, Redondo-Buil P. Sarcoidosis mimicking lytic osseous metastases. *CCJM.* (2017) 84:753–4. doi: 10.3949/ccjm.84a.16108
105. Park HJ, Jung JI, Chung MH, Song SW, Kim HL, Baik JH, et al. Typical and atypical manifestations of intrathoracic sarcoidosis. *Korean J Radiol.* (2009) 10:623. doi: 10.3348/kjr.2009.10.6.623
106. Criado E, Sánchez M, Ramírez J, Arguis P, de Caralt TM, Perea RJ, et al. Pulmonary sarcoidosis: typical and atypical manifestations at high-resolution CT with pathologic correlation. *RadioGraphics.* (2010) 30:1567–86. doi: 10.1148/rg.306105512
107. Bouvry D, Uzunhan Y, Naccache J-M, Nunes H, Brillet P-Y, Valeyre D. Sarcoïdose à présentation atypique. *La Revue de Médecine Interne.* (2008) 29:46–53. doi: 10.1016/j.revmed.2007.10.005
108. El Jammal T, Jamilloux Y, Gerfaud-Valentin M, Valeyre D, Sève P. Refractory sarcoidosis: a review. *TCRM.* (2020) 16:323–45. doi: 10.2147/TCRM.S192922
109. Du J, Zhang Y, Liu D, Zhu G, Zhang Q. Hodgkin's lymphoma with marked granulomatous reaction: a diagnostic pitfall. *Int J Clin Exp Pathol.* (2019) 12:2772–4.
110. Hou W, Wei P, Xie J, Zheng Y, Zhou X. Classical Hodgkin lymphoma with necrotic granuloma-like morphological features. *Int J Clin Exp Med.* (2018) 11:9593–602.
111. Bhatlapenumarthi V, Patwari A, Pascual SK. diagnostic dilemma: an unusual case of angioimmunoblastic T-cell lymphoma manifesting as bone marrow non-caseating granuloma. *J Hematol.* (2020) 9:37–40. doi: 10.14740/jh607
112. Song JY, Pittaluga S, Dunleavy K, Grant N, White T, Jiang L, et al. Lymphomatoid granulomatosis—a single institute experience: pathologic findings and clinical correlations. *Am J Surg Pathol.* (2015) 39:141–56. doi: 10.1097/PAS.0000000000000328
113. Fauci A, Haynes B, Costa J, Katz P, Wolff S. Lymphomatoid granulomatosis: prospective clinical and therapeutic experience over 10 years. *N Engl J Med.* (1982) 306: 68–7.
114. Akaike G, Itani M, Shah H, Ahuja J, Yilmaz Gunes B, Assaker R, et al. PET/CT in the diagnosis and workup of sarcoidosis: focus on atypical manifestations. *RadioGraphics.* (2018) 38:1536–49. doi: 10.1148/rg.2018180053
115. Fallanca F, Picchio M, Crivellaro C, Mapelli P, Samanes Gajate AM, Sabattini E, et al. Unusual presentation of sarcoid-like reaction on bone marrow level associated with mediastinal lymphadenopathy on 18F-FDG-PET/CT resembling an early recurrence of Hodgkin's Lymphoma. *Revista*

Española de Medicina Nuclear e Imagen Molecular. (2012) 31:207–9. doi: 10.1016/j.remn.2012.03.002

116. Lococo F, Muoio B, Chiappetta M, Nachira D, Petracca Ciavarella L, Margaritora S, et al. Diagnostic performance of PET or PET/CT with different radiotracers in patients with suspicious lung cancer or pleural tumours according to published meta-analyses. *Contrast Med Mol Imaging.* (2020) 2020:1–7. doi: 10.1155/2020/5282698
117. Chan W-L, Ramsay SC, Szeto ER, Freund J, Pohlen JM, Tarlinton LC, et al. Dual-time-point 18F-FDG-PET/CT imaging in the assessment of suspected malignancy: Dual-time-point PET/CT in malignancy. *J Med Imaging Radiat Oncol.* (2011) 55:379–90. doi: 10.1111/j.1754-9485.2011.02287.x
118. Gamperl I, Enzinger C, Pichler A, Feichtinger M, Schlager T, Fertl E. Can pulmonary sarcoidosis trigger a progressive multifocal leukoencephalopathy? Considerations from a case series and a review of literature. *Clin Case Rep.* (2018) 6:2121–5. doi: 10.1002/ccr3.1816
119. Conte G, Zugni F, Bellomi M, Petralia G. Sarcoidosis with bone involvement mimicking metastatic disease at 18F-FDG PET/CT: problem solving by diffusion whole-body MRI. *ecancer.* (2015) 9:537. doi: 10.3332/ecancer.2015.537

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.

frontiers in Medicine

BRIEF RESEARCH REPORT
published: 25 November 2020
doi: 10.3389/fmed.2020.606461

Gene Co-expression Networks Identifies Common Hub Genes Between Cutaneous Sarcoidosis and Discoid Lupus Erythematosus

Melissa A. Nickles[1], Kai Huang[1], Yi-Shin Chang[1], Maria M. Tsoukas[2], Nadera J. Sweiss[3], David L. Perkins[1] and Patricia W. Finn[1]**

[1] Department of Medicine, University of Illinois at Chicago, Chicago, IL, United States, [2] Department of Dermatology, University of Illinois at Chicago, Chicago, IL, United States, [3] Division of Rheumatology, University of Illinois at Chicago, Chicago, IL, United States

OPEN ACCESS

Edited by:
George Bertsias,
University of Crete, Greece

Reviewed by:
Anastasia Filia,
Biomedical Research Foundation of the Academy of Athens (BRFAA), Greece
Nikolaos Panousis,
Wellcome Sanger Institute (WT), United Kingdom

***Correspondence:**
Kai Huang
khuang49@uic.edu
Patricia W. Finn
pwfinn@uic.edu

Specialty section:
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

Received: 15 September 2020
Accepted: 28 October 2020
Published: 25 November 2020

Citation:
Nickles MA, Huang K, Chang Y-S, Tsoukas MM, Sweiss NJ, Perkins DL and Finn PW (2020) Gene Co-expression Networks Identifies Common Hub Genes Between Cutaneous Sarcoidosis and Discoid Lupus Erythematosus. Front. Med. 7:606461. doi: 10.3389/fmed.2020.606461

In this study we analyzed gene co-expression networks of three immune-related skin diseases: cutaneous sarcoidosis (CS), discoid lupus erythematosus (DLE), and psoriasis. We propose that investigation of gene co-expression networks may provide insights into underlying disease mechanisms. Microarray expression data from two cohorts of patients with CS, DLE, or psoriasis skin lesions were analyzed. We applied weighted gene correlation network analysis (WGCNA) to construct gene-gene similarity networks and cluster genes into modules based on similar expression profiles. A module of interest that was preserved between datasets and corresponded with case/control status was identified. This module was related to immune activation, specifically leukocyte activation, and was significantly increased in both CS lesions and DLE lesions compared to their respective controls. Protein-protein interaction (PPI) networks constructed for this module revealed seven common hub genes between CS lesions and DLE lesions: TLR1, ITGAL, TNFRSF1B, CD86, SPI1, BTK, and IL10RA. Common hub genes were highly upregulated in CS lesions and DLE lesions compared to their respective controls in a differential expression analysis. Our results indicate common gene expression patterns in the immune processes of CS and DLE, which may have indications for future therapeutic targets and serve as Th1-mediated disease biomarkers. Additionally, we identified hub genes unique to CS and DLE, which can help differentiate these diseases from one another and may serve as unique therapeutic targets and biomarkers. Notably, we find common gene expression patterns in the immune processes of CS and DLE through utilization of WGCNA.

Keywords: WGCNA, co-expression network, cutaneous sarcoidosis, discoid lupus erythematosus, hub genes

INTRODUCTION

Cutaneous sarcoidosis (CS), discoid lupus erythematosus (DLE), and psoriasis are immune-related cutaneous disorders with different pathologies and clinical presentations. CS occurs in up to one third of patients with systemic sarcoidosis, an inflammatory disease characterized by non-caseating granulomas (1). CS is often considered the "great imitator" in dermatology due to its large range of

morphologies, including papules, plaques, lupus pernio, and scar psoriaform and ulcerative lesions (1). DLE is the most prevalent type of chronic cutaneous lupus erythematosus characterized by pathogenic autoantibodies and immune complexes (2). The most common presentation of DLE is coin-shaped plaques on the scalp and ears (2, 3). DLE can occur as a skin manifestation of systemic lupus erythematosus (SLE) in up to 20% of patients (2, 3). Psoriasis is a common skin condition that affects over 7 million Americans (4). The hallmark of immune dysfunction in psoriasis is uncontrolled keratocyte proliferation and differentiation (5). Plaque psoriasis is the most common subtype, presenting with well-defined areas of erythematous plaques with silvery scales (5). The severity of psoriasis can greatly vary, with the joints being affected in 20–30% of patients (6). While psoriasis was traditionally considered a Th1-mediated disease, recent studies suggests that psoriasis may be predominantly Th17-mediated (7). In contrast, both CS and DLE may be predominantly Th1-mediated diseases (8, 9).

Within the past 20 years, biological treatments for several skin diseases, including psoriasis, atopic dermatitis, urticaria, and pemphigus vulgaris have emerged as major therapeutic breakthroughs (10). First-line therapy for cutaneous sarcoidosis consists of corticosteroids and second-line therapies consist of tetracyclines, hydroxychloroquine, and methotrexate. Biologics, e.g., anti-TNF, have been used to treat chronic or resistant cutaneous sarcoidosis with improvement or worsening of disease (11). A limited number of studies support the use of both infliximab and adalimumab as third-line therapies for cutaneous sarcoidosis, with some reports of etanercept, rituximab, golimumab, and ustekinumab being successful as well (12). For DLE, first line therapies include photoprotection in conjunction with topical or oral corticosteroids, topical calcineurin inhibitors, and systemic antimalarial therapy (2, 3, 13). Refractory lesions may be treated with intralesional corticosteroid injections (2, 13). Chronic DLE lesions that are not responsive to topical corticosteroids or topical calcineurin inhibitors may be responsive to intralesional corticosteroid injections (2). Intravenous immunoglobin, rituximab, and dapsone have successfully treated cutaneous lupus lesions in a limited number of studies, as well as toxilizumab and anti-CD4 antibody in single reports (14). For mild to moderate psoriasis, first-line therapies include topical therapies including corticosteroids, vitamin D3 analogs, and combination products (15). Moderate to severe psoriasis can be treated with systemic therapies such as phototherapy, acitretin, methotrexate, and cyclosporine (15). For patients who do not respond to systemic therapies, biologics may be used. Infliximab has been found to be the most effective, followed by ustekinumab, adalimumab, and etanercept (15). These therapies do not address the presence of concomitant diseases of sarcoidosis and psoriasis. For example, anti-TNF in sarcoidosis may induce psoriasis skin lesions. Thus, we undertook this study to dissect the common and differentially expressed pathways among these three diseases.

Biological therapies must target a specific immune component that plays a key role in disease pathogenesis. Ideally, treatment should be directed to a patient-specific target (16). We have previously used gene co-expression networks to identify genes and molecular pathways of a disease state associated with clinical traits (17), as well as identifying similar immunological mechanisms between sarcoidosis and idiopathic pulmonary (18). In this study, we characterized commonly altered biological pathways in cutaneous sarcoidosis (CS), discoid lupus erythematosus (DLE), and psoriasis using gene coexpression networks. We created gene co-expression networks of microarray data from two previous studies. The first study found that active CS skin lesions showed several thousand differentially expression genes compared to non-lesional skin in CS patients and healthy controls. These differentially expressed genes showed a strong Th1 profile of sarcoidosis and expression of interleukin (IL)-23 and IL-23R with limited expression of other Th17 pathway genes (8). The second study found that DLE skin lesions demonstrated a predominance of IFN-γ-producing Th1 cells and an absence of IL-17-producing Th17 cells compared to psoriasis skin lesions (9).

In this study, we hypothesized that there would be common gene expression patterns between CS and DLE due to the similarities between the two diseases. Both CS and DLE are related to systemic disease, have a greater prevalence in African American populations (2, 19), and are predominantly Th1-mediated (8, 9). To investigate this hypothesis, microarray expression data with weighted gene co-network analysis (WGCNA) was applied. Since genes with similar expression patterns are likely to be functionally related, WGCNA clusters genes with correlated expression profiles into groups known as modules. WGCNA was used to identify the most relevant module in immune-related skin disorders. Hub genes within the module were identified using intramodular connectivity and protein-protein interaction (PPI) networks. The hub genes were further characterized by differential gene expression (DGE) analysis. We propose that characterization of commonly altered biologic pathways in CS, DLE, and psoriasis may uncover immunological targets and/or biomarkers.

METHODS

Data Collection and Preprocessing

Microarray data and associated clinical data was obtained from the NCBI Gene Expression Omnibus (GEO). Dataset 1 (GSE32887) (8) included 15 skin samples from CS lesions, 11 skin samples of non-lesional skin (NLS) on the same patients with CS, and 5 skin samples from healthy volunteers (control 1). Dataset 2 (GSE52471) (9) included 7 skin samples from DLE lesions, 18 skin samples from psoriasis lesions, and 13 skin samples from healthy volunteers (control 2). Both datasets were generated using Affymetrix Human Genome U133A 2.0 Array. The *collapseRows()* function was used to filter the probes to include only unique genes that were present in both datasets.

Co-expression Network Construction

The WGCNA package on R (20) was used to construct co-expression networks of both datasets. We utilized code from (21). Since both datasets used the same platform, they were comparable. To create a scale-free network, an appropriate soft

power was selected to promote strong connections between genes and filter out weak ones. Pearson correlation was used to measure the concordance of pair-wise genes. The Pearson correlation matrix was transformed into a weighted network for each dataset using the *adjacency()* function. Dataset 1 was used to construct modules. The dynamic tree-cutting function with a cut height of 0.99, a minimum cluster size of 30, and deep split of 3 identified modules with similar expression patterns. Modules are given arbitrary color names for easier tracking.

Identification of Module of Interest and GO Enrichment Analysis

The modules constructed from dataset 1 were mapped onto dataset 2. A preservation Z-score summary for each module was calculated using the *modulePreservation()* function in order to identify highly preserved modules between the two datasets. A Z-score of greater than five was used as the threshold for module preservation (21). The*GOenrichmentAnalysis()* function in WGCNA was used to annotate each module with significant biological functions. Module eigengenes (ME), which can be interpreted as the level of expression of each module within each sample, were obtained for all samples across studies. We performed a 3-group comparison using a single-factor ANOVA to compare MEs of preserved modules between the CS lesions, NLS, and controls in dataset 1. We repeated this procedure for DLE, psoriasis, and controls for dataset 2. For modules with significant ANOVA results, we performed follow-up pairwise *t*-tests assuming unequal variance between each subgroup in each dataset. The module which demonstrated a significant difference between case/control status in both datasets was investigated further.

Identification of Hub Genes in Module of Interest

The genes in the module of interest were ranked by intramodular connectivity. The top 5% of genes in dataset 1 and dataset 2 were considered potential hub genes (22). The lists were cross-referenced to identify overlapping genes. We also used a second method to identify hub genes. The 2,000 genes (the maximum allowed) with the highest intramodular connectivity for each dataset were entered into the STRING (23) database to construct a protein-protein interaction (PPI) network. The minimum protein interaction score was set to high confidence (0.7). The output from the PPI network was imported into Cytoscape (24), an open source software platform for visualizing complex networks. The top 5% of genes with the highest degree were considered potential hub genes and the lists from the two datasets were cross referenced to identify overlapping genes (25). We termed genes that were common to both network types and common between datasets as "hub genes." Gene expression of hub genes was compared between disease groups using Kruskal-Wallis test and *post-hoc* Dunn's test with Bonferroni correction for each gene.

Validation of Hub Genes Using Differential Gene Expression (DGE) Analysis

The limma package (26) on R was used to identify differentially expressed genes between CS lesions and DLE lesions vs. their respective controls to validate our results. No covariates were used in the limma model because case/control status was the only variable. The *lmFit()* function and empirical Bayes method in the limma package was used to analyze the genes. The *topTable()* function summarized the results from the linear fit. We used the criteria of $p < 0.01$ and log2 (fold-change) to define differentially expressed genes. We adjusted our *p*-values via the Benjamini-Hochberg Procedure.

RESULTS

Co-expression Network Construction Identifies a Module of Interest

Our filtering process resulted in 12,991 genes to use in network construction. The co-expression network resulted in 14 modules, six of which were preserved between datasets. Of the six preserved modules, two modules were related to cellular division, one was related to biosynthesis, one was related to the nucleus, one was related to sensory perception, and one was related to leukocyte activation. The top 10 GO terms for these preserved modules can be found in File 1. All *P*-values reported have been adjusted for multiple comparisons. While all six preserved modules in dataset 2 revealed significant differences between case/control status ($p < 0.01$), only the leukocyte activation module significantly differed between case/control status in dataset 1 ($p < 0.001$). We therefore selected the leukocyte activation module as our module of interest. The module of interest was significantly increased in both the CS lesions and DLE lesions compared to their respective controls ($p < 0.001$). There was no significant difference between NLS and control in dataset 1. Psoriasis showed decreased expression of the leukocyte activation module compared to both DLE ($p < 0.001$) and controls in dataset 2 ($p < 0.05$).

Hub Genes Involved in Sarcoidosis and Lupus Pathogenesis Are Identified

The leukocyte activation module contained a total of 3,511 genes. From the co-expression network, the two datasets had 21 potential hub genes in common. From the PPI network, the two datasets had 74 potential hub genes in common. We found seven genes that were hubs in both network construction methods: TLR1, ITGAL, TNFRSF1B, CD86, SPI1, BTK, and IL10RA (**Figure 1**). All seven hub genes were upregulated in both the CS and DLE compared to their respective controls ($p < 0.05$). Additionally, all seven genes were significantly upregulated in the CS lesions compared to NLS, which did not show any significant differences from their controls. The psoriasis lesion samples showed decreased expression of BTK and TNFRSF1B compared to controls (**Table 1**). The seven hub genes were unique to the leukocyte activation module and were not found in any other preserved module. The expression level of each hub gene based on case/control status is demonstrated in **Figure 2**.

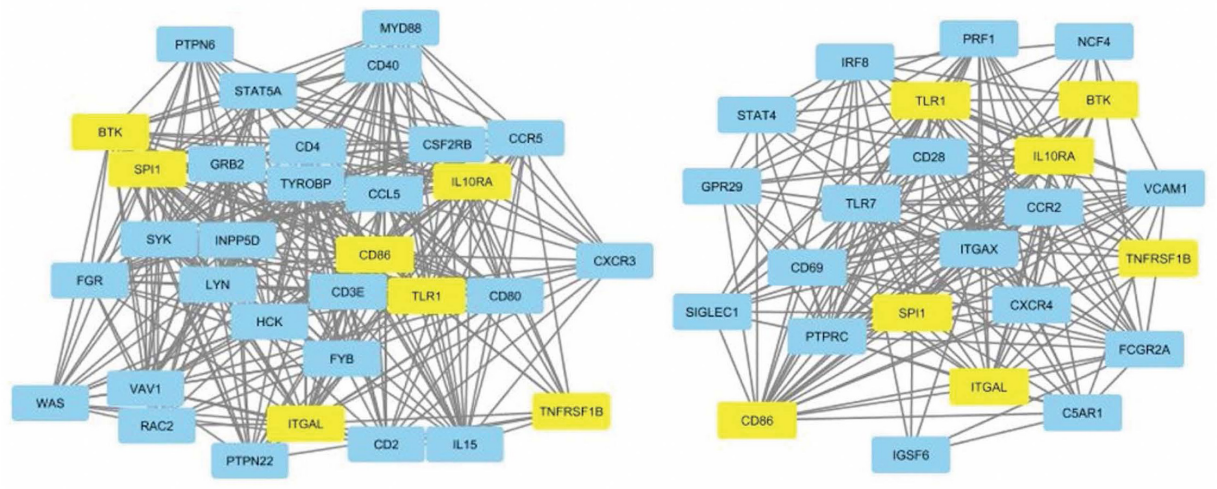


FIGURE 1 | Protein-protein interaction network for dataset 1 showing nodes with 20+ connections (left). Protein-protein interaction network for dataset 2 showing nodes with 10+ connections (right). Common hub genes are highlighted in yellow. The network is derived from the leukocyte activation module.

TABLE 1 | Adjusted *P*-values demonstrate significant differences in hub gene expression levels between groups in each dataset.

Gene name	Adjusted *P*-values of hub gene expression values					
	Dataset 1			Dataset 2		
	CS vs. control	NLS vs. control	CS vs. NLS	DLE vs. control	Psoriasis vs. control	DLE vs. psoriasis
BTK	0.008*	NS	0.0115*	0.0129*	0.0357**	<0.0001*
CD86	0.0024*	NS	0.0015*	0.0001*	NS	0.0005*
ITGAL	0.0057*	NS	0.0015*	0.0002*	NS	0.0004*
IL10RA	0.0011*	NS	0.0023*	0.0004*	NS	0.0002*
SPI1	0.0008*	NS	0.0035*	0.0004*	NS	0.0003*
TLR1	0.0071*	NS	0.0011*	0.0153*	NS	<0.0001*
TNFRSF1B	0.0017*	NS	0.0027*	0.0007*	0.0252**	0.0001*

**Increased expression level; **Decreased expression level.*
CS, cutaneous sarcoidosis; NLS, non-lesional sarcoidosis lesion; DLE, discoid lupus erythematosus.

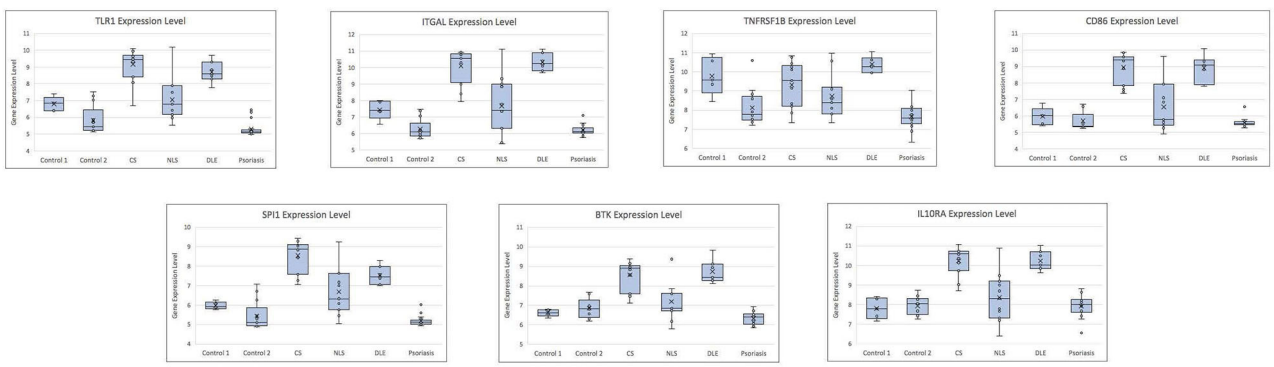


FIGURE 2 | Box plots showing gene expression level for each hub gene by case/control status. CS, cutaneous sarcoidosis; NLS, non-lesional skin of patients with cutaneous sarcoidosis; DLE, discoid lupus erythematosus.

TABLE 2 | Hub genes that are unique to disease-type identified by intramodular connectivity and protein-protein interaction (PPI) networks.

Cutaneous sarcoidosis hub genes	SYK, CD40*, CD80*, CCR5*, CCL5, MYD88, IL15, LYN, RAC2, GRB2*, STAT5A*, VAV1, CXCR3*, PTPN6, CD33*, HCK, FGR
Discoid lupus erythematosus hub genes	PTPRC, TLR7, ITGAX, FCGR2A, CCR2, PRF1

**Indicates that the gene was unique to that disease-type in the differential gene expression analysis.*

Seventeen hub genes were found only in dataset 1, including CD40, CCR5, CCL5, MYD88, IL15, and CXCR3. Six hub genes were found only in the dataset 2, of note TLR7, FCGR2A, and CCR2. The listed genes have primarily been indicated in the pathogenesis of sarcoidosis or lupus in the literature (27–30). Full gene lists can be found in **Table 2**.

Common Hub Genes Are Highly Upregulated in Differential Expression Analysis

The common hub genes were found to be individually upregulated in disease states compared to controls. The DGE analysis demonstrates that as a group, the common hub genes are highly upregulated compared to other DEGs in the datasets for both CS and DLE vs. respective controls. Volcano plots of DEGs with labeled hub genes are shown in **Figure 3**.

All 17 hub genes unique to dataset 1 showed significant upregulation in the DGE analysis of CS lesions vs. control. Ten of the 17 hub genes unique to dataset 1 were also significantly upregulated DEGs in the DLE vs. control analysis. The remaining seven were upregulated DEGs only in the CS vs. control analysis (**Table 2**). Of the six hub genes significant only to dataset 2, all six were upregulated in DLE lesions vs. control, as well as the CS vs. control analysis. Thus, in the DGE analysis there were only seven hub genes that were unique to CS and no hub genes that were unique to DLE.

DISCUSSION

Our findings suggest that there may be common gene expression patterns in the immune processes of CS and DLE. Since there were no significant differences between control and NLS, it appears that the gene dysregulation is confined to the sarcoidosis lesions. Additionally, psoriasis does not demonstrate the same patterns of gene expression differences as demonstrated in CS and DLE, suggesting that similarities between CS and DLE may extend beyond being immune-related skin disorders. The common hub genes we identified may be further investigated as biomarkers of Th1-driven inflammatory disorders. These genes may represent underlying drivers of Th1-skewed immune disease and could serve as a therapeutic target in CS and DLE. We also identified hub genes that are unique to CS and DLE, which can help differentiate these diseases from one another and may serve as unique markers rather than general Th1-mediated disease markers.

Closer examination of the seven identified hub genes reveals involvement of pathways that activate the immune system. Some of these genes encode for proteins that are already therapeutic targets. For example, TNFRSF1B encodes a protein that is a member of the TNF-receptor superfamily, which mediates the recruitment of anti-apoptotic proteins (31, 32). TNF-α inhibitors are currently used to treat a variety of immune-related disorders including sarcoidosis, lupus, and plaque psoriasis (33). However, TNF-α inhibitors do not consistently work for sarcoidosis (34) and are also complicated in SLE treatment due to the fact that TNF-α inhibitors can induce lupus (35). Future studies must utilize gene-regulatory networks to stratify phenotypes of sarcoidosis and lupus that are responsive to specific therapies.

Other hub genes identified encode for immune system related proteins that have shown promise as biomarkers or targets. CD86 encodes a protein that is expressed on antigen presenting cells (APCs) and serves as the costimulatory signal for T-cell activation (31). In sarcoidosis patients, alveolar macrophages (AM) act as APCs and express high levels of CD86 (31) to stimulate T-cell activation. BTK, which plays a crucial role in B-cell development, has been identified as having multiple roles in the production of autoantibodies and the pathogenesis of lupus (36). BTK inhibitors serve as a promising new therapeutic target and are currently being tested to treat lupus in animal models (37). TLR1 is a member of the toll-like receptor (TLR) family, which plays a fundamental role in pathogen recognition and innate immunity. Toll-like receptors recognize pathogen-associated molecular pathways (PAMPS) that are expressed on infectious agents (38). TLR1 in particular is expressed at higher levels than other TLRs and has been associated with infectious diseases that affect the skin, including Leprosy (39) and Lyme Disease (40). TLRs are thought to play a role in both sarcoidosis and lupus pathogenesis (41, 42), and has been suggested as a potential therapeutic target for lupus (43).

Hub genes may be useful as biomarkers for disease activity as well. IL10RA is a receptor for interleukin 10, an anti-inflammatory cytokine. IL-10 is increased in active pulmonary sarcoidosis as a compensatory response to increased expression of proinflammatory cytokines (44). IL-10 mediates disease activity of SLE (45). Together, the genes identified are those known to influence inflammatory responses.

The hub genes unique to CS and DLE are also potentially useful as disease markers and treatment targets. Of the 17 hub genes unique to the sarcoidosis samples, many are involved in T-cell activation and proliferation, such as CD40, CD80, CCR5, CCL5, and IL15, or cellular signaling, such as SYK, MYD88, and CXCR3. Some of the hub genes have been indicated in the pathogenesis of sarcoidosis. For example, CD40 which is required to activate APCs, is expressed in higher levels on AMs in patients with sarcoidosis (46). The expression of CD40 correlates with CD86, a common hub gene (46).

The C-C chemokine receptor 5 (CCR5) binds to chemokine ligand RANTES (CCL5) and is expressed on T-cells and macrophages. CCR5 mRNA is increased in bronchoalveolar lavage fluid (BALF) of sarcoidosis patients and may serve as a marker of pulmonary disease (47). Furthermore, certain CCR5 haplotypes are associated with sarcoidosis. The CCR5 haplotype

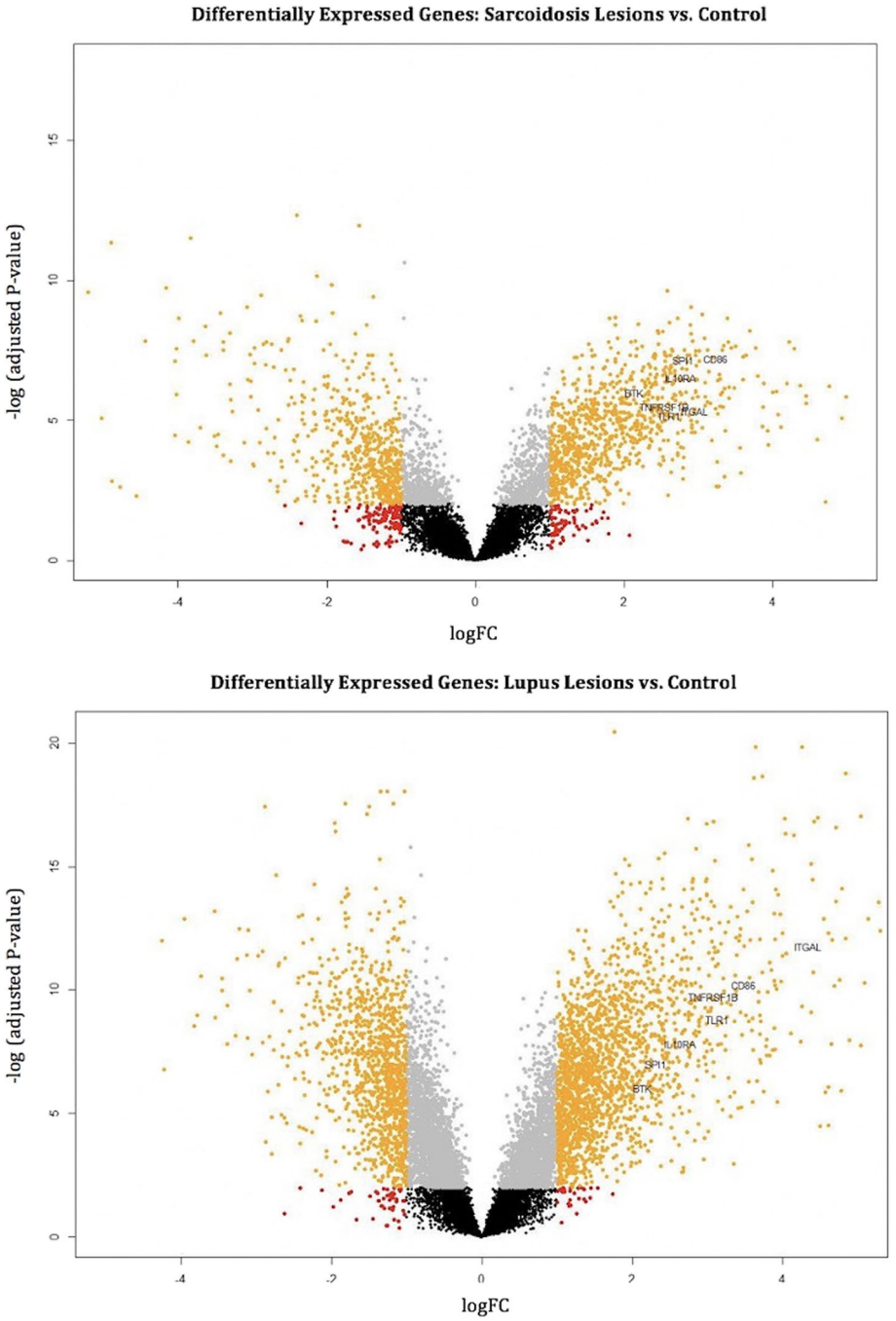


FIGURE 3 | Volcano plots mark differentially expressed genes (orange dots) in cutaneous sarcoidosis lesions vs. control (top) and discoid lupus erythematosus lesions vs. control (bottom). The orange dots represent differentially expressed genes using the criteria of $p < 0.01$ and log2 (fold-change). Black, gray, and red dots are genes that do not meet the criteria of a differentially expressed gene. The common hub genes (TLR1, ITGAL, TNFRSF1B, CD86, SPI1, BTK, and IL10RA) are marked.

HHC is strongly correlated with parenchymal lung disease in sarcoidosis, however, appears not to increase susceptibility to sarcoidosis and is only relevant after disease induction (48).

As a whole, we identified sarcoidosis genes that reflect immune cell activation. In contrast, the unique genes of the lupus group are more involved in pathogen recognition and degradation, such as TLR7, and FCGR2A. Some FCGR2A polymorphisms may increase susceptibility and development of SLE in certain ethnic populations (30). Data from mouse models have shown that TLR7, which is involved with PAMP recognition, serves a pathogenic role in the development of SLE, while TLR9 serves a protective role (28). Altered expression

of TLR7 and TLR9 has been suggested as a biomarker to identify a subset of SLE patients that may respond to a targeted therapeutic approach (29). CCR2+ T-cells, which aid in monocyte chemotaxis, are found to be selectively decreased during SLE flares and could potentially serve as a biomarker (27).

Together, our study indicates that our methodology may be informative in proposing key gene regulatory points in the immune processes of sarcoidosis and lupus. Notably, we find common gene expression patterns in the immune processes of CS and DLE. We have utilized WGCNA to identify genes that may underlie the pathogenesis of Th1-mediated skin disorders. The study limitations include lack of clinical data regarding therapy or systemic involvement, as well as sample collection at a single time point. Future analysis of the skin lesions at multiple points with functional interruption would be necessary to characterize the specific roles of hub genes in disease pathogenesis. We underscore that our demonstration of high intramolecular connectivity of the hub genes strongly suggests regulatory roles. We have identified hub genes that have been previously identified, as well as novel gene candidates for lupus and sarcoidosis. Lupus is a systemic autoimmune disease with skin manifestations, while sarcoidosis has been viewed as a predominantly pulmonary disorder with skin manifestations. Our results suggest that sarcoidosis may also be a systemic disease with immune dysregulation. Studies that stratify samples by therapy, organ involvement or disease progression are warranted. Future studies that utilize gene-regulatory networks and identify new genes may enhance our understanding of lupus or sarcoidosis as systemic disorders with implications for biomarkers or therapies.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: <NCBI GEO GSE32887 and GSE52471>.

AUTHOR CONTRIBUTIONS

MN, KH, Y-SC, DP, and PF designed the experiments. MN, KH, and Y-SC performed the data analysis. MN wrote the manuscript. NS provided expertise related to sarcoidosis. MT provided expertise related to dermatological disorders. All authors reviewed and approved the manuscript.

FUNDING

This work was funded by the National Heart, Lung, and Blood Institute 1R01HL138628-01.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.606461/full#supplementary-material

REFERENCES

1. Katta R. Cutaneous sarcoidosis: a dermatologic masquerader. *Am Fam Physician*. (2002) 65:1581–4.
2. Tanner BMSSLS. *Discoid lupus erythematosus.* StatPearls [Internet] (accessed July 3, 2020).
3. Okon LG, Werth VP. Cutaneous lupus erythematosus: diagnosis and treatment. *Best Pract Res Clin Rheumatol.* (2013) 27:391–404. doi: 10.1016/j.berh.2013.07.008
4. Schleicher SM. Psoriasis: pathogenesis, assessment, and therapeutic update. *Clin Podiatr Med Surg.* (2016) 33:355–66. doi: 10.1016/j.cpm.2016.02.004
5. Rendon A, Schäkel K. Psoriasis pathogenesis and treatment. *Int J Mol Sci.* (2019) 20:1475. doi: 10.3390/ijms20061475
6. Ocampo DV, Gladman D. Psoriatic arthritis. *F1000Res.* (2019) 8:F1000 Faculty Rev-1665. doi: 10.12688/f1000research.19144.1
7. Cai Y, Fleming C, Yan J. New insights of T cells in the pathogenesis of psoriasis. *Cell Mol Immunol.* (2012) 9:302–9. doi: 10.1038/cmi.2012.15
8. Judson MA, Marchell RM, Mascelli M, Piantone A, Barnathan ES, Petty KJ, et al. Molecular profiling and gene expression analysis in cutaneous sarcoidosis: the role of interleukin-12, interleukin-23, and the T-helper 17 pathway. *J Am Acad Dermatol.* (2012) 66: e901–2. doi: 10.1016/j.jaad.2011.06.017
9. Jabbari A, Suárez-Fariñas M, Fuentes-Duculan J, Gonzalez J, Cueto I, Franks AGJr, et al. Dominant Th1 and minimal Th17 skewing in discoid lupus revealed by transcriptomic comparison with psoriasis. *J Invest Dermatol.* (2014) 134:87–95. doi: 10.1038/jid.2013.269
10. Sehgal VN, Pandhi D, Khurana A. Biologics in dermatology: an integrated review. *Indian J Dermatol.* (2014) 59:425–41. doi: 10.4103/0019-5154.139859
11. Sweiss NJ, Baughman RP. Tumor necrosis factor inhibition in the treatment of refractory sarcoidosis: slaying the dragon? *J Rheumatol.* (2007) 34:2129–31.
12. Dai C, Shih S, Ansari A, Kwak Y, Sami N. Biologic therapy in the treatment of cutaneous sarcoidosis: a literature review. *Am J Clin Dermatol.* (2019) 20:409–22. doi: 10.1007/s40257-019-00428-8
13. Chang AY, Werth VP. Treatment of cutaneous lupus. *Curr Rheumatol Rep.* (2011) 13:300–7. doi: 10.1007/s11926-011-0180-z
14. Winkelmann RR, Kim GK, Del Rosso JQ. Treatment of cutaneous lupus erythematosus: review and assessment of treatment benefits based on oxford centre for evidence-based medicine criteria. *J Clin Aesthet Dermatol.* (2013) 6:27–38.
15. Kim WB, Jerome D, Yeung J. Diagnosis and management of psoriasis. *Can Family Physician.* (2017) 63:278–85.
16. Yavuz C. Biologics in dermatology: what does the future hold? *Dermatol Ther.* (2019) 32:e12932. doi: 10.1111/dth.12932
17. Ascoli C, Huang Y, Schott C, Turturice BA, Metwally A, Perkins DL, et al. A circulating micro-RNA signature serves as a diagnostic and prognostic indicator in sarcoidosis. *Am J Respir Cell Mol Biol.* (2017) 58:40–54. doi: 10.1165/rcmb.2017-0207OC
18. Schott CA, Ascoli C, Huang Y, Perkins DL, Finn PW. Declining pulmonary function in interstitial lung disease linked to lymphocyte dysfunction. *Am J Respir Crit Care Med.* (2020) 201:610–3. doi: 10.1164/rccm.201910-1909LE
19. Gerke AK, Judson MA, Cozier YC, Culver DA, Koth LL. Disease burden and variability in sarcoidosis. *Annals Am Thoracic Soc.* (2017) 14:S421–8. doi: 10.1513/AnnalsATS.201707-564OT
20. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinform.* (2008) 9:559. doi: 10.1186/1471-2105-9-559
21. Miller J. *Meta-Analyses of Data From Two (or More) Microarray Data Sets* (2011).

22. Liu J, Jing L, Tu X. Weighted gene co-expression network analysis identifies specific modules and hub genes related to coronary artery disease. *BMC Cardiovascul Disord.* (2016) 16:54–54. doi: 10.1186/s12872-016-0217-3
23. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Res.* (2017) 45:D362–8. doi: 10.1093/nar/gkw937
24. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* (2003) 13:2498–504. doi: 10.1101/gr.1239303
25. Xia WX, Yu Q, Li GH, Liu YW, Xiao FH, Yang LQ, et al. Identification of four hub genes associated with adrenocortical carcinoma progression by WGCNA. *PeerJ.* (2019) 7:e6555. doi: 10.7717/peerj.6555
26. Phipson B, Lee S, Majewski IJ, Alexander WS, Smyth GK. Robust hyperparameter estimation protects against hypervariable genes and improves power to detect differential expression. *Ann Appl Stat.* (2016) 10:946–63. doi: 10.1214/16-AOAS920
27. Amoura Z, Combadiere C, Faure S, Parizot C, Miyara M, Raphaël D, et al. Roles of CCR2 and CXCR3 in the T cell-mediated response occurring during lupus flares. *Arthritis Rheum.* (2003) 48:3487–96. doi: 10.1002/art.11350
28. Celhar T, Magalhães R, Fairhurst AM. TLR7 and TLR9 in SLE: when sensing self goes wrong. *Immunol Res.* (2012) 53:58–77. doi: 10.1007/s12026-012-8270-1
29. Celhar T, Fairhurst A-M. Toll-like receptors in systemic lupus erythematosus: potential for personalized treatment. *Front Pharmacol.* (2014) 5:265. doi: 10.3389/fphar.2014.00265
30. Li R, Peng H, Chen GM, Feng CC, Zhang YJ, Wen PF, et al. Association of FCGR2A-R/H131 polymorphism with susceptibility to systemic lupus erythematosus among Asian population: a meta-analysis of 20 studies. *Arch Dermatol Res.* (2014) 306:781–91. doi: 10.1007/s00403-014-1483-5
31. Genecards. CD86 Gene [Online]. *Weizmann Institutue of Science* (accessed July 12, 2020).
32. Nih. *TNFRSF1B gene* [Online]. *NIH U.S. National Library of Medicine* (accessed July 12, 2020).
33. Lis K, Kuzawińska O, Bałkowiec-Iskra E. Tumor necrosis factor inhibitors - state of knowledge. *Arch Med Sci.* (2014) 10:1175–85. doi: 10.5114/aoms.2014.47827
34. Callejas-Rubio JL, López-Pérez L, Ortego-Centeno N. Tumor necrosis factor-alpha inhibitor treatment for sarcoidosis. *Therapeut Clin Risk Manage.* (2008) 4:1305–13. doi: 10.2147/TCRM.S967
35. Almoallim H, Al-Ghamdi Y, Almaghrabi H, Alyasi O. Anti-tumor necrosis factor-α induced systemic lupus erythematosus(). *Open Rheumatol J.* (2012) 6:315–9. doi: 10.2174/1874312901206010315
36. Satterthwaite AB. Bruton's tyrosine kinase, a component of b cell signaling pathways, has multiple roles in the pathogenesis of lupus. *Front Immunol.* (2017) 8:1986. doi: 10.3389/fimmu.2017.01986
37. Chalmers SA, Wen J, Doerner J, Stock A, Cuda CM, Makinde HM, et al. Highly selective inhibition of Bruton's tyrosine kinase attenuates skin and brain disease in murine lupus. *Arthritis Res Ther.* (2018) 20:10. doi: 10.1186/s13075-017-1500-0
38. Kawasaki T, Kawai T. Toll-like receptor signaling pathways. *Front Immunol.* (2014) 5:461. doi: 10.3389/fimmu.2014.00461
39. Pinheiro RO, Schmitz V, Silva BJA, Dias AA, De Souza BJ, De Mattos Barbosa MG, et al. Innate immune responses in leprosy. *Front Immunol.* (2018) 9:518. doi: 10.3389/fimmu.2018.00518
40. Rahman S, Shering M, Ogden NH, Lindsay R, Badawi A. Toll-like receptor cascade and gene polymorphism in host-pathogen interaction in Lyme disease. *J Inflamm Res.* (2016) 9:91–102. doi: 10.2147/JIR.S104790
41. Margaritopoulos GA, Antoniou KM, Karagiannis K, Samara KD, Lasithiotaki I, Vassalou E, et al. Investigation of Toll-like receptors in the pathogenesis of fibrotic and granulomatous disorders: a bronchoalveolar lavage study. *Fibrogenesis Tissue Repair.* (2010) 3:20. doi: 10.1186/1755-1536-3-20
42. Devarapu SK, Anders HJ. Toll-like receptors in lupus nephritis. *J Biomed Sci.* (2018) 25:35. doi: 10.1186/s12929-018-0436-2
43. Wu YW, Tang W, Zuo JP. Toll-like receptors: potential targets for lupus treatment. *Acta Pharmacol Sin.* (2015) 36:1395–407. doi: 10.1038/aps.2015.91
44. Oltmanns U, Schmidt B, Hoernig S, Witt C, John M. Increased spontaneous interleukin-10 release from alveolar macrophages in active pulmonary sarcoidosis. *Exp Lung Res.* (2003) 29:315–28. doi: 10.1080/01902140303786
45. Godsell J, Rudloff I, Kandane-Rathnayake R, Hoi A, Nold MF, Morand EF, et al. Clinical associations of IL-10 and IL-37 in systemic lupus erythematosus. *Sci Rep.* (2016) 6:34604. doi: 10.1038/srep34604
46. Nicod LP, Isler P. Alveolar macrophages in sarcoidosis coexpress high levels of CD86 (B7.2), CD40, and CD30L. *Am J Respir Cell Mol Biol.* (1997) 17:91–6. doi: 10.1165/ajrcmb.17.1.2781
47. Petrek M, Gibejova A, Drabek J, Mrazek F, Kolek V, Weigl E, et al. CC chemokine receptor 5 (CCR5) mRNA expression in pulmonary sarcoidosis. *Immunol Lett.* (2002) 80:189–93. doi: 10.1016/S0165-2478(01)00324-8
48. Spagnolo P, Renzoni EA, Wells AU, Copley SJ, Desai SR, Sato H, et al. C-C chemokine receptor 5 gene variants in relation to lung disease in sarcoidosis. *Am J Respir Crit Care Med.* (2005) 172:721–8. doi: 10.1164/rccm.200412-1707OC

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.

frontiers in Medicine

CASE REPORT
published: 30 November 2020
doi: 10.3389/fmed.2020.541629

Case Report: All That Glisters Is Not* Cancer

Claudio Tirelli[1], *Chandra Bortolotto*[2], *Patrizia Morbini*[3] *and Giulia Maria Stella*[1]*

[1] *Unit of Respiratory System Diseases, Department of Medical Sciences and Infectious Diseases, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo Foundation, Pavia, Italy,* [2] *Unit of Radiology, Department of Intensive Medicine, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo Foundation and University of Pavia Medical School, Pavia, Italy,* [3] *Unit of Pathology, Department of Molecular Medicine, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo Foundation and University of Pavia Medical School, Pavia, Italy*

Properly performed staging in non-small-cell lung cancer (NSCLC) is necessary to avoid wrong therapeutic decisions. Here we present a case which manifested as advanced NSCLC but ultimately was composed of two different and rare pathologies. The first is a TTF-1 positive axillary lymph node that could be defined either as an unusual isolated differentiated cancer of unknown primary or as an even rarer case of ectopic lung epithelium which underwent malignant transformation. The second is sarcoidosis, a sarcoid-like alteration, in remission after oral steroids. The main implication of a correct diagnosis regards patient outcome and the avoidance of toxic inappropriate systemic chemotherapy.

Keywords: lung cancer, sarcoidosis, metastases, staging, origin

OPEN ACCESS

Edited by:
Nadera J. Sweiss, University of Illinois at Chicago, United States

Reviewed by:
Gunnar N. Hillerdal, Karolinska University Hospital, Sweden
Christian Ascoli, University of Illinois at Chicago, United States

***Correspondence:**
Giulia Maria Stella
g.stella@smatteo.pv.it

Shakespeare W, The Merchant of Venice (1594)

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: *09 March 2020*
Accepted: *02 November 2020*
Published: *30 November 2020*

Citation:
Tirelli C, Bortolotto C, Morbini P and Stella GM (2020) Case Report: All That Glisters Is Not Cancer. Front. Med. 7:541629. doi: 10.3389/fmed.2020.541629*

INTRODUCTION

Lung cancer is a major cause of death in both men and women worldwide. It is frequently associated to distant metastasis, most often discovered at the time of diagnosis; thus, correct disease staging, including molecular profile analysis, is fundamental in order to properly subtype cancer and for the subsequent choice of the most effective treatment.

CASE REPORT

In 2014, a 60-year-old non-smoker female with past medical history of recurrent bronchitis came to visit for 3 months of dry cough and exertional dyspnea, which remained unchanged after treatment with clarithromycin. At chest examination, bilateral diffuse crackles could be appreciated, without wheezing. No touchable lymphadenopathies were present. Echocardiogram was normal, while on chest X-ray (CXR; **Figure 1**) parenchymal infiltrates were documented, which were more evident in the left lung. A total body CT scan (TB CT; **Figure 2**) revealed diffuse parenchymal infiltrates with mediastinal and hilar lymphadenopathies, suggestive of sarcoidosis but also compatible with a malignant origin. A vascularized pathologic (3 cm) lymph node was also detected in the right axillary region and surgically removed 8 days after the CXR and 2 days after the CT scan, together with other nodes of the same station, for diagnostic purposes. At histologic examination, localization/metastasis of poorly differentiated epithelial neoplasia was found in only one of the resected nodes (**Figures 3A–C**), whereas in the others giant cell granulomatous epithelioid sarcoid-like reaction was detected (**Figure 3D**). An exhaustive and multidisciplinary diagnostic workup was thus initiated to

determine the putative primary origin of cancer cells. Their immunophenotype (cytokeratin 7+, AE1/AE3+, TTF1+, and p63-) was coherent with axillary lymph node localization of lung adenocarcinoma (1, 2). In detail, the cytokeratin panel expression confirmed the epithelial origin, while the positivity of thyroid transcription factor 1 (TTF-1) was a highly specific marker for lung as primary origin and suggested the adenocarcinomatous lineage of differentiation. The absence of p63 expression allowed to exclude squamous differentiation as well as neuroendocrine carcinomas. Subsequent molecular analysis documented the absence of actionable genetic targets since mutational analysis revealed *EGFR* wt, *KRAS* wt, *ALK* not translocated, and *HER2/neu* not amplified. We then proceeded with a first bronchoscopy to study deeper the lung parenchyma and find the primary site of malignant growth. A transbronchial biopsy (TBB) was then performed, revealing bilateral superior bronchial stenosis in the absence of histologic neoplastic infiltration (**Figures 3E,F**). To complete the disease stage and/or eventually to detect a distant site of origin of the neoplastic cells, a whole-body positron emission tomography was performed and evidenced supra- and subdiaphragmatic pathologic lymphadenopathies in the absence of putative primary mass detection (**Figure 4**). Since no tumor masses were demonstrated by imaging, a second bronchoscopy with TBB was thus repeated to deeply analyze the lung parenchyma. Quite unsuspectedly, the TBB obtained specimens that were free from neoplastic cells but rich of sarcoid non-necrotizing epithelioid granulomas. Steroid treatment (prednisone, 1 mg/kg) was started, with rapid improvement on symptomatology. After 1 month, a TB CT showed reduction in parenchymal infiltrates and lymphadenopathies without extra-thoracic neoplastic or sarcoid localizations. Being in the presence of carcinoma featuring lung epithelial lineage of origin vs metastatic lung cancer without detectable primary mass, in the absence of the expression of actionable targets, platinum-based conventional chemotherapy (cisplatin-pemetrexed) was started, and steroids were tapered until 10 mg/day. The TB CT at the end of three cycles of adjuvant chemotherapy confirmed the presence of some fibrotic branches, compatible with stage IV pulmonary sarcoidosis, without oncologic localizations (**Figure 5**). A timeline with all relevant data from this clinical case is available in **Figure 6**. The steroid regimen was gradually reduced to a maintenance of 5 mg/day over a period of 1 year. At 5 years from diagnosis,

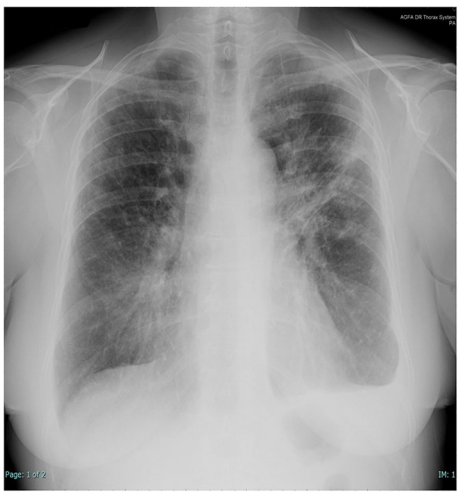


FIGURE 1 | Chest X-rays at presentation. Left subclavian pulmonary thickening associated with patchy bilateral peripheral opacity particularly represented in the left lung where there are also band-like consolidations and pleural effusion.

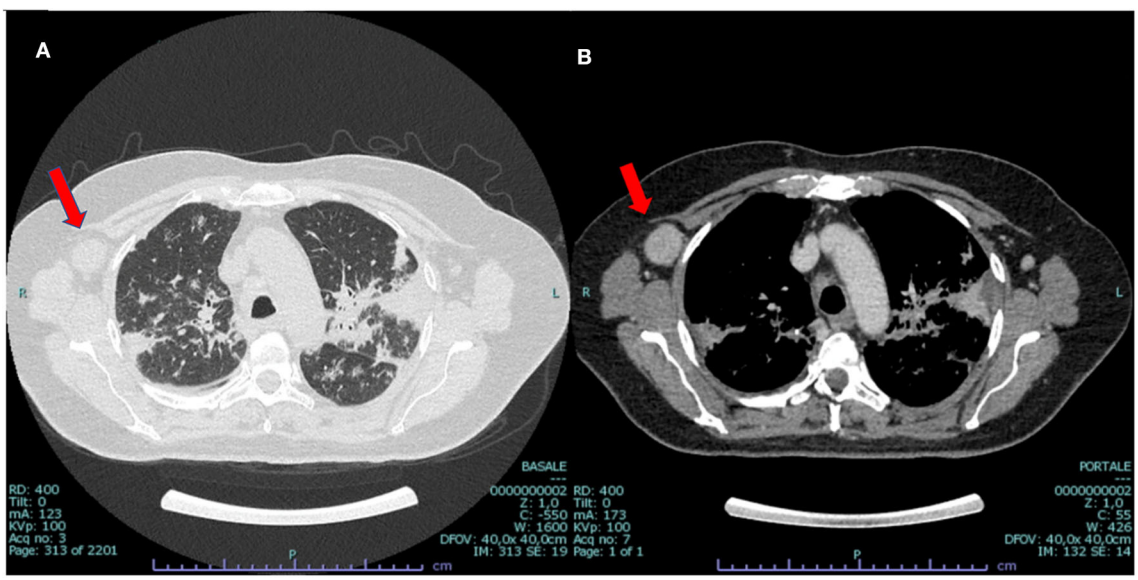


FIGURE 2 | CT scan at presentation: bilateral parenchymal infiltrates **(A)** and mediastinal hilar lymph node enlargement **(B)**, suggestive of sarcoidosis but also compatible with malignant origin. Enlarged vascularized pathologic (3 cm) axillar node detected in the right axillary region (red arrow).

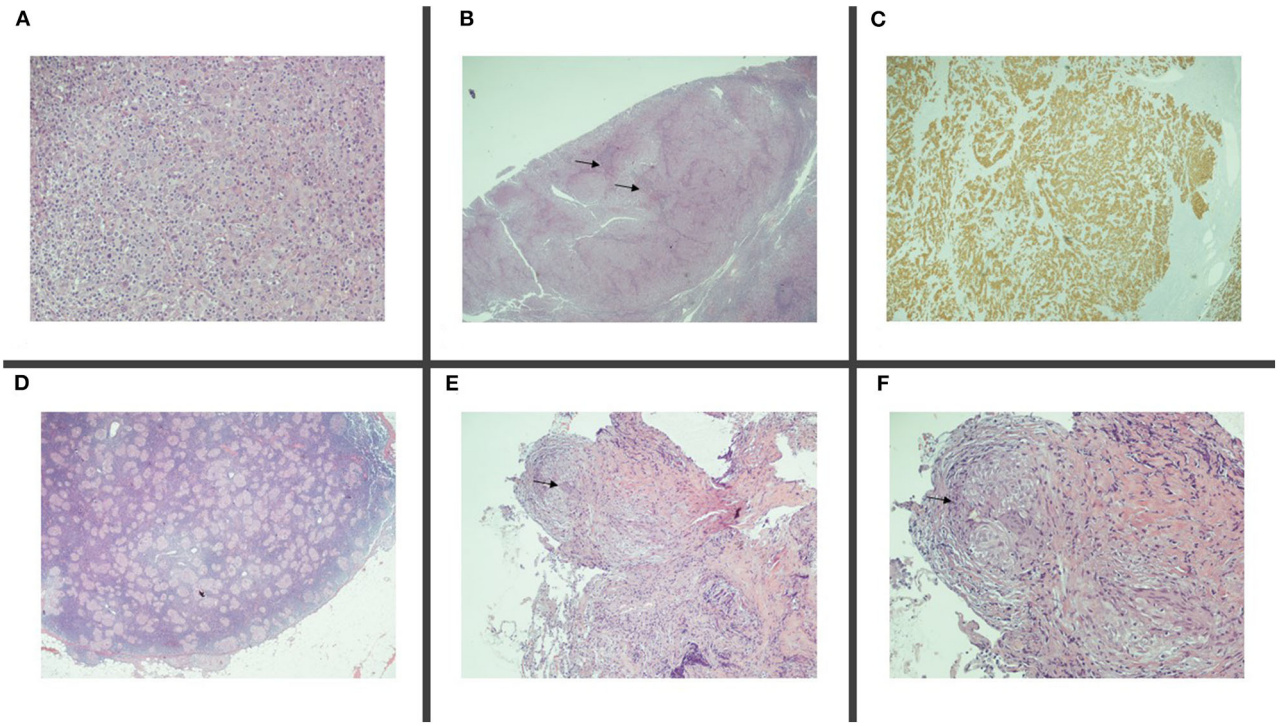


FIGURE 3 | Histologic samples. Surgical sample of lymph node localization of adenocarcinoma, ×20 **(A)**, ×5 (**B**, →), featuring positive staining for cytokeratin 7 **(C)**, and concomitant epithelioid sarcoid-like granuloma, ×5 **(D)**. Transbroronchial biopsy ×10 **(E)** and ×20 **(F)** displaying sarcoid non-necrotizing epithelioid granulomas (→).

the result at follow-up is still negative, with no neoplasm recurrence and with good control of sarcoidosis in the absence of steroid treatment.

COMMENTS

We report a case of coexistent ectopic/metastatic lung cancer (in the absence of detectable primary mass in the lung parenchyma) and sarcoidosis. The latter most often occurs in younger people, but it can be present at any age. Females are overrepresented in the age group 50–60 years, and the disease is then often chronic and already advanced at presentation (3), as in the present case. The relationship between cancer and sarcoidosis is still controversial (4, 5), although a moderate but significantly increased cancer risk in patients with sarcoidosis has been reported (6). On this basis, sarcoidosis has been proposed as a risk factor for lung cancer in never-smokers, but no conclusive data are available until now (7). Three hypotheses have been proposed regarding the relationship between cancer and sarcoidosis: (1) sarcocentric: sarcoidosis develops before lung cancer and could induce neoplastic transformation through cell-mediated immune abnormalities in the absence of activation of known oncogenic drivers, (2) oncocentric: sarcoidosis might represent an immunological reaction to disperse cancer antigens, and (3) the two entities might be independent. The mechanistic explanation of the pathogenesis of cancer associated with sarcoid-like granulomas and/or sarcoidosis is still unknown. A quite recent hypothesis suggested that molecular mimicry could occur because of the altered activity of oncogenes and ultimately lead to crossed-type mediated body reactions targeting structurally similar sections or regions from the tissue homeostasis (8). Interestingly, it should be noted that the occurrence of sarcoidosis-like granulomas is involved also in response to novel cancer therapies since they might represent important immune checkpoint inhibitor-related reactions (9).

Notably, the patient far surpassed the expected survival for a stage IV non-small-cell lung cancer (NSCLC). This point allows us to conclude that malignant transformation of ectopic lung epithelium was more probable than metastatic lung disease; thus, surgical removal was, in that case, radical. However, it cannot be excluded that a small primary lung mass was initially present and that it subsequently evolved in dormancy or regression. Indeed the process of metastatic dissemination begins when malignant cells start to migrate and leave the primary mass (10). The latter define a rare but non-negligible disease (over 300,000 new cases per year worldwide) which is called cancer of unknown primary (2). The disease shares common traits: (i) early metastatic dissemination, (ii) unpredictable metastatic organ distribution, (iii) lack of organ- or tissue-specific differentiation, and (iv) extremely aggressive potential and poor prognosis in case of disseminated disease. The present case is worth to be described since it is paradigmatic of how adequate staging in NSCLC is mandatory to avoid wrong therapeutic decisions. What

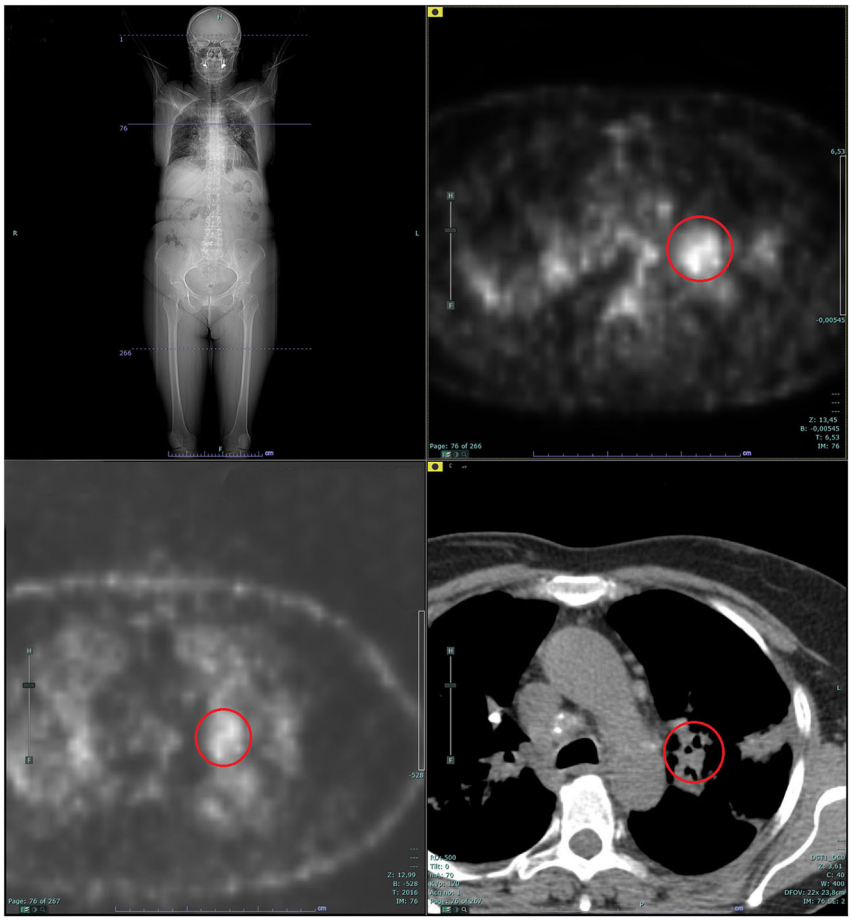


FIGURE 4 | Whole-body positron emission tomography (PET): supra- and subdiaphragmatic pathologic lymphadenopathies in the absence of putative primary mass detection. The red circle shows the pathologic standardized uptake value (SUV) at left hilar lymph node (L10 station).

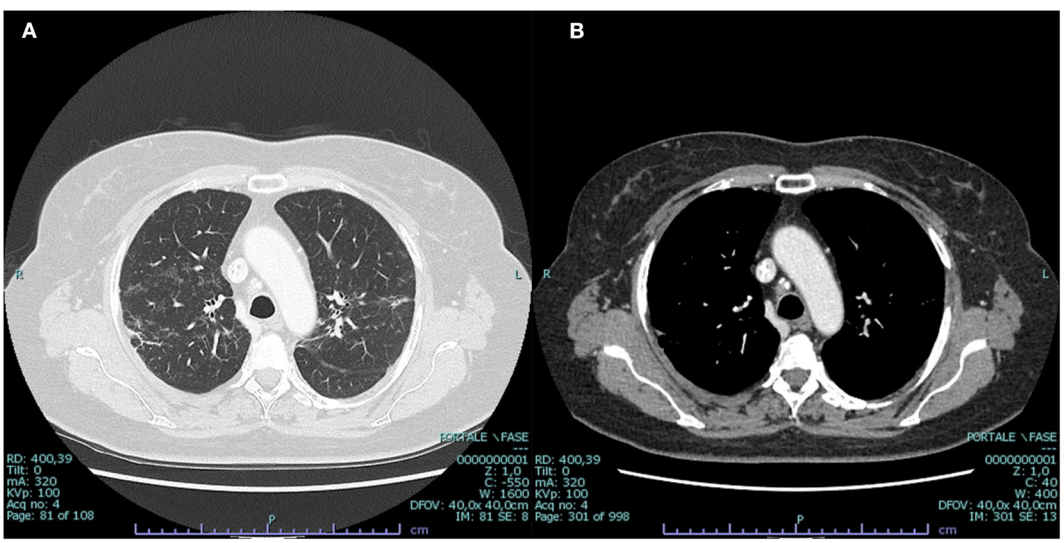


FIGURE 5 | CT scan after treatment: remaining parenchymal fibrosis in upper lobes compatible with sarcoidosis grade IV **(A)**, without mediastinal or extra-thoracic lymph node enlargement **(B)**.

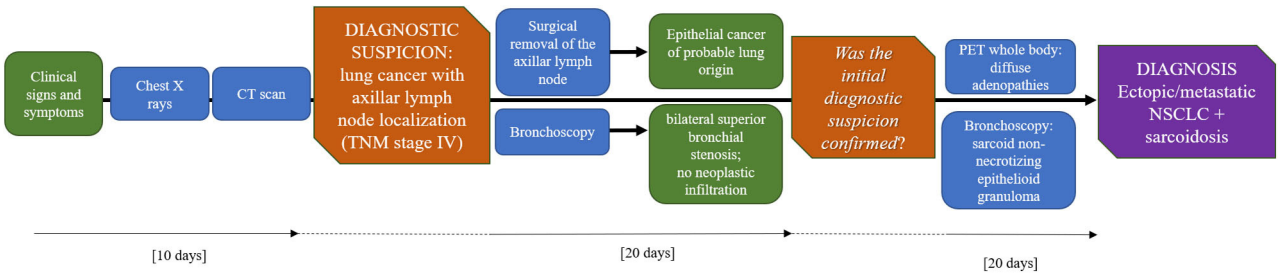


FIGURE 6 | Timeline with the most relevant data of the clinical case.

seemed to be consistent with a stage IV lung cancer requiring quite obvious therapeutic approach was instead composed of two different pathologies. The first is a TTF-1 positive axillary lymph node that could be defined either as an unusual isolated differentiated metastasis without a sure primary site of origin or as an even rarer case of ectopic lung epithelium which underwent malignant transformation. It was successfully removed in the absence of neoplastic recurrence. The second is sarcoidosis or, a sarcoid-like alteration, remaining after surgery but in persistent remission with a cycle of oral steroids. Given the improvement/reversibility and the presence of lung parenchymal and lymph node involvement and based also on Scadding staging on X-rays, there is a possibility that this was stage II sarcoidosis (11, 12). Overall, this quite unexpected diagnosis was allowed by invasive diagnostic procedures on both the hypothetical nodal metastasis and the primary site of the disease. The main implication of this approach regards patient prognosis and therapeutic management by avoiding the toxicity of an inappropriate treatment for advanced cancer.

ETHICS STATEMENT

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

CT was responsible for data collection and wrote the paper. CB and PM collected data. GS supervised and wrote the paper. All authors contributed to the article and approved the submitted version.

FUNDING

The study was supported by funds Ricerca Corrente from Italian Ministry of Health granted to GS, IRCCS Policlinico San Matteo, Pavia, Italy.

REFERENCES

1. Stella GM, Kolling S, Benvenuti S, Bortolotto C. Lung-seeking metastases. *Cancers (Basel).* (2019) 11:1010. doi: 10.3390/cancers11071010
2. Stella GM, Senetta R, Cassenti A, Ronco M, Cassoni P. Cancers of unknown primary origin: current perspectives and future therapeutic strategies. *J Transl Med.* (2012) 10:12. doi: 10.1186/1479-5876-10-12
3. Spagnolo P, Rossi G, Trisolini R, Sverzellati N, Baughman RP, Wells AU. Pulmonary sarcoidosis. *Lancet Respir Med.* (2018) 6:389–402. doi: 10.1016/S2213-2600(18)30064-X
4. Kachalia AG, Ochieng P, Kachalia K, Rahman H. Rare coexistence of sarcoidosis and lung adenocarcinoma. *Resp Med Case Rep.* (2014) 12:4–6. doi: 10.1016/j.rmcr.2013.12.008
5. Chopra A, Judson MA. How are cancer and connective tissue diseases related to sarcoidosis? *Curr Opin Pulm Med.* (2015) 21:517–24. doi: 10.1097/MCP.0000000000000186
6. Bonifazi M, Bravi F, Gasparini S, La Vecchia C, Gabrielli A, Wells AU, et al. Sarcoidosis and cancer risk: systematic review and meta-analysis of observational studies. *Chest.* (2015) 147:778–91. doi: 10.1378/chest.14-1475
7. Corrales L, Rosell R, Cardona AF, Martín C, Zatarain-Barrón ZL, Arrieta O. Lung cancer in never smokers: the role of different risk factors other than tobacco smoking. *Crit Rev Oncol Hematol.* (2020) 148:102895. doi: 10.1016/j.critrevonc.2020.102895
8. Tchernev G, Wollina U. Sarcoidosis, cancer and molecular mimicry. *Int J Immunopathol Pharmacol.* (2013) 26:753–5. doi: 10.1177/039463201302600319
9. Rambhia PH, Reichert B, Scott JF, Feneran AN, Kazakov JA, Honda K, et al. Immune checkpoint inhibitor-induced sarcoidosis-like granulomas. *Int J Clin Oncol.* (2019) 24:1171–81. doi: 10.1007/s10147-019-01490-2
10. Popper HH. Progression and metastasis of lung cancer. *Cancer Metast. Rev.* (2016) 35:75–91. doi: 10.1007/s10555-016-9618-0
11. Larici AR, Glaudemans AW, Del Ciello A, Slart RH, Calandriello L, Gheysens O. Radiological and nuclear medicine imaging of sarcoidosis. *Q J Nucl Med Mol Imaging.* (2018) 62:14–33. doi: 10.23736/S1824-4785.17.03046-1
12. Judson MA, Thompson BW, Rabin DL, Steimel J, Knattereud GL, Lackland DT, et al. The diagnostic pathway to sarcoidosis. *Chest.* (2003). 123:406–12. doi: 10.1378/chest.123.2.406

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.

frontiers in Medicine

ORIGINAL RESEARCH
published: 10 December 2020
doi: 10.3389/fmed.2020.565420

Acute and Chronic Sarcoid Arthropathies: Characteristics and Treatments From a Retrospective Nationwide French Study

*Carlotta Cacciatore[1], Pierre Belnou[2], Sara Thietart[1], Carole Desthieux[1], Mathilde Versini[3], Noemie Abisror[1], Sébastien Ottaviani[4], Gregoire Cormier[5], Alban Deroux[6], Azeddine Dellal[7], Nicolas Belhomme[8], Nathalie Saidenberg Kermanac'H[9,10], Philippe Khafagy[11], Martin Michaud[12], Sylvain Lanot[13], Fabrice Carrat[2,14], Olivier Fain[1] and Arsène Mékinian[1]**

[1] Sorbonne Université, Service de médecine interne, Hôpital Saint-Antoine, DHU I2B: Inflammation, Immunopathologie, Biothérapie, APHP, Paris, France, [2] Sorbonne Université, Service de santé publique, Hôpital Saint-Antoine, APHP, Paris, France, [3] Service de Médecine interne, Centre Hospitalier Universitaire de Nice, Nice, France, [4] Service de rhumatologie, Hôpital Bichat, APHP, Paris, France, [5] Service de rhumatologie, CHD Vendée, La Roche-sur-Yon, France, [6] Service de médecine interne, CHU Grenoble, La Tronche, France, [7] Service de rhumatologie, Hôpital Montfermeil, Montfermeil, France, [8] Service de médecine interne et immunologie clinique, CHU Rennes, Rennes, France, [9] Service de rhumatologie, Groupe hospitalier Avicenne-Jean Verdier-René Muret, APHP, Bobigny, France, [10] Sorbonne Paris Cité, Université Paris 13, INSERM U1125, Bobigny, France, [11] Service de radiologie, Hôpital Montfermeil, Montfermeil, France, [12] Service de médecine interne, Hôpital Joseph Ducuing, Toulouse, France, [13] Service de rhumatologie, C.H intercommunal Alençon-Mamers, Alençon, France, [14] Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, Paris, France

OPEN ACCESS

Edited by:
Mehdi Mirsaeidi, University of Miami, United States

Reviewed by:
Mauro Waldemar Keiserman, Hospital São Lucas da PUCRS, Brazil
João Eurico Fonseca, University of Lisbon, Portugal

***Correspondence:**
Arsène Mékinian
arsene.mekinian@aphp.fr

Specialty section:
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

Received: *25 May 2020*
Accepted: *16 October 2020*
Published: *10 December 2020*

Citation:
Cacciatore C, Belnou P, Thietart S, Desthieux C, Versini M, Abisror N, Ottaviani S, Cormier G, Deroux A, Dellal A, Belhomme N, Kermanac'H NS, Khafagy P, Michaud M, Lanot S, Carrat F, Fain O and Mékinian A (2020) Acute and Chronic Sarcoid Arthropathies: Characteristics and Treatments From a Retrospective Nationwide French Study. Front. Med. 7:565420. doi: 10.3389/fmed.2020.565420

Introduction: We aimed to analyze patients with acute and chronic joint involvements in sarcoidosis.

Methods: This is a retrospective multicenter analysis of patients with proven sarcoidosis, as defined by clinical, radiological, and histological criteria, with at least one clinical and/or ultrasonographic synovitis.

Results: Thirty-nine patients with sarcoid arthropathy were included, and among them 19 had acute sarcoidosis (Lofgren's syndrome). Joint involvement and DAS44-CRP were not significantly different in acute and chronic sarcoid arthropathies. Acute forms were more frequent than chronic sarcoid arthropathy in Caucasians, without any difference of sex or age between these 2 forms. Joint involvement was frequently more symmetrical in acute than chronic forms (100 vs. 70%; $p < 0.05$), with a more frequent involvement in wrists and ankles in acute forms, whereas the tender and swollen joint counts and the DAS44-CRP were similar between the 2 groups. Skin lesions were significantly more frequent in patients with acute forms [17 (89%) vs. 5 (25%); $p < 0.05$] and were erythema nodosum in all patients with Löfgren's syndrome and sarcoid skin lesions in those with chronic sarcoidosis. Among 20 patients with chronic sarcoidosis, treatment was used in 17 (85%) cases, and consisted in NSAIDs alone ($n = 5$; 25%), steroids alone ($n = 5$; 25%), hydroxychloroquine ($n = 2$; 20%), methotrexate ($n = 3$; 15%), and TNF inhibitors ($n = 2$; 10%). A complete/partial joint response was noted in 14 (70%) cases with a DAS44-CRP reduction of 2.07 [1.85–2.44] (from 3.13 [2.76–3.42] to 1.06 [0.9–1.17]; $p < 0.05$).

Conclusion: Sarcoid arthropathies have different clinical phenotypes in acute and chronic forms and various treatment regimens such as hydroxychloroquine and methotrexate could be used in chronic forms.

Keywords: sarcoid arthropathy, outcome, methotrexate, infliximab, sarcoidosis

INTRODUCTION

Sarcoidosis is a heterogeneous systemic granulomatous disease affecting mostly lung and lymphatic nodes. Non-caseating granulomas are the characteristic histopathological feature. Joint involvement, also known as sarcoid arthropathy, is observed in 6–35% of patients, and asymptomatic bone involvement in 3–13% of patients. Acute-onset arthritis is generally characterized by symmetric arthritis, and in Lofgren's syndrome is associated with bilateral hilar adenopathies and erythema nodosum, with remission usually occurring within 6–10 weeks (1–5). Chronic sarcoid arthropathy is characterized by persistent oligo or poly-arthritis in 20% of patients, with 40% of arthralgia (1, 2). Few studies have described the prevalence and features of sarcoid arthropathies, and association with other organ involvements (2, 4, 6, 7). Non-steroidal anti-inflammatory drugs (NSAIDs) are effective in acute sarcoidosis, but little is known about the efficacy of other therapies in steroid-dependent and refractory forms of sarcoid arthropathy. We aimed to describe clinical characteristics of sarcoid arthropathies and describe the treatment.

PATIENTS AND METHODS

Study Scheme

The study was observational with a retrospective analysis. A call for observation was sent to members of the "French Inflammatory Joint Disease Working Group" (Club Rhumatismes et Inflammation) and the National French Society of Internal Medicine (SNFMI) from July 2017 to November 2018. Physicians were asked to fill in a defined table. The study complied with the recommendations of the Declaration of Helsinki, and being observational and retrospective, ethics committee approval was not necessary according to the French local law. 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 from the patients was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Patients

Patients were included if they were aged of 18 years or more, had histologically-proven sarcoidosis (except for typical acute forms of Löfgren syndrome) and at least one episode of arthritis, defined as the presence of clinical and/or ultrasonographic synovitis (8–11). The definition of ultrasonographic synovitis was used as usually done in patients with rheumatoid arthritis or other inflammatory arthritis. Exclusion criteria were: patients with other granulomatous diseases, associated rheumatoid arthritis, systemic lupus erythematosus, mixed connective tissue disease, microcrystalline arthritis or infectious arthritis (3). All patients' medical records were reviewed by 2 investigators (Carlotta Cacciatore and Arsène Mekinian).

Data Collection

Clinical data was collected as follows: age, ethnicity, sex, date of diagnosis, disease duration, and presence of skin, lung, heart, ocular, and neurological involvements. Articular assessment, which was collected at diagnosis and during follow-up, included: tender joint count, swollen joint count, localization of joint involvement and DAS44-CRP. Synovitis was diagnosed by a clinical examination and/or joint ultrasonography: among 39 included patients with clinical synovitis, 31 have undergone joint ultrasonography and have all ultrasound-confirmed synovitis. Synovitis was defined as synovial hypertrophy $\geq$2, or synovial hypertrophy $\geq$1+PD signal $\geq$1, according to the OMERACT definitions (12). Joint activity was quantified using DAS28-CRP at diagnosis and during the follow up. The use of non-steroidal anti-inflammatory drugs (NSAIDs), steroids and/or synthetic disease-modifying antirheumatic drugs (DMARDs), hydroxychloroquine and TNF antagonists was collected. Laboratory data included serum hemoglobin, platelet, neutrophil and lymphocyte counts, calcium, creatinine, alanine aminotransferase, aspartate aminotransferase, C-reactive protein (CRP), erythrocyte sedimentation rates (ESR), gamma-globulin, angiotensin converting enzyme (ACE), rheumatoid factor, antinuclear and anti-citrullinated protein antibodies (ACPA). Sarcoidosis was considered as acute if remission was completely reached within 12 weeks. Löfgren's syndrome was defined in patients with acute sarcoidosis in the presence of arthritis, bilateral hilar adenopathy and erythema nodosum, and fever (6, 13). All remaining cases were defined as chronic sarcoid arthropathy. Efficacy of each drug was compared to each other, and all treatment lines were analyzed. Complete articular response to treatment was defined as total disappearance of arthralgia and synovitis, with a DAS44-CRP $<$1.6 (14, 15). Partial response was defined as an improvement of at least 50% of swollen joint count (i.e., number of synovitis). Non-response was defined as all remaining cases. For each line of treatment, the reasons of interruption has been codified among inefficacy, adverse events and remission.

Statistical Analysis

Data is expressed as medians with ranges and numbers with frequencies. Student *t*-test or Wilcoxon-Mann Whitney test were used to compare quantitative data and Chi-square or Fisher's exact tests for qualitative variables. All analyses were done using

TABLE 1 | Characteristics of patients with joint involvement and acute/chronic sarcoidosis.

Characteristics	All patients *N* = 39	Acute sarcoidosis *N* = 19	Chronic sarcoidosis *N* = 20
Age (years), medians [ranges]	38 [23–70]	34.5 [23–70]	42 [25–65]
Female sex (*n*;%)	25 (64)	13 (68)	12 (60)
Caucasians (*n*;%)	19 (48.7)	12 (63)	7 (35)*
Extra-articular involvement			
Skin involvement (*n*;%)	22 (56)	17 (89)	5 (25)*
Ocular involvement (*n*;%)	4 (10)	0	4 (20)*
Hilar and mediastinal adenopathies (*n*;%)	19 (49)	8 (42)	11 (55)*
Lung involvement (*n*;%)	32 (82)	16 (84)	16 (80)
Heart involvement (*n*;%)	2 (5)	0	2 (10)
CNS involvement (*n*;%)	1 (2.5)	0	1 (5)
Joint involvement			
Symmetrical (*n*;%)	33 (85)	19 (100)	14 (70)*
Wrist (*n*;%)	18 (46)	5 (26)	10 (50)*
Ankles (*n*;%)	32 (82)	17 (89)	13 (65)*
Metacarpo-phalangeal (*n*;%)	12 (31)	4 (21)	6 (30)
Knees (*n*;%)	16 (41)	6 (32)	10 (50)
Tender joints medians [ranges]	6 [1–28]	4 [2–28]	6 [1–12]
Swollen joints medians [ranges]	2 [1–6]	2 [1–4]	2 [1–6]
DAS 44-CRP medians [ranges]	3.4 [2.3–5.9]	3.7 [2.3–5.9]	3.4 [2.3–3.5]
Laboratory data			
Lymphocytes (G/l) medians [ranges]	1.410 [0.66–3.35]	1.8 [0.66–2.50]	1.11 [0.71–3.35]
Gammaglobulins (g/l) medians [ranges]	11.6 [7.5–24.7]	11.4 [7.5–13.3]	12.3 [7.8–24.7]
Calcium levels (mg/l) medians [ranges]	2.34 [2.16–2.57]	2.3 [2.16–2.53]	2.36 [2.26–2.57]
ACE (UI/l) medians [ranges]	65 [21.2–300]	55 [21.20–111]	76.5 [36–300]
First line treatments	34 (87)	17 (89)	17 (85)
NSAIDs alone (*n*;%)	15 (38)	10 (53)	5 (25)*
Steroids alone (*n*;%)	9 (23)	4 (21)	5 (25)
Hydroxychloroquine (*n*;%)	4 (10)	2 (10)	2 (10)
With NSAIDs	3 (7.5)	2 (10)	1 (5)
With steroids	0	0	0
Methotrexate (*n*;%)	4 (10)	1 (5)	3 (15)
With NSAIDs	1 (2.5)	1 (5)	0
With steroids	2 (5)	0	2 (10)
TNF inhibitors (*n*;%)	2 (5)	0	5 (25)
With steroids		0	5 (10)
Follow-up (months) medians [ranges]	18 [3–264]	20.5 [3–57]	62.5 [3–264]*

Values are medians with interquartiles and numbers with frequencies.

*$p < 0.05$.

ACPA, anti Citrullinated Peptide antibodies; ANA, anti-nuclear antibodies; CRP, C-reactive protein; ESR, erythrocyte-sedimentation rate; NSAIDs, non-steroidal anti-inflammatories; RF, rheumatoid factor.

R software (R Foundation, Vienna, Austria version 3.0.2) and a $p < 0.05$ was considered as significant.

RESULTS

Thirty-nine patients (64% women) were included with a median age of 41 years [25–75] (**Table 1**). Among 39 patients with clinical arthritis, 31 (82%) had done ultrasound echography and all these 31 cases had ultrasound-confirmed synovitis. The pattern of arthritis was mostly symmetric polyarthritis affecting ankles in 33 (85%) cases, wrists in 18 (46%) cases and metacarpo-phalangeal joints in 12 (31%) cases. None reported spine involvement or dactylitis. Median tender and swollen joint count at diagnosis was 6 [1–28] and 2 [1–6], respectively. C-reactive protein levels were at 22 mg/l [1–271] and ACE at 65 U/L [21.2–300]. Median DAS44-CRP at diagnosis was 3.4 [2.3–5.9]. No patients had positive rheumatoid factor or ACPA, nor radiological structural damage. Acute forms were diagnosed in 19 (49%) patients and among them 17 (89%) have Löfgren syndrome. The remaining 20 (51%) patients have chronic sarcoid arthropathy. Acute

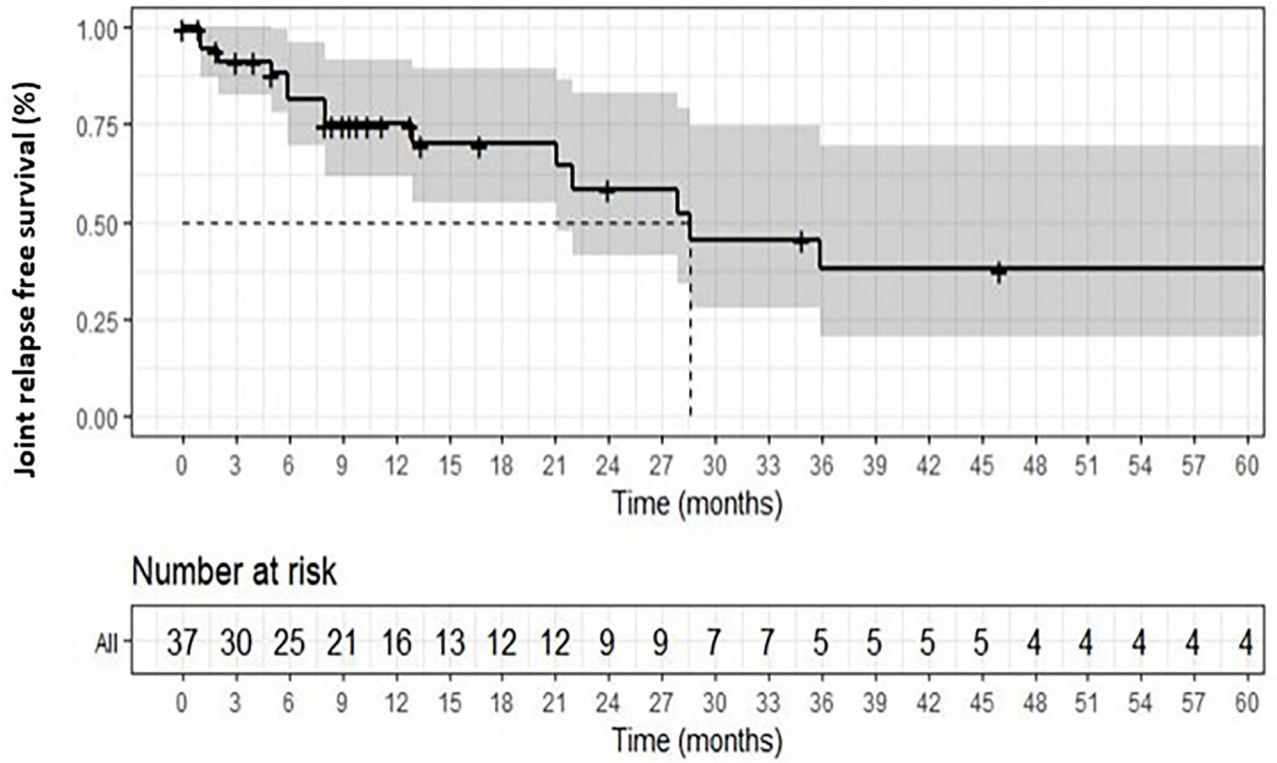


FIGURE 1 | Overall relapse-free survival in sarcoid arthropathy.

forms were more frequent than chronic sarcoid arthropathies in caucasians, without any difference of sex or age between these 2 forms. Joint involvement was frequently more symmetrical in acute than chronic forms (100 vs. 70%; $p < 0.05$), with a more frequent involvement in wrists and ankles in acute forms, whereas the tender and swollen joint counts and the DAS44-CRP were similar between the 2 groups (**Table 1**). Skin lesions were significantly more frequent in patients with acute forms [17 (89%) vs. 5 (25%); $p < 0.05$] and were erythema nodosum in all patients with Löfgren's syndrome and sarcoid skin lesions in those with chronic sarcoidosis. Ocular involvement was present only in patients with chronic sarcoidosis ($n = 4$, 20%): one chorioretinitis and anterior uveitis ($n = 3$). There were no significant differences in calcium levels, ACE, gammaglobulin, and lymphocyte levels at diagnosis between patients with acute and chronic sarcoid arthropathies (data not shown).

During the median follow-up of 18 [3–264] months, 34 patients (87%) received at least one therapy among NSAIDs, steroids, methotrexate, hydroxychloroquine or TNF inhibitors. Among the 19 patients with acute sarcoidosis, treatment was used in 17 (89%) cases, and consisted of NSAIDs alone ($n = 10$; 53%), steroids alone ($n = 4$; 21%), hydroxychloroquine ($n = 2$; 10%), and methotrexate ($n = 1$; 5%) (**Table 1**). A complete/partial joint response was noted in 15 (79%) cases with a DAS44-CRP reduction of 2.45 [1.66–2.66] (from 3.37 [2.62–3.48] to 0.92 [0.89–1.58]; $p < 0.05$).

Among 20 patients with chronic sarcoidosis, a first-line treatment was used in 17 (85%) cases, and consisted of NSAIDs alone ($n = 5$; 25%), steroids alone ($n = 5$; 25%), hydroxychloroquine ($n = 2$; 20%), methotrexate ($n = 3$; 15%), and TNF inhibitors ($n = 5$; 25%) (**Table 1**). A complete/partial joint response was noted in 14 (70%) cases with a DAS44-CRP reduction of DAS44-CRP reduction of 2.07 [1.85–2.44] (from 3.13 [2.76–3.42] to 1.06 [0.9–1.17]; $p < 0.05$). A second-line therapy for chronic sarcoidosis was used in 8 cases: methotrexate ($n = 6$), steroids alone and hydroxychloroquine ($n = 1$ each) with a joint response in 6 cases. A third-line therapy for sarcoid chronic arthropathy was used in 5 cases and among them TNF inhibitors in 5 cases with joint responses in all cases.

NSAIDs was the most frequent first-line option in acute sarcoidosis [10 (53%) vs. 5 (25%); $p < 0.05$], but other first-line therapy frequencies were not significantly different between the 2 groups. The median time of treatment was at 2.5 months [0.6–7]. The joint relapse-free survival was at 26.6 months in the overall group (**Figure 1**).

DISCUSSION

This study reports clinical and laboratory features of acute and chronic sarcoid arthropathies, and shows some significant differences in joint and extra-articular involvements. The acute forms have a more symmetrical joint distribution

with predominant skin involvement, whereas chronic sarcoid arthropathies have more frequent ocular involvements (16).

Joint involvement in sarcoidosis ranges from 6 to 35% (2, 11, 13–15, 17–19). Clinical features of acute and chronic sarcoid arthropathies have rarely been analyzed (20, 21). The chronic form is usually associated with parenchymal lung or other organ involvements and is relatively rare (up to 7%) (4, 18, 22, 23). In our study, swollen joint count and DAS44 CRP were comparable in both sarcoid arthropathies, but acute forms were frequently more symmetrical and affected ankles (4). DAS-28 score is not validated in the setup of sarcoidosis, and in particular does not include the ankle involvement which could be frequent in acute forms, but has already previously been used in other inflammatory arthritis and sarcoid arthritis (24, 25). Consistently with previous studies, skin involvement (mostly erythema nodosum) was particularly frequent in acute sarcoid arthropathy, whereas ocular involvement occurred just in chronic subsets (26). The presence and features of hilar and lung involvements were not discriminatory between acute and chronic sarcoid forms.

Management of sarcoid arthropathy is poorly studied (11, 27): most data involves acute forms, with chronic forms only described in small case-series. In this study, we compared the efficacy of various treatment regimens on sarcoid arthropathy with clinical synovitis. Using pooled lines of various therapies, we showed no significant differences in joint responses using hydroxychloroquine, methotrexate and TNF antagonists. Previous studies confirmed the efficacy of NSAIDs and steroids for acute sarcoid arthropathy (13, 18, 28), as we reported in the present study. For chronic sarcoid arthropathy, small case-series showed the benefit of methotrexate (29, 30), whereas in a retrospective study among 10 patients treated with TNF antagonists, despite initial rapid efficacy, no clinical amelioration was noted after 1 year of treatment (24). Judson et al. conducted the only trial evaluating sarcoidosis joint manifestations under infliximab. No satisfactory effect on joint disease was observed at 24 and 48 weeks, even though significant attenuation of involvement for all combined organs was observed at 28 weeks (22). TNF- antagonists could also have a steroid-sparing effect, in particular in chronic sarcoidosis (22, 24, 25).

Our study has several biases that limit definitive conclusions. Firstly, it is a retrospective study with small sample sizes, and the small number of patients treated by TNF antagonists does not allow definitive conclusions about efficacy of this therapy in joint involvement. We assessed disease activity with DAS44-CRP score usually used for rheumatoid arthritis, as some other previous studies (24), because no validated score exists to evaluate joint activity in sarcoidosis.

CONCLUSION

Sarcoid arthropathies have different clinical phenotypes in acute and chronic forms and various treatment regimens such as hydroxychloroquine and methotrexate could be used in chronic forms.

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 from the patients was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.565420/full#supplementary-material

REFERENCES

1. Nessrine A, Zahra AF, Taoufik H, Nessrine A, Zahra AF, Taoufik H. Musculoskeletal involvement in sarcoidosis. *J Bras Pneumol.* (2014) 40:175–82. doi: 10.1590/S1806-37132014000200012
2. Visser H, Vos K, Zanelli E, Verduyn W, Schreuder GM, Speyer I, et al. Sarcoid arthritis: clinical characteristics, diagnostic aspects, and risk factors. *Ann Rheum Dis*. (2002) 61:499–504. doi: 10.1136/ard.61.6.499
3. Glennås A, Kvien TK, Melby K, Refvem OK, Andrup O, Karstensen B, et al. Acute sarcoid arthritis: occurrence, seasonal onset, clinical features and outcome. *Br J Rheumatol.* (1995) 34:45–50. doi: 10.1093/rheumatology/34.1.45
4. Kobak S, Sever F, Usluer O, Goksel T, Orman M. The clinical characteristics of sarcoid arthropathy based on a prospective cohort study. *Ther Adv Musculoskelet Dis*. (2016) 8:220–4. doi: 10.1177/1759720X16670598
5. Al-Kofahi K, Korsten P, Ascoli C, Virupannavar S, Mirsaeidi M, Chang I, et al. Management of extrapulmonary sarcoidosis: challenges and solutions. *Ther Clin Risk Manag*. (2016) 12:1623–34. doi: 10.2147/TCRM.S74476
6. Ungprasert P, Crowson CS, Matteson EL. Clinical characteristics of sarcoid arthropathy: a population-based study. *Arthritis Care Res*. (2016) 68:695–9. doi: 10.1002/acr.22737
7. Muñoz C, Restrepo-Escobar M, Martínez-Muñoz M, Echeverri A, Márquez J, Pinto LF. Differences between patients with sarcoidosis with and without joint involvement treated for fifteen years in a third level hospital. *Reumatol Clin.* (2020) 16:45–8. doi: 10.1016/j.reumae.2018.01.004
8. Statement on Sarcoidosis. *Am J Respir Crit Care Med.* (1999) 160:736–55. doi: 10.1164/ajrccm.160.2.ats4-99

9. Le Bras E, Ehrenstein B, Fleck M, Hartung W. Evaluation of ankle swelling due to Lofgren's syndrome: a pilot study using B-mode and power Doppler ultrasonography. *Arthritis Care Res*. (2014) 66:318–22. doi: 10.1002/acr.22099
10. Kellner H, Späthling S, Herzer P. Ultrasound findings in Löfgren's syndrome: is ankle swelling caused by arthritis, tenosynovitis or periarthritis? *J Rheumatol*. (1992) 19:38–41.
11. Rao DA, Dellaripa PF. Extrapulmonary manifestations of sarcoidosis. *Rheum Dis Clin North Am*. (2013) 39:277–97. doi: 10.1016/j.rdc.2013.02.007
12. D'Agostino MA, Terslev L, Aegerter P, Backhaus M, Balint P, Bruyn GA, et al. Scoring ultrasound synovitis in rheumatoid arthritis: a EULAR-OMERACT ultrasound taskforce-part 1: definition and development of a standardised, consensus-based scoring system. *RMD Open*. (2017) 3:e000428. doi: 10.1136/rmdopen-2016-000428
13. Byun CW, Yang SN, Yoon JS, Kim SH. Lofgren's syndrome-acute onset sarcoidosis and polyarthralgia: a case report. *Ann Rehabil Med*. (2013) 37:295–9. doi: 10.5535/arm.2013.37.2.295
14. Fleischmann RM, van der Heijde D, Gardiner PV, Szumski A, Marshall L, Bananis E. DAS28-CRP and DAS28-ESR cut-offs for high disease activity in rheumatoid arthritis are not interchangeable. *RMD Open*. (2017) 3:e000382. doi: 10.1136/rmdopen-2016-000382
15. Burmester GR, Ferraccioli G, Flipo R-M, Monteagudo-Sáez I, Unnebrink K, Kary S, et al. Clinical remission and/or minimal disease activity in patients receiving adalimumab treatment in a multinational, open-label, twelve-week study. *Arthritis Rheum*. (2008) 59:32–41. doi: 10.1002/art.23247
16. Sweiss NJ, Patterson K, Sawaqed R, Jabbar U, Korsten P, Hogarth K, et al. Rheumatologic manifestations of sarcoidosis. *Semin Respir Crit Care Med*. (2010) 31:463–73. doi: 10.1055/s-0030-1262214
17. Hirabayashi Y, Ishii T. The DAS28-ESR cutoff value necessary to achieve remission under the new Boolean-based remission criteria in patients receiving tocilizumab. *Clin Rheumatol*. (2013) 32:123–7. doi: 10.1007/s10067-012-2103-4
18. Ungprasert P, Carmona EM, Utz JP, Ryu JH, Crowson CS, Matteson EL. Epidemiology of sarcoidosis 1946-2013: a population-based study. *Mayo Clin Proc*. (2016) 91:183–8. doi: 10.1016/j.mayocp.2015.10.024
19. Cozier YC, Berman JS, Palmer JR, Boggs DA, Serlin DM, Rosenberg L. Sarcoidosis in Black women in the United States. *Chest*. (2011) 139:144–50. doi: 10.1378/chest.10-0413
20. Rubio-Rivas M, Franco J, Corbella X. Sarcoidosis presenting with and without Löfgren's syndrome: Clinical, radiological and behavioral differences observed in a group of 691patients. *Joint Bone Spine*. (2020) 87:141–7. doi: 10.1016/j.jbspin.2019.10.001
21. Bechman K, Christidis D, Walsh S, Birring SS, Galloway J. A review of the musculoskeletal manifestations of sarcoidosis. *Rheumatology*. (2018) 57:777–83. doi: 10.1093/rheumatology/kex317
22. Judson MA, Baughman RP, Costabel U, Flavin S, Lo KH, Kavuru MS, et al. Efficacy of infliximab in extrapulmonary sarcoidosis: results from a randomised trial. *Eur Respir J*. (2008) 31:1189–96. doi: 10.1183/09031936.00051907
23. Eschard JP, Etienne JC. [Osteoarticular manifestations of sarcoidosis]. *Rev Med Interne*. (1994) 15(Suppl. 3):305–7S.
24. Banse C, Bisson-Vaivre A, Kozyreff-Meurice M, Vittecoq O, Goëb V. No impact of tumor necrosis-factor antagonists on the joint manifestations of sarcoidosis. *Int J Gen Med*. (2013) 6:605–11. doi: 10.2147/IJGM.S44542
25. Banse C, Goëb V. Do not forget the joint involvement of sarcoidosis. *Immunotherapy*. (2015) 7:599–600. doi: 10.2217/imt.15.24
26. Schupp JC, Freitag-Wolf S, Bargagli E, Mihailović-Vučinić V, Rottoli P, Grubanovic A, et al. Phenotypes of organ involvement in sarcoidosis. *Eur Respir J*. (2018) 51:1700991.
27. Awada H, Abi-Karam G, Fayad F. Musculoskeletal and other extrapulmonary disorders in sarcoidosis. *Best Pract Res Clin Rheumatol*. (2003) 17:971–87. doi: 10.1016/j.berh.2003.09.005
28. Matsui K, Adachi M, Kawasaki Y, Matsuda K, Shinohara K. Sarcoidosis acutely involving the musculoskeletal system. *Intern Med*. (2007) 46:1471–4. doi: 10.2169/internalmedicine.46.6375
29. Suda T, Sato A, Toyoshima M, Imokawa S, Yoshitomi A, Tamura R, et al. Weekly low-dose methotrexate therapy for sarcoidosis. *Intern Med Tokyo JPN*. (1994) 33:437–40. doi: 10.2169/internalmedicine.33.437
30. Kaye O, Palazzo E, Grossin M, Bourgeois P, Kahn MF, Malaise MG. Low-dose methotrexate: an effective corticosteroid-sparing agent in the musculoskeletal manifestations of sarcoidosis. *Rheumatology*. (1995) 34:642–4. doi: 10.1093/rheumatology/34.7.642

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.

frontiers in Medicine

REVIEW
published: 11 January 2021
doi: 10.3389/fmed.2020.594275

Sarcoidosis: Pitfalls and Challenging Mimickers

Naureen Narula and Michael Iannuzzi*

Staten Island University Hospital, New York, NY, United States

Sarcoidosis, a systemic granulomatous disease of unknown etiology, may mimic other conditions at presentation often resulting in delayed diagnosis. These conditions include infections, neoplasms, autoimmune, cardiovascular, and drug-induced diseases. This review highlights the most common sarcoidosis mimics that often lead to pitfalls in diagnosis and delay in appropriate treatment. Prior to invasive testing and initiating immunosuppressants (commonly corticosteroids), it is important to exclude sarcoid mimickers.

Keywords: sarcoidosis, cardiac sarcoidosis (CS), neurosarcoidosis, hypercalcemia (HCM), hypersentivity pneumonitis, sarcoidosis associated pulmonary hypertension, aseptic meningitis

INTRODUCTION

Jonathon Hutchinson, an English physician, first described sarcoidosis in 1877 (1). Over the last few decades, substantial progress has been made to better define the clinical, radiological, immunological, and pathological features of sarcoidosis. The diagnosis of sarcoidosis is based on three major criteria: clinical presentation compatible with sarcoidosis, presence of non-necrotizing granulomatous inflammation in one or more tissue samples, and the exclusion of alternative causes of granulomatous disease. The American Thoracic Society recently published an official clinical practice guideline in which a panel of experts discuss and summarize evidence-based diagnosis of sarcoidosis (2). Despite advancements clinicians often remain challenged in reaching a diagnosis of sarcoidosis.

Infections

Nearly all infectious causes of granulomas may present similarly to sarcoidosis (**Table 1**) (3–7) thus excluding an infectious etiology must be routine and relies more on laboratory studies rather than differentiating clinical features. **Table 1** highlights the recommended laboratory studies to be considered during the workup of a patient. Sarcoidosis is an often overlooked cause of fever of unknown origin (FUO) (8). About one-third of patients with sarcoidosis exhibit non-specific constitutional findings such as fever, lethargy, night sweats, and weight loss.

In developing countries with high TB burdens, diagnosing sarcoidosis can be particularly challenging. TB is classically characterized by caseating granulomas whereas sarcoidosis has non-caseating epithelioid cell granulomas. However, when caseous necrosis is not seen and acid-fast staining of biopsy specimens is negative then a patient with suspected TB infection can be mistakenly diagnosed with sarcoidosis (3). In an endemic area, clinical judgment is crucial. Radiological findings of upper lobe predominant cavitary lesions favor the diagnosis of TB, as cavity formation occurs in only 3% of sarcoidosis cases (9).

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) to obtain TB-PCR samples makes ruling out TB more certain. In a study by Eom et al., 86 specimens were examined in 46 patients and the sensitivity, specificity, positive predictive value, negative predictive

OPEN ACCESS

Edited by:
Peter Korsten,
University Medical Center Göttingen, Germany

Reviewed by:
Cheng-De Yang,
Shanghai Jiao Tong University, China
Wonder Drake,
Vanderbilt University, United States

****Correspondence:***
Naureen Narula
drnaureennarula@gmail.com

Specialty section:
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

Received: *12 August 2020*
Accepted: *30 October 2020*
Published: *11 January 2021*

Citation:
Narula N and Iannuzzi M (2021) Sarcoidosis: Pitfalls and Challenging Mimickers. Front. Med. 7:594275. doi: 10.3389/fmed.2020.594275

TABLE 1 | Infectious causes of granulomas and the laboratory studies to consider to rule out infection.

1. **Bacteria**
Actinomyces
Bartonella
Borrelia burgdorferi
Brucellosis
Mycobacterium tuberculosis
Non-tuberculous mycobacterium
Nocardia
Q fever
Treponema pallidum
Whipples disease
2. **Fungi**
Aspergillosis
Blastomycosis
Candida
Cryptococcus
Histoplasma
3. **Parasitic**
Leshmaniosis
Schistosomiasis
4. **Viral**
Cytomegalovirus
Epstein barr
Laboratory studies to rule out infection:
1. Histopathologic examination
Ziehl–Neelsen stain or Auramine–rhodamine fluorescence for mycobacteria
Grocott methenamine silver stain for fungi
TB PCR
2. Culture for bacteria (aerobic and anaerobic), fungi, mycobacteria
3. Serology for histoplasma, syphilis, lyme disease

value, and diagnostic accuracy were analyzed. EBUS-TBNA TB PCR was found to be 56%, 100%, for sensitivity and specificity, respectively. Positive and negative predictive values were 100%, and 81%. Diagnostic accuracy was 85% (10). Characterization of sonographic features of lymph nodes by an EBUS can also aid in differentiating TB associated lymphadenopathy (LAD) from sarcoidosis. Dhooria et al. analyzed 250 EBUS-guided TBNA procedures in patients with intrathoracic LAD and found that sonographic features of heterogeneous echotexture and coagulation necrosis are suggestive of TB rather than sarcoidosis. A combination of a positive tuberculin skin test (TST) and either heterogeneous echotexture or coagulation necrosis sign had a specificity of 98% and a positive predictive value of 91% for a diagnosis of tuberculosis (11).

Heterogeneous echotexture (53.4 vs. 12.6%, $P < 0.001$) and coagulation necrosis (26.1 vs. 3.3%; $P < 0.001$) are suggestive of TB rather than sarcoidosis. A combination of a positive tuberculin skin test (TST) and either heterogeneous echotexture or coagulation necrosis sign had a specificity of 98% and positive predictive value of 91% for a diagnosis of tuberculosis (11). Use of an interferon-γ (IFN-γ) release assay has been reported to demonstrate a better predictive ability than tuberculin skin tests (12). Culture although time-consuming is still considered as a gold standard test for the diagnosis of Tuberculosis (13).

An accurate and timely diagnosis of sarcoidosis helps prevent unnecessary antituberculosis therapy (ATT) drug exposure. An accurate diagnosis of TB prevents exposure to immunosuppressive agents. Concomitant tuberculosis and sarcoidosis is rare (14).

The presence of exudative pleural effusions may favor other diagnoses. Pleural effusions associated with sarcoidosis are uncommon (8.2%) and can be present at the time of diagnosis or at a later time, coinciding with an exacerbation (15, 16). These effusions are typically right sided, exudative, and lymphocytic predominant. Eosinophilic and neutrophilic effusions have been reported, but are less common. Pleural fluid in sarcoidosis is characterized by having a high CD4/CD8 ratio and frequently sarcoid related pleural effusions resolve spontaneously but still may require treatment with corticosteroids (17).

The presence of an exudative effusion in the setting of sarcoidosis warrants infectious workup e.g., parapneumonic effusion, or empyema. Patients on immunosuppressive agents are particularly susceptible to opportunistic infections (18–20). The failure of pleural effusions to respond to corticosteroid treatment should raise suspicion for an underlying opportunistic infection or other complications such as pulmonary embolism. **Table 2** highlights the characteristic differentiating features in patients with common infectious etiologies which can mimic sarcoidosis.

GRANULOMATOUS CHRONIC INTERSTITIAL LUNG DISEASE

Along with sarcoidosis, granuloma formation is a feature of other chronic interstitial lung diseases such as chronic beryllium disease (CBD) and hypersensitivity pneumonitis (HP).

Chronic Beryllium Disease (CBD)

Chronic beryllium disease (CBD) is clinically and pathologically indistinguishable from sarcoidosis. CBD risk is associated with fluorescent light manufacturing and in industries such as nuclear energy, ceramics, aerospace, automotive, electronics, and telecommunications (42). Non-occupational exposure to CBD has been reported in family members of factory workers likely due to second-hand exposure and in residents of communities near the beryllium factories.

Symptoms of CBD include dry cough, progressive dyspnea on exertion, fatigue, and night sweats. The radiological features vary with common chest CT findings including small nodules, ground-glass opacification, mild hilar adenopathy, and septal lines. A diagnosis of CBD is favored over sarcoidosis when there is documented occupational exposure to beryllium. Beryllium exposure should be considered at the time of sarcoidosis diagnosis as well as in chronic corticosteroid-resistant sarcoidosis to ensure they are removed from ongoing exposure to immunosuppressants.

A diagnosis of CBD can be aided by the following three criteria: (1) symptomatic disease with a histopathological demonstration of non-caseating granuloma, pulmonary function impairment, and abnormal chest radiographs; (2) proof of beryllium sensitization by two independently positive beryllium

TABLE 2 | Highlights the characteristic differentiating features in patients with common infectious etiologies which can mimic sarcoidosis.

	Features	Sarcoidosis	*Mycobacterium tuberculosis*	Histoplasmosis
A	**Lab findings** 1. ACE level 2. Abnormal calcium metabolism 3. Kveim-Siltzbach test 4. Mantoux test 5. Other diagnostic tests	Elevated ACE (50–80%) (19–21) Elevated vitamin D Hypercalcemia Hypercalciuria (22) Positive in 60% (23) Anergic; Negative in 90% (24)	Elevated ACE in 9% (22, 25) Hypovitaminosis D Hypercalcemia rare but can be seen in MTB IRD cases (26) Negative Positive in 65–94% (27, 28) PCR MTB	Elevated in 25% (29, 30) Hypercalcemia rare but can be seen (31) Negative Negative Histoplasma antigen in urine (sensitivity 93–100%) (Gold standard test) and serum (32) - PCR assays for the Hcp 100 gene - Histoplasmin sensitivity skin test
B	BAL findings	- CD4/CD8 ratio >3.5 (33) - Elevated ACE (34, 35)	BAL AFB smear (sensitivity 38.1%) BAL culture (sensitivity 74.5%) (35, 36) Elevated $CD4^+/CD8^+$ ratio (37)	Histoplasmosis Antigen in BAL (38)
C	Histopathology	Non-caseating granulomas	Caseating granulomas - AFB^+ bacilli - Isolation of MTB	-Non-caseating granulomas - Isolation of *H. capsulatum* from tissue specimens (gold standard) - methenamine silver or PAS stains revealing narrow-based budding yeasts
D	1. Radiological Hilar and mediastinal LAD 2. EBUS	Bilateral Hilar LAD when present is symmetrical Nodules Reticulonodular opacities Cavitation rare Granular appearance in lymph nodes -highest specificity (99.3%) for the diagnosis of sarcoidosis (39).	Asymmetrical, unilateral Upper-lobe infiltrates with cavitation, tree-in-bud, macro-nodular infiltrates (3). Cavitation is more common (9) Heterogeneous echotexture (53.4% $P < 0.001$) and coagulation necrosis (26.1% $P < 0.001$) (11)	Asymmetrical, unilateral (40) Fibrosing mediastinitis Reticulonodular opacities Cavitation is rare (41) Non-specific
E	Treatment	Immunosuppressant like corticosteroids	ATT	Itraconazole, AMB Streptomycin

AFB, Acid Fast Bacilli; ATT, Anti-Tuberculosis Treatment; AMB, Amphotericin B; MTB, Mycobacterium tuberculosis; LAD, Lymphadenopathy; PCR, Polymerase Chain Reaction; MTB-IRD, Mycobacterium tuberculosis Immune Reconstitution Disease.

lymphocyte proliferation assays (BeLPTs) in the absence of treatment with systemic corticosteroids for preceding 3 months, and (3) proof of beryllium exposure (43, 44). Beryllium sensitization is diagnosed when either criteria 2 or 3 are fulfilled without criterium 1.

Management of suspected CBD involves stopping all further exposure and continued clinical surveillance. Therapy with corticosteroids may be beneficial in selected patients, but the response is variable. Oral prednisone (doses ranging from 20 to 40 mg/day) for 3–6 months followed by a gradual taper to the lowest effective dose is deemed acceptable. Unfortunately, CBD is typically a progressive disease and lifelong treatment may be required. BeLPT should be offered to any patient with sarcoidosis who has worked around metal-dust or fumes (45).

Hypersensitivity Pneumonitis

Hypersensitivity pneumonitis (HP) has clinical, radiological, and histopathological features that overlap with sarcoidosis. HP pathogenesis results from a combination of immune complex-mediated (type III) and delayed-type (type IV) hypersensitivity reactions to antigen inhalation in a susceptible person. Cryptogenic HP is a subtype of HP in which despite typical HP features an inciting exposure is not identified (46–48).

Table 3 illustrates comparative features between hypersensitivity pneumonitis and sarcoidosis.

HP can be categorized by disease duration into acute, subacute, and chronic subtypes (60). Raghu et al. recently proposed categorization based on the presence or absence of radiological and/or histopathological fibrosis into either fibrotic HP or non-fibrotic HP, which has been widely accepted (61).

The median age at diagnosis is 65 years which is somewhat older than what is typical of sarcoidosis (20–39 years). Common presenting symptoms of HP include dyspnea, cough, wheezing, and, less frequently, constitutional symptoms such as weight loss. Physical examination may reveal the presence of rales. An acute presentation is more consistent with the non-fibrotic subtype and an insidious presentation is more consistent with fibrotic HP (47, 62, 63).

The radiological features of HP depend upon the subtype. Typical radiologic features of non-fibrotic HP include high-resolution CT (HRCT) showing parenchymal infiltration with ground-glass opacities (GGO), and mosaic attenuation. HRCT may also demonstrate small airway disease, which can be described as ill-defined, small (<5 mm) centrilobular nodules on inspiratory images and air trapping on expiratory images, both in a diffuse distribution. Typical radiological features associated

TABLE 3 | Compares the characteristics of CBD with sarcoidosis across various clinical characteristics, lab abnormalities, and imaging studies.

	Sarcoidosis	Hypersensitivity pneumonitis (HP)	CBD
Exposure history	Unknown	May have a history of an allergen exposure	Exposure to beryllium; Occupational history
Age of diagnosis	Pea peak incidence at age of 20–39 years (49)	The median age of diagnosis 65 years	Mean age at time of diagnosis of CBD 43.9 (25–80) years (50)
Laboratory	ACE level elevated (>80%)	Serum IgG to potential antigens associated with HP (sensitivity and specificity of 83 and 68%) (48)	ACE may be elevated in 22% case (44, 51). BeLPT: abnormal (peripheral blood and/or BAL) (52) Patch testing ($BeSO_4$ or BeF): abnormal (peripheral blood and/or BAL)
Radiological	- Symmetrical and bilateral Hilar LAD - Nodules and/or Reticulonodular opacities	Features depend on subtype-non-fibrotic or fibrotic HP - GGO, mosaic attenuation, centrilobular nodules, fibrosis (irregular linear opacities; traction bronchiectasis and honeycombing) - Fibrosis is most severe in the mid or mid and lower lung zones or equally distributed in the three lung zones with relative basal sparing. Head cheese sign/three-density pattern (47)	The most common radiographic abnormalities include diffuse small round and reticular opacities. Hilar adenopathy, linear scars, lung distortion, bullae, and pleural thickening are found less commonly associated (53)
Histopathology	Well-formed, non-necrotizing granulomas, showing a lymphangitic distribution.	Small and poorly formed granulomas, comprising loose, poorly circumscribed clusters of epithelioid and multinucleated cells (54). Bronchiolocentric inflammation In Fibrotic HP-subpleural and centriacinar fibrosis, with or without bridging fibrosis. May have features that overlap with a UIP pattern (55)	Well-formed, non-necrotizing granulomas, and/or mononuclear cell interstitial cell infiltrates on endobronchial or transbronchial biopsy Calcific inclusions reported
BALF	Lymphocytic $CD4^+$:$CD8^+$ ratio >3.5	Lymphocytic (>30% for non- and ex-smokers and >20% for current smokers) (56) Low $CD4^+/CD8^+$ ratio (mean values of 0.5–1.5) Increased expression of CD80/CD86 (48, 57)	Lymphocytosis 41–53% (44, 58) BeLPT BAL
Spontaneous resolution	Depends upon the stage	Rare	Rare
Treatment	Immunosuppressants (corticosteroids)	Removal from exposure - Corticosteroids (59)	Removal from further exposure. In symptomatic cases, corticosteroids/immunosuppressants

ACE, angiotensin converting enzyme; BALF, bronchoalveolar fluid; BeLPT, beryllium lymphocyte proliferation assay; CBD, chronic beryllium disease.

with fibrotic HP include an HRCT pattern of irregular linear opacities and or/coarse reticulation with lung distortion; traction bronchiectasis and honeycombing, all typical of lung fibrosis, and at least one abnormality that indicates small airway disease. The presence of three different lung densities, previously referred by radiologists as "head-cheese sign" and recently being referred to as the "three-density pattern," is characteristically associated with HP (61). Radiologically, granulomas associated with HP are interstitial and do not follow the lymphatics as in sarcoid. Also, sarcoidosis normally spares the lung bases. For both HP and advanced sarcoidosis, a prognosis-predicting factor is the extent of the fibrotic score calculated on HRCT scans (64, 65). Further, HRCT can help differentiate active inflammation from fibrosis in patients with advanced-stage sarcoidosis (66). Honeycombing is uncommon in sarcoidosis but if present usually involves the middle and upper lung zones with relative sparing of the lung bases. The presence of lower lung honeycombing would make it difficult to distinguish between HP, sarcoidosis, and idiopathic pulmonary fibrosis (59).

Bronchoalveolar lavage fluid (BALF) analysis of patients with HP reveals a predominance of lymphocytes, like sarcoidosis. A meta-analysis of 53 studies (3,112 patients) by Raghu et al. demonstrated that patients with HP had a higher proportion of BALF lymphocytes than patients with sarcoidosis (MD, 19%; 95% CI, 17–21%). This was found regardless of whether the study enrolled patients with non-fibrotic HP (17 studies; MD, 25%; 95% CI, 22–27%), fibrotic HP (16 studies; MD, 16%; 95% CI, 11–20%), or mixed populations with both non-fibrotic and fibrotic

HP (21 studies; MD, 18%; 95% CI, 15–20%). For distinguishing non-fibrotic HP from sarcoidosis, BALF lymphocyte thresholds of 20, 30, and 40% yielded decreased sensitivities for HP of 95, 88, and 76%, respectively, but increased specificities of 26, 43, and 61%, respectively, with an area under the curve of 0.71 (95% CI, 0.67–0.74) (61).

A diagnosis of HP requires:

1. The presence of an exposure, defined as a positive exposure history, serum IgG testing against potential antigens associated with HP, and/or specific inhalation challenge
2. BALF revealing lymphocytosis or histopathological findings on biopsy
3. Typical radiographic features as described above (GGO and mosaic attenuation for non-fibrotic HP and fibrotic changes with small airway involvement for fibrotic HP).

A diagnosis of HP can be made with high confidence in patients with an identified provocative exposure and who have a typical HP radiological pattern on HRCT and with BALF revealing predominant lymphocytosis. For less straightforward cases, a multidisciplinary team consisting of a pulmonologist experienced in interstitial lung disease (ILD), a chest radiologist, and when necessary, a pathologist familiar with histopathological features of ILD and HP.

Treatment involves prompt and complete avoidance of further exposure to the inducer. Systemic corticosteroids are used in treatment however, their use has no effect on long-term outcomes and is often reserved for patients with more severe symptoms (67, 68). Fibrotic HP is associated with worse prognosis particularly with persistent exposure to the inciting agent, cigarette smoking, lower baseline vital capacity, and lack of BALF lymphocytosis (49, 62, 69, 70). Patients with the progressive disease should be evaluated for lung transplantation (55).

THROMBOSIS; PULMONARY HYPERTENSION

When presented with a patient with sarcoidosis and worsening shortness of breath, an assessment for pulmonary embolism (PE) and pulmonary hypertension (PH) is warranted. PE and sarcoidosis associated pulmonary hypertension (SAPH) may often be misdiagnosed as an exacerbation of sarcoidosis.

VENOUS THROMBOEMBOLISM (VTE) ASSOCIATED WITH SARCOIDOSIS

Sarcoidosis, as with other chronic inflammatory conditions, has been associated with an increased risk of venous thromboembolism (VTE) (71–73). Swigris et al. reported a greater than 2-fold higher risk of pulmonary embolism in patients with sarcoidosis when compared to the general population (74). In addition to PE, thrombosis secondary to localized inflammation has also been reported in the literature. Mural thrombosis in myocardial sarcoidosis, cerebral vein thrombosis in neurosarcoidosis and thoracic vein thrombosis in mediastinal sarcoidosis have all been reported (75–77). While the precise mechanism is not yet defined, chronic inflammation associated with sarcoidosis likely predisposes to endothelial cell injury with the inflammatory cytokines activating the coagulation cascade (78–80). Extrinsic vascular compression at a mediastinal or hilar level may lead to venous stasis thus progressing to localized thrombosis (77). Up to 38% of sarcoidosis patients demonstrate the presence of antiphospholipid antibodies (81). The use of glucocorticoids further increases the risk of VTE (82).

SARCOIDOSIS-ASSOCIATED PULMONARY HYPERTENSION (SAPH)

Fischer et al. reported elevated pulmonary-artery pressure in 6–23% of patients at rest and in as many as 43% with exertion (83). SAPH has been reported in up to 74% of patients with advanced sarcoidosis (84, 85). In one case-series, Schorr et al. reported a 7-fold increased risk for death over a 3-year follow-up in patients with SAPH (86). Pulmonary fibrosis leading to obliteration of the pulmonary vessels is considered the most common mechanism for developing PH (84). SAPH is classified as World Health Organization (WHO) group 5 due to its complex and multifactorial mechanisms (87). The optimal management for pulmonary hypertension in sarcoidosis is not well-defined. Along with treatment directed at active sarcoid inflammation (corticosteroid and steroid-sparing agents), therapy should focus on correcting hypoxia when present and managing comorbidities such as sleep apnea and cardiac dysfunction. Reduced diffusing capacity of the lung for carbon monoxide (DLCO) correlates with the severity of SAPH (88). Currently, the use of pulmonary hypertension specific therapy for the management of SAPH is controversial, and thus far pulmonary arterial hypertension specific therapies have not been approved (89, 90). Lung transplantation may also be considered in this high risk group. A reduced *D*LCO (<35% predicted) and a 6MWD of <300 m are associated with worse transplant-free survival. Oksana et al. identified preservation of FEV1/FVC ratio as an independent risk factor for worsened outcomes (91).

NEUROSARCOIDOSIS AND ITS POTENTIAL MIMICS-ASEPTIC MENINGITIS AND MULTIPLE SCLEROSIS

Any part of the nervous system may be involved in sarcoidosis. Neurological involvement occurs in 5–15% of patients and often precedes the diagnosis of sarcoidosis in up to 74%. Neurological Involvement most commonly affects cranial nerves (Cranial nerve VII and II are most common), the hypothalamus and the pituitary gland followed by the meninges, brainstem, spinal cord are less frequently involved (92–96).

Neurosarcoidosis can mimic other neurologic diseases including neoplasm (lymphoma, metastasis) (97), infectious etiologies (meningoencephalitis) (98) and other inflammatory diseases (angiitis/vasculitis, demyelinating disorders). Neurosarcoidosis can present as multiple supratentorial and/or infratentorial masses (35%) or solitary masses (15%) (99). The differential diagnosis includes gliomas, primary B

cell lymphoma, metastatic disease, infarct, and demyelinating disease. Motor dysfunction is present in up to 50% of cases. Patients can demonstrate neuropsychiatric manifestations such as depression, psychoses, dementia, poor concentration, and hallucinations (100–102).

CENTRAL INVOLVEMENT

Aseptic Meningitis Associated With Sarcoidosis

Cerebrospinal Fluid (CSF) findings in neurosarcoidosis may reveal elevated protein (50–70% of patients), elevated CSF pressure with a lymphocytic pleocytosis (57–72% of patients), and a reduced glucose level (up to 18% patients). None of these abnormalities are specific for neurosarcoidosis. Several studies evaluated the role of elevated CSF angiotensin-converting enzyme (ACE) level for diagnosing neurosarcoidosis (103, 104). The sensitivity of CSF ACE varies depending on the location of the central nervous system (CNS) involvement. For example, higher levels are rarely seen with spinal cord involvement (105). The ACE assay is not a specific test as it can be elevated in bacterial and viral encephalitis, neurosyphilis, malignant CNS tumors, Huntington's disease, multiple sclerosis and neuroleptic-treated schizophrenic patients (106). Steroid therapy can decrease the ACE level (103).

In suspected neurosarcoidosis associated aseptic meningitis, CSF should be sent for routine microbiological studies, fungal, and mycobacterium cultures, mycobacterium TB polymerase chain reaction (PCR). Cytology and flow cytometry should also be considered. Once the infection has been ruled out, corticosteroids can be initiated as first-line treatment. In sarcoidosis, both aseptic meningitis and isolated cranial nerve abnormalities usually respond to steroids. Corticosteroids can be started at 1 mg/kg or as a pulse of methylprednisolone (1,000 mg/day for 3 days). Steroids may be gradually reduced over the next 6–9 months. In steroid-refractory cases, methotrexate, azathioprine, cyclophosphamide, or mycophenolate should be considered. Radiotherapy has been reported in patients with refractory sarcoid meningitis but has been used infrequently (92, 107, 108).

Multiple Sclerosis

One of the most challenging presentations is in patients, typically young women, with optic neuritis and finding a few demyelinating lesions on brain MRI. The clinical presentation of both neurosarcoidosis and multiple sclerosis (MS) include both these features. While over 90% of MS patients have oligoclonal bands on CSF analysis, oligoclonal bands can be seen in up to 25–50% of neurosarcoidosis patients making distinguishing sarcoidosis from multiple sclerosis more difficult (109–112). Neurosarcoidosis involvement on MRI demonstrates non-enhancing T2 and FLAIR white matter lesions often in a periventricular distribution mimicking the demyelinating lesions seen in MS (111, 113). Both MS and neurosarcoidosis can follow a relapsing or progressive course. A timely and correct diagnosis is essential because the misdiagnosis of neurosarcoidosis would prevent the patient from receiving highly effective MS therapies. Additionally, exposure to neurosarcoidosis specific therapies such as TNF-alpha antagonists may worsen MS (114, 115).

Table 4 illustrates the characteristic clinical and radiological features of Neurosarcoidosis, aseptic Meningitis and MS.

The diagnostic workup in a patient with a suspicion of neurosarcoidosis mimicking MS should include chest CT to search for lymphadenopathy and a full-body positron emission tomography (PET) scan to search for other organ involvement that supports the diagnosis of sarcoidosis, e.g., FDG uptake in thoracic lymph nodes, lung, spleen, and bone. PET scans can also direct tissue biopsy for histologic confirmation (118).

Diagnostic criteria proposed by Zajicek et al. categorizes neurosarcoidosis into definitive, probable, and possible neurosarcoidosis (130). Definite neurosarcoidosis is defined as clinical presentation suggestive of neurosarcoidosis after the exclusion of other possible diagnoses with the histological evidence of sarcoid in the nervous system. Probable neurosarcoidosis is defined as a suggestive clinical presentation of neurosarcoidosis in a patient with systemic sarcoid and after alternative diagnoses have been excluded. Possible neurosarcoidosis is defined as clinical presentation suggestive of neurosarcoidosis after alternative diagnoses have been excluded.

MRI is the preferred mode of imaging for diagnosis and follow up of neurosarcoidosis as it carries a high sensitivity. Leptomeningeal enhancement favors a diagnosis of sarcoidosis over neoplasm (123, 130–132). Enhancing parenchymal masses are an infrequent but important manifestation of sarcoidosis, but may also occur in primary lymphoma of the brain (97, 133). A CNS biopsy may be indicated when there is no extra-neurological disease, particularly if a lack of response to immunosuppressive treatment occurs. MRI can reveal accessible locations for biopsy, preferably from the leptomeninges (113).

Immunosuppression has been the mainstay of treatment for neurosarcoidosis with corticosteroids being the first-line. Other steroid-sparing therapeutic agents include methotrexate, mycophenolate mofetil, azathioprine, cyclosporine, cyclophosphamide, chlorambucil, pentoxifylline, hydroxychloroquine, thalidomide, infliximab, and adalimumab (134).

Peripheral Involvement

Peripheral nervous system involvement includes mononeuropathy, mononeuritis multiplex, sensory, sensorimotor, and motor polyneuropathies. Symptoms may be acute, subacute, or chronic. An acute generalized demyelinating motor neuropathy similar to the Guillain-Barré syndrome also has been described (135). Small fiber neuropathy resulting in distal limb pain and impaired perception of temperature, hyperesthesia, and autonomic dysfunction have been recognized (136).

Steroid-induced remissions coupled with frequent relapses observed in patients with neurosarcoidosis can lead to a clinical picture that resembles an inflammatory demyelinating disease. Neurologic involvement may be more difficult to confirm as tissue biopsy may be impractical (137–139).

TABLE 4 | Highlights the features of aseptic meningitis secondary to viral etiology and neuro-sarcoidosis associated meningitis.

	Sarcoidosis	Multiple sclerosis	Aseptic meningitis associated with viral infection
Laboratory	Elevated ACE level Abnormal calcium metabolism (hypercalciuria and/or hypercalcemia) (7, 12)	Non-specific laboratory findings	Enteroviruses most common, arboviruses, herpesviruses, influenza, mumps, HIV (109, 116, 117).
Eye involvement	Anterior uveitis Acute Bilateral optic neuritis (118, 119)	Unilateral optic neuritis, pain on eye movement, partial and mainly central visual blurring, normal disc or mild disc swelling	Ocular involvement not common.
Myelopathy	Can progress to spastic paraparesis MRI spine-meningeal and nerve root involvement (120)	Can progress to spastic paraparesis MRI spine-no meningeal enhancement has been reported.	Myelopathy is not common.
CSF findings	Lymphocytic Pleocytosis (57–72% patients) Elevated Protein (50–70% patients) Low glucose (18% patients) CSF ACE elevated 50% patients Oligoclonal bands Elevated CSF pressure finding of Kveim specific IgG in CSF (89, 121, 122)	Mild Lymphocytic Pleocytosis (<50/mm^3) Protein may be elevated in 1/3 cases. Normal glucose and pressure (123, 124) Oligoclonal bands or elevated IgG index in CSF (125)	Mononuclear pleocytosis (may be neutrophilic in early stage) Protein concentration can be normal to elevated (typically 0.4–0.8 g/l). Normal to low glucose CSF PCR specific for virus (enterovirus, HSV, VZV) (109)
MRI findings	Variable contrast enhancement of the leptomeninges with a nodular or diffuse pattern, non-specific white matter lesions, and hydrocephalus.	Oval, asymmetric white matter lesions perpendicular to the ventricles (Dawson fingers) or in the periventricular, juxtacortical, infratentorial or spinal cord region, and having a diameter >6 mm are considered typical of MS (54, 126–129)	MRI demonstrates abnormalities specific to the viral etiological agent (107)
Treatment	Corticosteroids	Corticosteroids, DMDs	Supportive management Acyclovir in HSV

CSF, Cerebrospinal fluid; MRI, Magnetic resonance imaging; PCR, Polymerase chain reaction; DMDs, Disease-modifying drugs; ACE, Angiotensin converting enzyme; MRI, magnetic resonance imaging.

ABNORMAL CALCIUM METABOLISM

Harrel et al. first described hypercalcemia associated with sarcoidosis in 1939 and since then abnormal calcium metabolism is considered a key feature of sarcoidosis. Hypercalcemia in sarcoidosis is secondary to an uncontrolled synthesis of 1,25-dihydroxy vitamin D3 by macrophages present in granulomas. The increased 1,25 dihydroxyvitamin D3 leads to increased intestinal absorption of calcium that leads to increased resorption of calcium in the bone (140, 141). The most common manifestation of abnormal calcium metabolism is hypercalciuria which is prevalent in ∼40–62% (119). Patients often provide a history of nephrolithiasis.

In the kidney, untreated hypercalcemia causes afferent arteriole vasoconstriction and inhibition of sodium-potassium ATPase causing a decrease in glomerular filtration rate and urinary sodium wasting, polyuria, and dehydration. Increased intracellular calcium overload and tubular obstruction by calcium precipitates may lead to tubular necrosis. Renal consequences of hypercalcemia and hypercalciuria are frequently reversible, however, long-standing hypercalciuria may lead to nephrocalcinosis and permanent changes (142, 143).

Once hypercalcemia is detected, serum albumin, ionized calcium, and 24-h urine collection for calcium excretion should be measured. In cases of progressive renal impairment, 24 h creatinine clearance, and abdominal ultrasound should also be performed to exclude nephrolithiasis or nephrocalcinosis. Primary hyperparathyroidism should routinely be ruled out. Histologic confirmation of sarcoidosis may be required to exclude lymphoma as it has the propensity to present with hypercalcemia associated with lymphadenopathy (144).

MANAGEMENT

Table 5 highlights the laboratory diagnostics and management of hypercalcemia associated with sarcoidosis.

Asymptomatic and mild hypercalcemia detected in a patient presenting with acute sarcoidosis requires no further assessment, and studies suggest that monitoring the response to corticosteroid therapy is an acceptable practice. Management is aimed to prevent long term renal and bone complications. Patients are advised to maintain a fluid intake of >2 l per day, minimize their exposure to sunlight, and avoid vitamin D. Severe hypercalcemia is fairly responsive to steroids. Corticosteroids achieve normocalcemia by inhibiting the enzyme 1-α-hydroxylase, reducing gastrointestinal calcium absorption, inhibiting osteoclast function, and decreasing the production of parathyroid hormone-related protein (PTHrP) by the macrophages (31, 145–148). Early recognition and treatment of abnormal calcium metabolism associated with sarcoidosis is important as long-standing untreated hypercalcemia can

TABLE 5 | Laboratory and radiological testing along with the management of hypercalcemia associated with sarcoidosis.

Laboratory and radiological tests	Management
24-h urine collection for calcium excretion.	**Hypercalciuria without stone formation**—observational approach; often requires no treatment. Close monitoring to prevent renal failure. Treatment options may include corticosteroids and/or diuretics.
Parathyroid Hormone (PTH) and PTH-related peptide (PTHrP)	**Hypercalciuria with stone formation—**Bisphosphonates or corticosteroids should be considered; shockwave lithotripsy
Serum creatinine (and/or cystatin C)	**Mild, asymptomatic**
Serum calcium and albumin levels should be measured and the ionized calcium calculated.	**Hypercalcemia—**Encourage fluid intake of >2 l per day and minimize their exposure to sunlight, avoid vitamin D and fish oil supplementation.
Renal Ultrasound to exclude nephrolithiasis	**Moderate hypercalcemia—**May consider addition of corticosteroids and/or ketoconazole or hydroxychloroquine.
Age appropriate malignancy work up (To rule out hypercalcemia secondary to malignancy)	**Severe hypercalcemia**—act immediately: rehydrate. Treatment options include corticosteroids, calcitonin, loop diuretics, and bisphosphonates (145)

progress to irreversible renal failure. It is imperative to consider the implication of the use of corticosteroids in patients as it further increases the risk of developing glucocorticoid-induced osteoporosis (149).

SARCOID-LIKE REACTIONS (SLRs)

Sarcoid-like reaction (SLR) is defined as the presence of non-caseating epithelioid cell granuloma lesions of sarcoidosis without accompanying systemic symptoms. Sarcoid-like reactions are histologically indistinguishable from systemic sarcoidosis (150).

SARCOID-LIKE REACTIONS (SLRs) ASSOCIATED WITH MALIGNANCY

SLRs can be observed in patients with various malignancies. The lesions can be adjacent to the primary tumor, in the tumor itself or adjacent to the local draining lymph nodes (137, 151). SLRs occur in 4.4% of carcinomas, in 13.8% of patients with Hodgkin's disease, and in 7.3% of cases of non-Hodgkin lymphomas (152). Developing SLRs adjacent to the tumor sites may be due to a local reaction to tumor products or immunological response to an antigenic trigger. SLRs far from tumor sites may represent a host immune response to the soluble circulating tumor antigenic factors perhaps by acting as a type of auto-Kveim reagent (137, 153, 154).

Both SLRs and malignancy demonstrate increased uptake of 18F-fluorodeoxyglucose (FDG) on PET scan, which makes it difficult to distinguish the two. Though PET scans may be useful in selecting possible biopsy sites to search for malignancy, they have no role in differentiating between these two entities. There are no specific markers or radiographic patterns to distinguish SLRs from systemic sarcoidosis or from malignancy (154–157). SLRs may have an improved prognosis as a few studies have reported that SLR is self-limited (152, 158, 159). The occurrence of malignancy and sarcoidosis concomitantly is unusual but a few cases have been reported (159, 160). The development of hilar and/or mediastinal lymphadenopathies in patients with a history of malignancy should prompt considering SLRs. Biopsies of affected tissue are often required to rule out cancer recurrence.

DRUG-INDUCED SARCOIDOSIS-LIKE REACTIONS (DISRs)

Drug-induced sarcoidosis-like reactions (DISR) can be defined as a granulomatous tissue reaction, indistinguishable from sarcoidosis, that occurs at the same time as initiation of a potential offending drug (161). DISR can be associated with typical sarcoid-like manifestation including bilateral hilar lymphadenopathy, uveitis, hypercalcemia, cutaneous lesions, elevated serum ACE levels, and FDG uptake on PET scans (162–166). A variety of drugs have been implicated as causing DISRs including immune checkpoint inhibitors, tumor necrosis factor (TNF)-α antagonists, interferons (IFN), and antiretroviral drugs. It is unclear if the etiology of DISR is that of a sarcoid-like reaction *per se* or a result of an impaired immune system leading to the development of sarcoidosis while on drug therapy (161).

Although TNF-α is known to play an important role in the formation and stabilization of sarcoid granulomas, surprisingly DISRs have been reported with the use of TNF-α antagonists therapy. Most commonly reported with etanercept, DISRs occur with any of the TNF-α antagonists. The average time to occurrence of DISRs is 24 months after drug initiation with almost 60% of patients requiring treatment (167). Treatment requirements range from 0 to 59.2% in reported cases of DISR, depending on the class of drug implicated (**Table 6**).

DISRs may mimic other clinical manifestations of sarcoidosis such as infections, drug and autoimmune reactions, and neoplasms. In select cases, DISR can resolve with discontinuation of the offending drug (161, 168). DISRs that are not associated with significant symptoms, organ dysfunction, or quality of life impairment, do not often require treatment. In cases where treatment is required, standard sarcoidosis treatment regimens can be utilized (161).

IMPLANT AND DEVICE-INDUCED SARCOID-LIKE REACTIONS

SLRs have been reported after joint replacement surgery and after silicone breast implant placement (181, 182). These reactions are postulated to be due to an autoimmune inflammatory syndrome induced by adjuvants (ASIA). The pathogenesis of the development of ASIA is not clearly defined but one proposed mechanism involves adjuvants (i.e., silicone, mineral oil, hyaluronic acid) chronically stimulating the immune pathway and preventing antigens from being degraded which can then

TABLE 6 | Highlights the drugs commonly associated with DISR.

	Drug class	Percentage of patients requiring anti-sarcoidosis treatment	References
1.	Interferon (interferon alpha and interferon beta)	42.9% (158)	(165, 167–169)
2.	Tumor necrosis factor-α antagonist	59.5% (158)	(170, 171)
3.	BRAF inhibitor	0%	(172, 173)
4.	Interleukin-1 receptor antagonist	0%	(174)
5.	Immune checkpoint inhibitors (nivolumab, pembrolizumab, ipilimumab)	57.2% (158)	(175, 176)
6.	Highly active antiretroviral therapy (HAART)	41.1% (158)	(177–179)
7.	Botulinum neurotoxin A	0% (158)	(180)

enhance the antigen exposure to the antigen-presenting cells (APC) (183, 184). Definitive treatment requires the removal of the prosthetic implant.

CARDIAC INVOLVEMENT WITH SARCOIDOSIS AND DIFFERENTIATING IT FROM POTENTIAL MIMICS

In 1929, Bernstein was the first to recognize cardiac involvement in sarcoidosis. Granulomas in cardiac sarcoidosis (CS) most often involve the conduction system and can potentially involve any part of the heart including the pericardium and myocardium (185). Depending upon the location and extent of granulomatous inflammation, clinical manifestation range from asymptomatic conduction abnormalities to fatal heart block and ventricular arrhythmia and congestive heart failure (CHF). In CS, CHF is the second most frequent cause of death after sudden cardiac death. Pericardial effusions are infrequent in CS and rarely progress to cardiac tamponade (185, 186).

A timely diagnosis of CS is often difficult as it may present with clinical features and radiographic findings overlapping with other cardiac disorders. Plain chest radiographs may appear normal. Some of the common mimickers of CS cardiomyopathy include amyloidosis and other infiltrative diseases, non-ischemic and ischemic cardiomyopathy, right ventricle infarction, lymphocytic myocarditis, connective tissue diseases associated cardiomyopathy, vasculitis (Takayasu arteritis and Wegener granulomatosis), Chagas disease, hypertrophic cardiomyopathy, and other infectious causes associated with cardiomyopathy (e.g., rheumatic fever, syphilis, fungal infections, and TB) (187–193).

Echocardiographic abnormalities, present in up to 24–77% of CS patients, range from non-specific findings to more sarcoid associated findings such as thinning of the basal anterior septum, regional wall aneurysm, diastolic dysfunction, or motion wall abnormalities in a non-coronary artery distribution (194). Impaired regional peak systolic longitudinal strain on strain echocardiography has been described and associated with cardiovascular events (195).

Thallium 201 scintigraphy can be used to help distinguish the ischemic disease from sarcoidosis or other infiltrative diseases. The reverse distribution phenomenon on rest and exercise thallium described as defects detected in the resting phase during thallium scanning that disappears or decreases in size during exercise or after dipyridamole infusion (196). Cardiac PET scanning may be combined with MRI to further define CS involvement. The sensitivities of cardiac PET and MRI with contrast are about the same for detecting myocardial involvement. Late gadolinium enhancement (LGE) on MRI generally cannot distinguish scar from inflammation while FDG uptake implies active inflammation (197, 198). Cardiac PET scanning is particularly useful in patients unable to undergo cardiac MRI because of the presence of implantable cardiac devices or renal failure where gadolinium is contraindicated.

Infrequently, granulomatous infiltration of the myocardium can result in myocardial thickening which can morphologically mimic hypertrophic cardiomyopathy but unlike CS, which is characterized by inflammation and edema, T2-weighted MRI features not typically seen in hypertrophic cardiomyopathy. Areas of Late gadolinium- enhancement (LGE) in CS are more likely to be patchy and mid myocardial.

For cardiac amyloid which is a consideration for unexplained infiltrative disease, both 99mTcpyrophosphate scanning and tissue biopsy (fat pad) showing apple-green birefringence at polarizing light microscopy are distinguishing features (199–201). In patients with CS and extracardiac involvement lymph node or lung biopsy is typically targeted but in cases of negative extracardiac biopsy or isolated CS, MRI, or PET directed endomyocardial biopsy may be required. Electro-anatomic mapping can also be used to choose cardiac sites for biopsy (202, 203).

CS can be managed with corticosteroids and when necessary inotropic, vasodilator, and antiarrhythmic medications. Automatic Implantable Cardioverter Defibrillator (AICD) or cardiac pacing may have a role. Cardiac transplantation although rare can be considered in younger patients with advanced end-stage cardiac failure. No difference in outcome has been reported when transplants are done for CS (204–207).

COMMON VARIABLE IMMUNODEFICIENCY SYNDROME (CVID)

Common variable immunodeficiency syndrome may be complicated by granulomatous lymphocytic interstitial lung

disease (GLILD) (206). The presence of sarcoidosis like lesions associated with hypogammaglobulinemia should suggest a diagnosis of CVID as up to 8–22% of patients with CVID can present with GILD (208–210). In a retrospective analysis more than two-thirds of CVID patients with radiographic evidence of interstitial lung disease had GLILD. GLILD was also frequently reported with dyspnea and splenomegaly. These patients can present with a clinical picture that mimics sarcoidosis and misdiagnosis can delay the treatment for CVID (IVIG), which in the presence of GLILD portends a poorer prognosis. Steroid therapy can further worsen CVID (209, 211–213).

Key clinical features that should alert one to the diagnosis of CVID include a history of recurrent infections (214) and a concurrent diagnosis of autoimmune diseases. Concurrent autoimmune disease occurs much less frequently in sarcoidosis than CVID. Sarcoidosis is more commonly associated with uveitis and skin disease whereas CVID patients more frequently have autoimmune cytopenias. Comparing GLID to sarcoidosis, chest CT scans had more air bronchogram, halo signs, and smooth bordered nodules in the CVID group. Bronchoalveolar lavage (BAL) revealed lower T-cell CD4: CD8 as well in the CVID group (215, 216).

With the aid of B cell immunophenotyping, granulomas associated with CVID reveal a severe reduction in switched memory B cells and a high level of CD21 low B cells. Immunoglobulin perfusions are considered the treatment of choice for CVID (217). Measurement of serum immunoglobulins should be considered when a history of recurrent infections is given and should be considered in any patient with granulomatous disease, even if the clinical and radiological presentation appears typical for sarcoidosis (218).

CONCLUSION

As illustrated by the numerous diseases that sarcoidosis can imitate, sarcoidosis deserves the reputation of "The Great Mimicker." The purpose of this review the most common diseases that should be considered when sarcoidosis enters the differential diagnoses. Aside from the time lost for adequate treatment of the disease present, both using immunosuppressive drugs in a state that appears to be sarcoidosis or conversely failing to adequately treat sarcoidosis with its proper treatment regimen will lead to adverse outcomes. The authors herein described common pitfalls in the diagnostic process and how to avoid them in order to provide, to the best of our ability, an accurate diagnosis for a condition that continues to baffle, elude, and mislead clinicians.

AUTHOR CONTRIBUTIONS

All authors contributed equally in data collection, interpretation, paper writing, editing, and review.

REFERENCES

1. Hutchinson J. Case of livid papillary psoriasis. In: *Illustrations of Clinical Surgery*, Vol. 1. London: J&A Churchill (1877). p. 42–3.
2. Crouser ED, Maier LA, Wilson KC, Bonham CA, Morgenthau AS, Patterson KC, et al. Diagnosis and detection of sarcoidosis. An official American thoracic society clinical practice guideline. *Am J Respir Crit Care Med.* (2020) 201:e26–51. doi: 10.1164/rccm.202002-0251ST
3. Badar F, Azfar SF, Ahmad I, Yasmeen S, Kirmani S. Diagnostic difficulties in differentiating sarcoidosis from tuberculosis. *Oman Med J.* (2011) 26:210–1. doi: 10.5001/omj.2011.53
4. Mortaz E, Adcock IM, Barnes PJ. Sarcoidosis: role of non-tuberculosis mycobacteria and Mycobacterium tuberculosis. *Int J Mycobacteriol.* (2014) 3:225–9. doi: 10.1016/j.ijmyco.2014.10.008
5. Moravvej H, Vesal P, Abolhasani E, Nahidi S, Mahboudi F. Comorbidity of leishmania major with cutaneous sarcoidosis. *Indian J Dermatol.* (2014) 59:316. doi: 10.4103/0019-5154.131453
6. Sicherman HJ, Andersen HA, DeRemee RA. Sarcoidosis or fungal disease. *Chest.* (1973) 64:36–7. doi: 10.1378/chest.64.1.36
7. Paramothayan S, Jones PW. Corticosteroid therapy in pulmonary sarcoidosis: a systematic review. *JAMA.* (2002) 287:1301–7. doi: 10.1001/jama.287.10.1301
8. Telenti A, Hermans PE. Idiopathic granulomatosis manifesting as fever of unknown origin. *Mayo Clin Proc.* 64:44–50. doi: 10.1016/S0025-6196(12)65302-6
9. Grenier P, Valeyre D, Cluzel P, Brauner MW, Lenoir S, Chastang C. Chronic diffuse interstitial lung disease: diagnostic value of chest radiography high-resolution CT. *Radiology.* (1991) 179:123–32. doi: 10.1148/radiology.179.1.2006262
10. Eom JS, Mok JH, Lee MK, Lee K, Kim MJ, Jang SM, et al. Efficacy of TB-PCR using EBUS-TBNA samples in patients with intrathoracic granulomatous lymphadenopathy. *BMC Pulm Med.* (2015) 15:166. doi: 10.1186/s12890-015-0162-4
11. Dhooria S, Agarwal R, Aggarwal AN, Bal A, Gupta N, Gupta D. Differentiating tuberculosis from sarcoidosis by sonographic characteristics of lymph nodes on endobronchial ultrasonography: a study of 165 patients. *J Thorac Cardiovasc Surg.* (2014) 148:662–7. doi: 10.1016/j.jtcvs.2014.01.028
12. Zhou G, Luo Q, Luo S, Teng Z, Ji Z, Yang J, et al. Interferon-γ release assays or tuberculin skin test for detection and management of latent tuberculosis infection: a systematic review and meta-analysis. *Lancet Infect Dis.* (2020). doi: 10.1016/S1473-3099(20)30276-0
13. Afsar I, Gunes M, Er H, Gamze Sener A. Comparison of culture, microscopic smear and molecular methods in diagnosis of tuberculosis. *Rev Esp Quimioter.* (2018) 31:435–8.
14. Wong CF, Yew WW, Wong PC, Lee J. A case of concomitant tuberculosis and sarcoidosis with mycobacterial DNA present in the sarcoid lesion. *Chest.* (1998) 114:626–9. doi: 10.1016/S0012-3692(15)47782-7
15. Szwarcberg JB, Glajchen N, Teirstein AS. Pleural involvement in chronic sarcoidosis detected by thoracic CT scanning. *Sarcoidosis Vasc Diffuse Lung Dis.* (2005) 22:58–62.
16. Soskel NT, Sharma OP. Pleural involvement in sarcoidosis. *Curr Opin Pulm Med.* (2000) 6:455–68. doi: 10.1097/00063198-200009000-00012
17. Huggins JT, Doelken P, Sahn SA, King L, Judson MA. Pleural effusions in a series of 181 outpatients with sarcoidosis. *Chest.* (2006) 129:1599–604. doi: 10.1378/chest.129.6.1599
18. Sadikot RT, Dore P, Arnold AG. Sarcoidosis and opportunistic infections. *South Med J.* (2001) 94:75–7. doi: 10.1097/00007611-200194010-00016
19. Nottebart HC, McGehee RF, Utz JP. Cryptococcosis complicating sarcoidosis. *Am Rev Respir Dis.* (1973) 107:1060–3.
20. Winterbauer RH, Kraemer KG. The infectious complications of sarcoidosis: a current perspective. *Arch Intern Med.* (1976) 136:1356–62. doi: 10.1001/archinte.1976.03630120008006
21. Ungprasert P, Carmona EM, Crowson CS, Matteson EL. Diagnostic utility of angiotensin-converting enzyme in sarcoidosis: a population-based study. *Lung.* (2016) 194:91–5. doi: 10.1007/s00408-015-9826-3
22. Sharma OP. Markers of sarcoidosis activity. *Chest.* (1986) 90:471–3. doi: 10.1378/chest.90.4.471
23. Shai F, Baker RK, Addrizzo JR, Wallach S. Hypercalcemia in mycobacterial infection. *J Clin Endocrinol Metab.* (1972) 34:251–6. doi: 10.1210/jcem-34-2-251

24. Khasawneh FA, Ahmed S, Halloush RA. Progressive disseminated histoplasmosis presenting with cachexia and hypercalcemia. *Int J Gen Med.* (2013) 6:79–83. doi: 10.2147/IJGM.S41520
25. Studdy PR, Bird R. Serum angiotensin converting enzyme in sarcoidosis—its value in present clinical practice. *Ann Clin Biochem.* (1989) 26:13–8. doi: 10.1177/000456328902600102
26. Thillai M, Eberhardt C, Lewin AM, Potiphar L, Hingley-Wilson S, Sridhar S, et al. Sarcoidosis and tuberculosis cytokine profiles: indistinguishable in bronchoalveolar lavage but different in blood. *PLoS ONE.* (2012) 7:e38083. doi: 10.1371/journal.pone.0038083
27. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. *N Engl J Med.* (2007) 357:2153–65. doi: 10.1056/NEJMra071714
28. Gupta D, Chetty M, Kumar N, Aggarwal AN, Jindal SK. Anergy to tuberculin in sarcoidosis is not influenced by high prevalence of tuberculin sensitivity in the population. *Sarcoidosis Vasc Diffuse Lung Dis.* (2003) 20:40–5.
29. Khan AH, Ghani F, Khan A, Khan MA, Khurshid M. Role of serum angiotensin converting enzyme in sarcoidosis. *J Pak Med Assoc.* (1998) 48:131–3.
30. Ryder KW, Jay SJ, Kiblawi SO, Hull MT. Serum angiotensin converting enzyme activity in patients with histoplasmosis. *JAMA.* (1983) 249:1888–9. doi: 10.1001/jama.1983.03330380076032
31. Young C, Burrows R, Katz J, Beynon H. Hypercalcemia in sarcoidosis. *Lancet.* (1999) 353:374. doi: 10.1016/S0140-6736(98)08251-8
32. Regatieri A, Abdelwahed Y, Perez MT, Bush LM. Testing for tuberculosis: the roles of tuberculin skin tests and interferon gamma release assays. *Lab Med.* (2011) 42:11–6. doi: 10.1309/LMU57KYINZ6WJTIT
33. Kantrow SP, Meyer KC, Kidd P, Raghu G. The CD4/CD8 ratio in BAL fluid is highly variable in sarcoidosis. *Eur Respir J.* (1997) 10:2716–21.
34. Pottumarthy S, Morris AJ, Harrison AC, Wells VC. Evaluation of the tuberculin gamma interferon assay: potential to replace the Mantoux skin test. *J Clin Microbiol.* (1999) 37:3229–32.
35. Connolly PA, Durkin MM, Lemonte AM, Hackett EJ, Wheat LJ. Detection of histoplasma antigen by a quantitative enzyme immunoassay. *Clin Vaccine Immunol.* (2007) 14:1587–91. doi: 10.1128/CVI.00071-07
36. Danila E, Norkuniene J, Jurgauskiene L, Malickaite R. Diagnostic role of BAL fluid CD4/CD8 ratio in different radiographic and clinical forms of pulmonary sarcoidosis. *Clin Respir J.* (2009) 3:214–21. doi: 10.1111/j.1752-699X.2008.00126.x
37. Kalawat U, Sharma KK, Reddy PN, Kumar AG. Study of bronchoalveolar lavage in clinically and radiologically suspected cases of pulmonary tuberculosis. *Lung India.* (2010) 27:122–4. doi: 10.4103/0970-2113.68307
38. Conde MB, Soares SL, Mello FC, Rezende VM, Almeida LL, Reingold AL, et al. Comparison of sputum induction with fiberoptic bronchoscopy in the diagnosis of tuberculosis: experience at an acquired immune deficiency syndrome reference center in Rio de Janeiro, Brazil. *Am J Respir Crit Care Med.* (2000) 162:2238–40. doi: 10.1164/ajrccm.162.6.2003125
39. Greco S, Marruchella A, Massari M, Saltini C. Predictive value of BAL cellular analysis in differentiating pulmonary tuberculosis and sarcoidosis. *Eur Respir J.* (2005) 26:360–2. doi: 10.1183/09031936.05.00042905
40. Glazer CS, Rose CS, Lynch DA. Clinical and radiologic manifestations of hypersensitivity pneumonitis. *J Thorac Imaging.* (2002) 17:261–72. doi: 10.1097/00005382-200210000-00003
41. Hage CA, Davis TE, Fuller D, Egan L, Witt JR 3rd, Wheat LJ, et al. Diagnosis of histoplasmosis by antigen detection in BAL fluid. *Chest.* (2010) 137:623–8. doi: 10.1378/chest.09-1702
42. Hardy HL, Tabershaw IR. Delayed chemical pneumonitis occurring in workers exposed to beryllium compounds. *J Ind Hyg Toxicol.* (1946) 28:197–211.
43. Newman LS, Kreiss K, King TE Jr, Seay S, Campbell PA. Pathologic and immunologic alterations in early stages of beryllium disease. Re-examination of disease definition and natural history. *Am Rev Respir Dis.* (1989) 139:1479–86. doi: 10.1164/ajrccm/139.6.1479
44. Balmes JR, Abraham JL, Dweik RA, Fireman E, Fontenot AP, Maier LA, et al. An official American thoracic society statement: diagnosis and management of beryllium sensitivity and chronic beryllium disease. *Am J Respir Crit Care Med.* (2014) 190:e34–e59. doi: 10.1164/rccm.201409-1722ST
45. Alberts WM. Lung disease and the lightest of metals. *Chest.* (2004) 126:1730–2. doi: 10.1378/chest.126.6.1730
46. Selman M. Hypersensitivity pneumonitis. In: *Interstitial Lung Disease*. 3rd ed. Hamilton, BC: Decker Inc. (1998). p. 393–422.
47. Lacasse Y, Selman M, Costabel U, Dalphin JC, Ando M, Morell F, et al. Clinical diagnosis of hypersensitivity pneumonitis. *Am J Respir Crit Care Med.* (2003) 168:952–8. doi: 10.1164/rccm.200301-137OC
48. Walters GI, Mokhlis JM, Moore VC, Robertson AS, Burge GA, Bhomra PS, et al. Characteristics of hypersensitivity pneumonitis diagnosed by interstitial and occupational lung disease multidisciplinary team consensus. *Respir Med.* (2019) 155:19–25. doi: 10.1016/j.rmed.2019.06.026
49. Vourlekis JS, Schwarz MI, Cherniack RM. The effect of pulmonary fibrosis on survival in patients with hypersensitivity pneumonitis. *Am J Med.* (2004) 116:662–8. doi: 10.1016/j.amjmed.2003.12.030
50. Müller-Quernheim KI, Gaede E, Fireman G. Zissel Diagnoses of chronic beryllium disease within cohorts of sarcoidosis patients. *J Eur Respir J.* (2006) 27:1190–5. doi: 10.1183/09031936.06.00112205
51. Newman LS, Orton R, Kreiss K. Serum angiotensin converting enzyme activity in chronic beryllium disease. *Am Rev Respir Dis.* (1992) 146:39–42. doi: 10.1164/ajrccm/146.1.39
52. Barna BP, Culver DA, Yen-Lieberman B, Dweik RA, Thomassen MJ. Clinical application of beryllium lymphocyte proliferation testing. *Clin Diagn Lab Immunol.* (2003) 10:990–4. doi: 10.1128/CDLI.10.6.990-994.2003
53. Aronchick JM, Rossman MD, Miller WT. Chronic beryllium disease: diagnosis, radiographic findings, and correlation with pulmonary function tests. *Radiology.* (1987) 163:677–82. doi: 10.1148/radiology.163.3.3575713
54. Castonguay MC, Ryu JH, Yi ES, Tazelaar HD. Granulomas and giant cells in hypersensitivity pneumonitis. *Hum Pathol.* (2015) 46:607–13. doi: 10.1016/j.humpath.2014.12.017
55. Kern RM, Singer JP, Koth L, Mooney J, Golden J, Hays S, et al. Lung transplantation for hypersensitivity pneumonitis. *Chest.* (2015) 147:1558–65. doi: 10.1378/chest.14-1543
56. The BAL Cooperative Group Steering Committee. Bronchoalveolar lavage constituents in healthy individuals, idiopathic pulmonary fibrosis, and selected comparison groups. *Am Rev Respir Dis.* (1990) 141:S169–202.
57. Rybicki BA, Major M, Popovich J Jr, Maliarik MJ, Iannuzzi MC. Racial differences in sarcoidosis incidence: a 5-year study in a health maintenance organization. *Am J Epidemiol.* (1997) 145:234–41. doi: 10.1093/oxfordjournals.aje.a009096
58. Ozgul MA, Cetinkaya E, Kirkil G, Ozgul G, Abul Y, Acat M, et al. Lymph node characteristics of sarcoidosis with endobronchial ultrasound. *Endosc Ultrasound.* (2014) 3:232–7. doi: 10.4103/2303-9027.144541
59. Hamper UM, Fishman EK, Khouri NF, Johns CJ, Wang KP, Siegelman SS. Typical and atypical CT manifestations of pulmonary sarcoidosis. *J Comput Assist Tomogr.* (1986) 10:928–36. doi: 10.1097/00004728-198611000-00006
60. Fink JN, Ortega HG, Reynolds HY, Cormier YF, Fan LL, Franks TJ, et al. Needs and opportunities for research in hypersensitivity pneumonitis. *Am J Respir Crit Care Med.* (2005) 171:792–8. doi: 10.1164/rccm.200409-1205WS
61. Raghu G, Remy-Jardin M, Ryerson CJ, Myers JL, Kreuter M, Vasakova M, et al. Diagnosis of hypersensitivity pneumonitis in adults. An official ATS/JRS/ALAT clinical practice guideline. *Am J Respir Crit Care Med.* (2020) 202:e36–69. doi: 10.1164/rccm.202005-2032ST
62. Fernández Pérez ER, Kong AM, Raimundo K, Koelsch TL, Kulkarni R, Cole AL. Epidemiology of hypersensitivity pneumonitis among an insured population in the United States: a claims-based cohort analysis. *Ann Am Thorac Soc.* (2018) 15:460–9. doi: 10.1513/AnnalsATS.201704-288OC
63. Ohtani Y, Saiki S, Sumi Y, Inase N, Miyake S, Costabel U, et al. Clinical features of recurrent and insidious chronic bird fancier's lung. *Ann Allergy Asthma Immunol.* (2003) 90:604–10. doi: 10.1016/S1081-1206(10)61863-7
64. Jeong YJ, Lee KS, Chung MP, Han J, Johkoh T, Ichikado K. Chronic hypersensitivity pneumonitis and pulmonary sarcoidosis: differentiation from usual interstitial pneumonia using high-resolution computed tomography. *Semin Ultrasound CT MR.* (2014) 35:47–58. doi: 10.1053/j.sult.2013.10.006
65. Sarno M, Hasleton PS, Spiteri M. Sarcoidosis. In: Hasleton PS, editor. *Spencer's Pathology of the Lung*. 5th ed. New York, NY: McGraw-Hill (1996). p. 507–35.

66. Nishimura K, Itoh H, Kitaichi M, Nagai S, Izumi T. CT and pathological correlation of pulmonary sarcoidosis. *Semin Ultrasound CT MR.* (1995) 16:361–70. doi: 10.1016/0887-2171(95)90025-X
67. Kokkarinen JI, Tukiainen HO, Terho EO. Effect of corticosteroid treatment on the recovery of pulmonary function in farmer's lung. *Am Rev Respir Dis.* (1992) 145:3–5. doi: 10.1164/ajrccm/145.1.3
68. Hsieh C. *Hypersensitivity Pneumonitis Treatment & Management*. Medscape Web Site. Available online at: http://emedicine.medscape.com/article/299174-treatment (accessed February 8, 2017).
69. Wang P, Jones KD, Urisman A, Elicker BM, Urbania T, Johannson KA, et al. Pathologic findings and prognosis in a large prospective cohort of chronic hypersensitivity pneumonitis. *Chest*. (2017) 152:502–9. doi: 10.1016/j.chest.2017.02.011
70. Ohtsuka Y, Munakata M, Tanimura K. Smoking promotes insidious and chronic farmer's lung disease, and deteriorates the clinical outcome. *Intern Med*. (1995) 34:966–71. doi: 10.2169/internalmedicine.34.966
71. Ungprasert P, Crowson CS, Matteson EL. Association of sarcoidosis with increased risk of VTE: a population-based study, 1976 to 2013. *Chest.* (2017) 151:425–30. doi: 10.1016/j.chest.2016.09.009
72. Ungprasert P, Srivali N, Wijarnpreecha K, Thongprayoon C. Sarcoidosis and risk of venous thromboembolism: a systematic review and meta-analysis. *Sarcoidosis Vasc Diffuse Lung Dis.* (2015) 32:182–7.
73. Yaqoob ZJ, Al-Kindi SG, Zein JG. Sarcoidosis and risk of VTE: validation with big data. *Chest.* (2017) 151:1398–9. doi: 10.1016/j.chest.2017.03.022
74. Swigris JJ, Olson AL, Huie TJ, Fernandez-Perez ER, Solomon JJ, Sprunger D, et al. Increased risk of pulmonary embolism among US decedents with sarcoidosis from 1988 to 2007. *Chest*. (2011) 140:1261–6. doi: 10.1378/chest.11-0324
75. Wynne JW, Ryerson GG, Dalovisio J. Myocardial sarcoidosis complicated by mural thrombosis. *Thorax*. (1979) 34:127–9. doi: 10.1136/thx.34.1.127
76. Selvi A, Diakou M, Giannopoulos S, Zikou AK, Argyropoulou MI, Kyritsis AP. Cerebral venous thrombosis in a patient with sarcoidosis. *Intern Med.* (2009) 48:723–5. doi: 10.2169/internalmedicine.48.1809
77. Marc K, Bourkadi JE, Benamor J, Iraqi G. Thoracic venous thrombosis in the course of sarcoidosis. *Rev Mal Respir.* (2008) 25:105–6. doi: 10.1016/S0761-8425(08)70477-7
78. Silvarino R, Danza A, Merola V. Venous thromboembolic disease is systemic autoimmune diseases: an association to keep in mind. *Autoimmun Rev.* (2012) 12:289–94. doi: 10.1016/j.autrev.2012.05.002
79. Reitsma PH, Rosendaal FR. Activation of innate immunity in patients with venous thrombosis: the leiden thrombophilia study. *J Thromb Haemost.* (2004) 2:619–22. doi: 10.1111/j.1538-7836.2004.00689.x
80. Takizawa H, Satoh M, Okazaki H, Matsuzaki G, Suzuki N, Ishii A, et al. Increased IL-6 and IL-8 in bronchoalveolar lavage fluids (BALF) from patients with sarcoidosis: correlation with the clinical parameters. *Clin Exp Immunol.* (1997) 107:175–81. doi: 10.1046/j.1365-2249.1997.d01-905.x
81. Ina Y, Takada K, Yamamoto M, Sato T, Ito S, Sato S. Antiphospholipid antibodies. A prognostic factor in sarcoidosis? *Chest.* (1994) 105:1179–83. doi: 10.1378/chest.105.4.1179
82. Johannesdottir SA, Horvath-Puho E, Dekkers OM. Use of glucocorticoids and risk of venous thromboembolism: a nationwide population-based case-control study. *JAMA Intern Med.* (2013) 173:743–52. doi: 10.1001/jamainternmed.2013.122
83. Fisher KA, Serlin DM, Wilson KC, Walter RE, Berman JS, Farber HW. Sarcoidosis-associated pulmonary hypertension: outcome with long-term epoprostenol treatment. *Chest.* (2006) 130:1481–8. doi: 10.1378/chest.130.5.1481
84. Handa T, Nagai S, Miki S, Fushimi Y, Ohta K, Mishima M, et al. Incidence of pulmonary hypertension and its clinical relevance in patients with sarcoidosis. *Chest.* (2006) 129:1246–52. doi: 10.1378/chest.129.5.1246
85. Shlobin OA, Nathan SD. Management of end-stage sarcoidosis: pulmonary hypertension and lung transplantation. *Eur Respir J.* (2012) 39:1520–33. doi: 10.1183/09031936.00175511
86. Shorr AF, Helman DL, Davies DB, Nathan SD. Pulmonary hypertension in advanced sarcoidosis: epidemiology and clinical characteristics. *Eur Respir J.* (2005) 25:783–8. doi: 10.1183/09031936.05.00083404
87. Simonneau G, Gatzoulis MA, Adatia I, Celermajer D, Denton C, Ghofrani A, et al. Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol.* (2013) 62:D34–41. doi: 10.1016/j.jacc.2013.10.029
88. Baughman RP, Shlobin OA, Wells AU, Alhamad EH, Culver DA, Barney J, et al. Clinical features of sarcoidosis associated pulmonary hypertension: Results of a multi-national registry. *Respir Med*. (2018) 139:72–8. doi: 10.1016/j.rmed.2018.04.015
89. Corte TJ, Wells AU, Nicholson AG, Hansell DM, Wort SJ. Pulmonary hypertension in sarcoidosis: a review. *Respirology.* (2011) 16:69–77. doi: 10.1111/j.1440-1843.2010.01872.x
90. Diaz-Guzman E, Farver C, Parambil J, Culver DA. Pulmonary hypertension caused by sarcoidosis. *Clin Chest Med.* (2008) 29:549–63. doi: 10.1016/j.ccm.2008.03.010
91. Shlobin OA, Kouranos V, Barnett SD, Alhamad EH, Culver DA, Barney J, et al. Physiological predictors of survival in patients with sarcoidosis-associated pulmonary hypertension: results from an international registry. *Eur Respir J.* (2020) 55:1901747. doi: 10.1183/13993003.01747-2019
92. Nozaki K, Judson MA. Neurosarcoidosis: clinical manifestations, diagnosis and treatment. *Presse Med.* (2012) 41:e331–48. doi: 10.1016/j.lpm.2011.12.017
93. Tabuchi S, Uno T. Hydrocephalus with panventricular enlargement as the primary manifestation of neurosarcoidosis: a case report. *J Med Case Rep.* (2013) 7:240. doi: 10.1186/1752-1947-7-240
94. Rao DA, Dellaripa PF. Extrapulmonary manifestations of sarcoidosis. *Rheum Dis Clin North Am.* (2013) 39:277–97. doi: 10.1016/j.rdc.2013.02.007
95. Lacomis D. Neurosarcoidosis. *Curr Neuropharmacol.* (2011) 9:429–36. doi: 10.2174/157015911796557975
96. Agnihotri SP, Singhal T, Stern BJ, et al. Neurosarcoidosis. *Semin Neurol.* (2014) 34:386–94. doi: 10.1055/s-0034-1390387
97. Tsao CY, Lo WD, Rusin JA, Henwood MJ, Boue DR. Isolated neurosarcoidosis presenting as headache and multiple brain and spinal cord lesions mimicking central nervous system metastases. *Brain Dev.* (2007) 29:514–8. doi: 10.1016/j.braindev.2006.12.011
98. Galnares-Olalde JA, Berebichez-Fridman R, Gómez-Garza G, Mercado M, Moreno-Sánchez F, Alegría-Loyola MA. Not everything is as it seems: neurosarcoidosis presenting as leptomeningitis. *Clin Case Rep.* (2018) 6:596–602. doi: 10.1002/ccr3.1418
99. Kumar G, Kang CA, Giannini C. Neurosarcoidosis presenting as a cerebellar mass. *J Gen Intern Med.* (2007) 22:1373–6. doi: 10.1007/s11606-007-0272-7
100. Durel CA, Marignier R, Maucort-Boulch D, Iwaz J, Berthoux E, Ruivard M, et al. Clinical features and prognostic factors of spinal cord sarcoidosis: a multicenter observational study of 20 BIOPSY-PROVEN patients. *J Neurol.* (2016) 263:981–90. doi: 10.1007/s00415-016-8092-5
101. Matsuda R, Nishimura F, Motoyama Y, Park YS, Nakase H. A case of intraventricular isolated neurosarcoidosis diagnosed by neuroendoscopic biopsy. *No Shinkei Geka.* (2015) 43:247–52. doi: 10.11477/mf.1436202995
102. Lyra TG, Lee HW, de Vellutini EAS, da Martin MGM, Cardoso APT, de Godoy LFS, et al. Gasserian ganglion neurosarcoidosis mimicking trigeminal schwannoma. *Arq Neuropsiquiatr*. (2015) 73:173–4. doi: 10.1590/0004-282X20140209
103. Oksanen V. Neurosarcoidosis: clinical presentations and course in 50 patients. *Acta Neurol Scand.* (1986) 73:283–90. doi: 10.1111/j.1600-0404.1986.tb03277.x
104. Tahmoush AJ, Amir MS, Connor WW, Farry JK, Didato S, Ulhoa-Cintra A, et al. CSF-ACE activity in probable CNS neurosarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* (2002) 19:191–7.
105. Sakushima K, Yabe I, Nakano F, Yoshida K, Tajima Y, Houzen H, et al. Clinical features of spinal cord sarcoidosis: analysis of 17 neurosarcoidosis patients. *J Neurol.* (2011) 258:2163–7. doi: 10.1007/s00415-011-6080-3
106. Wahlbeck K, Cheine M, Essali A, Adams C. Evidence of clozapine's effectiveness in schizophrenia: a systematic review and meta-analysis of randomized trials. *Am J Psychiatry.* (1999) 156:990–9.
107. Dutra LA, Braga-Neto P, Oliveira RA, Pedroso JL, Abrahão A, Barsottini OGP. Neurosarcoidosis: guidance for the general neurologist. *Arq Neuropsiquiatr.* (2012) 70:293–9. doi: 10.1590/S0004-282X2012000400014
108. Langrand C, Bihan H, Raverot G, Varron L, Androdias G, Borson-Chazot F, et al. Hypothalamo-pituitary sarcoidosis: a multicenter study of 24 patients. *Q J Med.* (2012) 105:981–95. doi: 10.1093/qjmed/hcs121

109. Pawate S, Moses H, Sriram S. Presentations and outcomes of neurosarcoidosis: a study of 54 cases. *QJM.* (2009) 102:449–60. doi: 10.1093/qjmed/hcp042
110. Borucki SJ, Nguyen BV, Ladoulis CT, McKendall RR. Cerebrospinal fluid immunoglobulin abnormalities in neurosarcoidosis. *Arch Neurol.* (1989) 46:270–3. doi: 10.1001/archneur.1989.00520390036012
111. Scott TF, Yandora K, Kunschner LJ, Schramke C. Neurosarcoidosis mimicry of multiple sclerosis: clinical, laboratory, and imaging characteristics. *Neurologist.* (2010) 16:386–9. doi: 10.1097/NRL.0b013e3181b287df
112. Gaines JD, Eckman PB, Remington JS. Low CSF glucose level in sarcoidosis involving the central nervous system. *Arch Intern Med.* (1970) 125:333–6. doi: 10.1001/archinte.1970.00310020139021
113. Shah R, Roberson GH, Cure JK. Correlation of MR imaging findings and clinical manifestations in neurosarcoidosis. *AJNR Am J Neuroradiol.* (2009) 30:953–61. doi: 10.3174/ajnr.A1470
114. Ivan Oosten BW, Barkhof F, Truyen L, Boringa JB, Bertelsmann FW, et al. Increased MRI activity and immune activation in two multiple sclerosis patients treated with the monoclonal anti-tumor necrosis factor antibody cA2. *Neurology.* (1996) 47:1531–4. doi: 10.1212/WNL.47.6.1531
115. The Lenercept Multiple Sclerosis Study Group and the University of British Columbia MS/MRI Analysis Group. TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. *Neurology.* (1999) 53:457–65. doi: 10.1212/WNL.53.3.457
116. Kastrup O, Wanke I, Maschke M. Neuroimaging of infections. *NeuroRx.* (2005) 2:324–32. doi: 10.1602/neurorx.2.2.324
117. Logan SA, MacMahon E. Viral meningitis. *BMJ.* (2008) 336:36–40. doi: 10.1136/bmj.39409.673657.AE
118. Hebel R, Dubaniewicz-Wybieralska M, Dubaniewicz A. Overview of neurosarcoidosis: recent advances. *J Neurol.* (2015) 262:258–67. doi: 10.1007/s00415-014-7482-9
119. Taylor R, Lynch H, Wysor WG. Seasonal influence of sunlight on the hypercalcemia of sarcoidosis. *Am J Med.* (1963) 34:221–7. doi: 10.1016/0002-9343(63)90055-X
120. Burman J, Raininko R, Fagius J. Bilateral and recurrent optic neuritis in multiple sclerosis. *Acta Neurol Scand.* (2011) 123:207–10. doi: 10.1111/j.1600-0404.2010.01388.x
121. Ratzan KR. Viral meningitis. *Med Clin North Am.* (1985) 69:399–413. doi: 10.1016/S0025-7125(16)31051-3
122. Kidd DP, Burton BJ, Graham EM, Plant GT. Optic neuropathy associated with systemic sarcoidosis. *Neurol Neuroimmunol Neuroinflamm.* (2016) 3:e270. doi: 10.1212/NXI.0000000000000270
123. Ginat DT, Dhillon G, Almast J. Magnetic resonance imaging of neurosarcoidosis. *J Clin Imaging Sci.* (2011) 1:15. doi: 10.4103/2156-7514.76693
124. Fieschi C, Gasperini C, Ristori G, Bastianello S, Girmenia F, Leuzzi V, et al. Diagnostic problems in "clinically definite" multiple sclerosis patients with normal CSF and multiple MRI abnormalities. *Eur J Neurol.* (1994) 1:127–33. doi: 10.1111/j.1468-1331.1994.tb00060.x
125. Reiber H, Felgenhauer K. Protein transfer at the blood-cerebrospinal fluid barrier and the quantitation of the humoral immune response within the central nervous system. *Clin Chim Acta.* (1987) 163:319–28. doi: 10.1016/0009-8981(87)90250-6
126. Kabat EA, Freedman DA, Murray JP, Knaub V. A study of the crystalline albumin, gamma globulin and total protein in the cerebrospinal fluid of one hundred cases of multiple sclerosis and in other diseases. *Am J Med Sci.* (1950) 219:55–64. doi: 10.1097/00000441-195001000-00009
127. Bergamaschi R, Tonietti S, Franciotta D, Candeloro E, Tavazzi E, Piccolo G, et al. Oligoclonal bands in devic's neuromyelitis optica and multiple sclerosis: differences in repeated cerebrospinal fluid examinations. *Mult Scler.* (2004) 10:2–4. doi: 10.1191/1352458504ms988oa
128. Barkhof F, Filippi M, Miller D, Scheltens P, Campi A, Polman CH, et al. Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. *Brain.* (1997) 120:2059–69. doi: 10.1093/brain/120.11.2059
129. Filippi M, Rocca MA, Bastianello S, Comi G, Gallo P, Gallucci M, et al. Guidelines from the Italian neurological and neuroradiological societies for the use of magnetic resonance imaging in daily life clinical practice of multiple sclerosis patients. *Neurol Sci.* (2013) 34:2085–93. doi: 10.1007/s10072-013-1485-7
130. Zajicek JP, Scolding NJ, Foster O, Rovaris M, Evanson J, Moseley IF, et al. Central nervous system sarcoidosis–diagnosis and management. *QJM.* (1999) 92:103–17. doi: 10.1093/qjmed/92.2.103
131. Terushkin V, Stern BJ, Judson MA, Hagiwara M, Pramanik B, Sanchez M, et al. Neurosarcoidosis: presentations and management. *Neurologist.* (2010) 16:2–15. doi: 10.1097/NRL.0b013e3181c92a72
132. Sherman JL, Stern BJ. Sarcoidosis of the CNS: comparison of unenhanced and enhanced MR images. *AJNR Am J Neuroradiol.* (1990) 11:915–23.
133. Uruha A, Koide R, Taniguchi M. Unusual presentation of sarcoidosis: solitary intracranial mass lesion mimicking a glioma. *J Neuroimaging.* (2009) 21:e180–2. doi: 10.1111/j.1552-6569.2009.00413.x
134. Stjepanovic MI, Vucinic VM, Jovanovic D, Mijajlović M, Trifunović VS, Stjepanović MM. Treatment of neurosarcoidosis–innovations and challenges. *Med Pregl.* (2014) 67:161–6. doi: 10.2298/MPNS1406161S
135. Saifee TA, Reilly MM, Ako E, Rugg-Gunn F, Brandner S, Lunn MP, et al. Sarcoidosis presenting as acute inflammatory demyelinating polyradiculoneuropathy. *Muscle Nerve.* (2011) 43:296–8. doi: 10.1002/mus.21890
136. Tavee JO, Karwa K, Ahmed Z, Thompson N, Parambil J, Culver DA. Sarcoidosis-associated small fiber neuropathy in a large cohort: Clinical aspects and response to IVIG and anti-TNF alpha treatment. *Respir Med.* (2017) 126:135–8. doi: 10.1016/j.rmed.2017.03.011
137. Baughman RP, Lower EE, Du Bois RM. Sarcoidosis. *Lancet.* (2003) 361:1111–8. doi: 10.1016/S0140-6736(03)12888-7
138. Spencer TS, Campellone JV, Maldonado I, Huang N, Usmani Q, Reginato AJ. Clinical and magnetic resonance imaging manifestations of neurosarcoidosis. *Semin Arthritis Rheum.* (2005) 34:649–61. doi: 10.1016/j.semarthrit.2004.07.011
139. Vargas DL, Stern BJ. Neurosarcoidosis: diagnosis and management. *Semin Respir Crit Care Med.* (2010) 31:419–27. doi: 10.1055/s-0030-1262210
140. Sharma OP. Vitamin D, calcium, and sarcoidosis. Sarcoidosis, hypercalcemia and primary hyperparathyroidism. The vicissitudes of diagnosis. *Chest.* (1996) 109:535–9. doi: 10.1378/chest.109.2.535
141. Correia FASC, Marchini GS, Torricelli FC, Danilovic A, Vicentini FC, Srougi M, et al. Renal manifestations of sarcoidosis: from accurate diagnosis to specific treatment. *Int Braz J Urol.* (2019) 46:15–25. doi: 10.1590/s1677-5538.ibju.2019.0042
142. Berliner AR, Haas M, Choi MJ. Sarcoidosis: the nephrologist's perspective. *Am J Kidney Dis.* (2006) 48:856–70. doi: 10.1053/j.ajkd.2006.07.022
143. Hilderson I, Van Laecke S, Wauters A, Donck J. Treatment of renal sarcoidosis: is there a guideline? Overview of the different treatment options. *Nephrol Dial Transplant.* (2014) 29:1841–7. doi: 10.1093/ndt/gft442
144. Schweitzer VG, Thompson NW, Clark KA, Nishiyama RH, Bigos ST. Sarcoidosis, hypercalcemia and primary hyperparathyroidism. The vicissitudes of diagnosis. *Am J Surg.* (1981) 142:499–503. doi: 10.1016/0002-9610(81)90383-4
145. Pinkson P, Saltini C, Muller-Quernheim J, Crystal RG. Corticosteroid therapy suppresses spontaneous IL-2 release and spontaneous proliferation of lung T-lymphocytes in patients with sarcoidosis. *J Immunol.* (1987) 139:755–60.
146. Rizzato G. Clinical impact of bone and calcium changes in sarcoidosis. *Thorax.* (1998) 53:425–9. doi: 10.1136/thx.53.5.425
147. Hamada K, Nagai S, Tsutsumi T, Izumi T. Ionized calcium and 1,25-dihydroxyvitamin D concentration in the serum of patients with sarcoidosis. *Eur Respir J.* (1998) 11:1015–20. doi: 10.1183/09031936.98.11051015
148. Potts JT. Diseases of the parathyroid gland and other disorders. In: Fauci AS, Braunwald E, editors. *Harrison's Principals of Internal Medicine*. 14th ed. New York, NY: McGraw-Hill (1998) 354:2236–42.
149. Conron M, Young C, Beynon HLC. Calcium metabolism in sarcoidosis and its clinical implications. *Rheumatology.* (2000) 39:707–13. doi: 10.1093/rheumatology/39.7.707
150. Martella S, Lohsiriwat V, Barbalho DM, Della Vigna P, Bottiglieri L, Brambullo T, et al. Sarcoid-like reaction in breast cancer: a long-term follow-up series of eight patients. *Surg Today.* (2012) 42:259–63. doi: 10.1007/s00595-011-0084-6

151. Tan QL, Leow LC, Haja Mohideen SM, Sewa DW. Sarcoid-like reaction associated with lung adenocarcinoma: a case report. *Proc Singapore Healthc.* (2019) 28:68–70. doi: 10.1177/2010105818766363
152. Brincker H. Sarcoid reactions in malignant tumours. *Cancer Treat Rev.* (1986) 13:147–56. doi: 10.1016/0305-7372(86)90002-2
153. Tolaney SM, Colson YL, Gill RR, Schulte S, Duggan MM, Shulman LN, et al. Sarcoidosis mimicking metastatic breast cancer. *Clin Breast Cancer.* (2007) 7:804–10. doi: 10.3816/CBC.2007.n.044
154. Hunt BM, Vallières E, Buduhan G, Aye R, Louie B. Sarcoidosis as a benign cause of lymphadenopathy in cancer patients. *Am J Surg.* (2009) 197:629–32. doi: 10.1016/j.amjsurg.2009.01.004
155. Spagnolo P, Luppi F, Roversi P. Sarcoidosis: challenging diagnostic aspects of an old disease. *Am J Med.* (2012) 125:118–25. doi: 10.1016/j.amjmed.2011.06.003
156. Ravaglia C, Gurioli C, Casoni GL, Romagnoli M, Tomassetti S, Gurioli C, et al. Sarcoid-like lesion is a frequent benign cause of lymphadenopathy in neoplastic patients. *Eur Respir J.* (2013) 41:754–5. doi: 10.1183/09031936.00141212
157. Chopra A, Judson MA. How are cancer and connective tissue diseases related to sarcoidosis? *Curr Opin Pulm Med.* (2015) 21:517–24. doi: 10.1097/MCP.0000000000000186
158. Steinfort DP, Tsui A, Grieve J, Hibbs ML, Anderson GP, Irving LB. Sarcoidal reactions in regional lymph nodes of patients with early stage non-small cell lung cancer predict improved disease-free survival: a pilot case–control study. *Hum Pathol.* (2012) 43:333–8. doi: 10.1016/j.humpath.2011.05.006
159. Sacks EL, Donaldson SS, Gordon J, Dorfman RF. Epithelioid granulomas associated with Hodgkin's disease: clinical correlations in 55 previously untreated patients. *Cancer.* (1978) 41:562–7. doi: 10.1002/1097-0142(197802)41:2<562::AID-CNCR2820410224>3.0.CO;2-X
160. Sakula A. Bronchial carcinoma and sarcoidosis. *Br J Cancer.* (1963) 17:206–12. doi: 10.1038/bjc.1963.29
161. Chopra A, Nautiyal A, Kalkanis A, Judson MA. Drug-induced sarcoidosis-like reactions. *Chest.* (2018) 154:664–77. doi: 10.1016/j.chest.2018.03.056
162. Danlos FX, Pages C, Baroudjian B, Vercellino L, Battistella M, Mimoun M, et al. Nivolumab-induced sarcoid-like granulomatous reaction in a patient with advanced melanoma. *Chest.* (2016) 149:e133–6. doi: 10.1016/j.chest.2015.10.082
163. Kim C, Gao J, Shannon VR, Siefker-Radtke A. Systemic sarcoidosis first manifesting in a tattoo in the setting of immune checkpoint inhibition. *BMJ Case Rep.* (2016) 2016:bcr2016216217. doi: 10.1136/bcr-2016-216217
164. Doycheva D, Deuter C, Stuebiger N, Zierhut M. Interferon-alpha-associated presumed ocular sarcoidosis. *Graefes Arch Clin Exp Ophthalmol.* (2009) 247:675–80. doi: 10.1007/s00417-008-1002-5
165. Nakajima R, Abe K, Nakajima A, Nishikawa T, Sakai S. Etanercept-induced sarcoidosis in rheumatoid arthritis: FDG PET findings. *Clin Nucl Med.* (2015) 40:58–61. doi: 10.1097/RLU.0000000000000582
166. Herbert VG, Blodorn-Schlicht N, Boer-Auer A. Cutaneous granulomatous reactions at botulinum neurotoxin a injection sites: first manifestation of systemic sarcoidosis. *Hautarzt.* (2015) 66:863–6. doi: 10.1007/s00105-015-3651-8
167. Callejas-Rubio JL, Lopez-Perez L, Ortego-Centeno N. Tumor necrosis factor-alpha inhibitor treatment for sarcoidosis. *Ther Clin Risk Manage.* (2008) 4:1305–13. doi: 10.2147/TCRM.S967
168. Zisman DA, McCune WJ, Tino G, Lynch JP III. Drug-induced pneumonitis: the role of methotrexate. *Sarcoidosis Vasc Diffuse Lung Dis.* (2001) 18:243–52.
169. Fantini F, Padalino C, Gualdi G, Monari P, Giannetti A. Cutaneous lesions as initial signs of interferon alpha-induced sarcoidosis: report of three new cases and review of the literature. *Dermatol Ther.* (2009) 22(Suppl. 1):S1–7. doi: 10.1111/j.1529-8019.2009.01263.x
170. Gitlin N. Manifestation of sarcoidosis during interferon and ribavirin therapy for chronic hepatitis C: a report of two cases. *Eur J Gastroenterol Hepatol.* (2002) 14:883–5. doi: 10.1097/00042737-200208000-00013
171. Shiki M, Hida T, Yamashita T. Development of sarcoidosis during β-interferon therapy for melanoma. *J Dermatol.* (2014) 41:862–3. doi: 10.1111/1346-8138.12581
172. Bobbio-Pallavicini E, Valsecchi C, Tacconi F, Moroni M, Porta C. Sarcoidosis following beta-interferon therapy for multiple myeloma. *Sarcoidosis.* (1995) 12:140–2.
173. Dhaille F, Viseux V, Caudron A, et al. Cutaneous sarcoidosis occurring during anti-TNF-alpha treatment: report of two cases. *Dermatology.* (2010) 220:234–7. doi: 10.1159/000275676
174. Skoie IM, Wildhagen K, Omdal R. Development of sarcoidosis following etanercept treatment: a report of three cases. *Rheumatol Int.* (2012) 32:1049–53. doi: 10.1007/s00296-009-1349-x
175. Garrido MC, Gutierrez C, Riveiro-Falkenbach E, Ortiz P, Rodriguez-Peralto JL. BRAF inhibitor-induced antitumoral granulomatous dermatitis Eruption in advanced melanoma. *Am J Dermatopathol.* (2015) 37:795–8. doi: 10.1097/DAD.0000000000000281
176. Park JJ, Hawryluk EB, Tahan SR, Flaherty K, Kim CC. Cutaneous granulomatous eruption and successful response to potent topical steroids in patients undergoing targeted BRAF inhibitor treatment for metastatic melanoma. *JAMA Dermatol.* (2014) 150:307–11. doi: 10.1001/jamadermatol.2013.7919
177. Friedman BE, English JC III. Drug-induced sarcoidosis in a patient treated with an interleukin-1 receptor antagonist for hidradenitis suppurativa. *JAAD Case Rep*. (2018) 4:543–5. doi: 10.1016/j.jdcr.2018.03.007
178. Firwana B, Ravilla R, Raval M, Hutchins L, Mahmoud F. Sarcoidosis-like syndrome and lymphadenopathy due to checkpoint inhibitors. *J Oncol Pharm Pract.* (2017) 23:620–4. doi: 10.1177/1078155216667635
179. Wilgenhof S, Morlion V, Seghers AC, Du Four S, Vanderlinden E, Hanon S, et al. Sarcoidosis in a patient with metastatic melanoma sequentially treated with anti-CTLA-4 monoclonal antibody and selective BRAF inhibitor. *Anticancer Res.* (2012) 32:1355–9.
180. Gomez V, Smith PR, Burack J, Daley R, Rosa U. Sarcoidosis after antiretroviral therapy in a patient with acquired immunodeficiency syndrome. *Clin Infect Dis.* (2000) 31:1278–80. doi: 10.1086/317422
181. Balbouzis T, Georgiadis T, Grigoris P. Granulomatous lung disease: a novel complication following metallosis from hip arthroplasty. *Hip Pelvis.* (2016) 28:249–53. doi: 10.5371/hp.2016.28.4.249
182. Jacobs JJ, Urban RM, Wall J, Black J, Reid JD, Veneman L. Unusual foreign-body reaction to a failed total knee replacement: simulation of a sarcoma clinically and a sarcoid histologically. A case report. *J Bone Joint Surg Am.* (1995) 77:444–51. doi: 10.2106/00004623-199503000-00015
183. Shoenfeld Y, Agmon-Levin N. 'ASIA' - autoimmune/inflammatory syndrome induced by adjuvants. *J Autoimmun*. (2011) 36:4–8. doi: 10.1016/j.jaut.2010.07.003
184. Yoshida T, Tanaka M, Okamoto K, Hirai S. Neurosarcoidosis following augmentation mammoplasty with silicone. *Neurol Res*. (1996) 18:319–20. doi: 10.1080/01616412.1996.11740428
185. Roberts WC, McAllister HA Jr, Ferrans VJ. Sarcoidosis of the heart. A clinicopathologic study of 35 necropsy patients (group 1) and review of 78 previously described necropsy patients (group 11). *Am J Med.* (1977) 63:86–108. doi: 10.1016/0002-9343(77)90121-8
186. Sekiguchi M, Numao Y, Imai M, Furuie T, Mikami R. Clinical and histopathological profile of sarcoidosis of the heart and acute idiopathic myocarditis. Concepts through a study employing endomyocardial biopsy. I. Sarcoidosis. *Jpn Circ J.* (1980) 44:249–63. doi: 10.1253/jcj.44.249
187. Bagwan IN, Hooper LV, Sheppard MN. Cardiac sarcoidosis and sudden death. The heart may look normal or mimic other cardiomyopathies. *Virchows Arch.* (2011) 458:671–8. doi: 10.1007/s00428-010-1003-8
188. Yazaki Y, Isobe M, Hiramitsu S, Morimoto S, Hiroe M, Omichi C, et al. Comparison of clinical features and prognosis of cardiac sarcoidosis and idiopathic dilated cardiomyopathy. *Am J Cardiol.* (1998) 82:537–40. doi: 10.1016/S0002-9149(98)00377-4
189. Yoshizawa S, Kato TS, Mancini D, Marboe CC. Outcome of patients having heart transplantation for lymphocytic myocarditis. *Am J Cardiol*. (2013) 112:405–10. doi: 10.1016/j.amjcard.2013.03.042
190. Kang EJ, Kim SM, Choe YH, Lee GY, Lee KN, Kim DK. Takayasu arteritis: assessment of coronary arterial abnormalities with 128-section dual-source CT angiography of the coronary arteries and aorta. *Radiology.* (2014) 270:74–81. doi: 10.1148/radiol.13122195

191. Mohty D, Damy T, Cosnay P, Echahidi N, Casset-Senon D, Virot P, et al. Cardiac amyloidosis: updates in diagnosis and management. *Arch Cardiovasc Dis.* (2013) 106:528–40. doi: 10.1016/j.acvd.2013.06.051
192. Nunes MC, Dones W, Morillo CA, Encina JJ, Ribeiro AL, Council on Chagas Disease of the Interamerican Society of Cardiology. Council on chagas disease of the interamerican society of cardiology. Chagas disease: an overview of clinical and epidemiological aspects. *J Am Coll Cardiol.* (2013) 62:767–76. doi: 10.1016/j.jacc.2013.05.046
193. Lynch JP 3rd, Hwang J, Bradfield J, Fishbein M, Shivkumar K, Tung R. Cardiac involvement in sarcoidosis: evolving concepts in diagnosis and treatment. *Semin Respir Crit Care Med.* (2014) 35:372–90. doi: 10.1055/s-0034-1376889
194. Houston BA, Mukherjee M. Cardiac sarcoidosis: clinical manifestations, imaging characteristics, and therapeutic approach. *Clin Med Insights Cardiol.* (2014) 8:31–7. doi: 10.4137/CMC.S15713
195. Chen J, Lei J, Scalzetti E, McGrath M, Feiglin D, Voelker R, et al. Myocardial contractile patterns predict future cardiac events in sarcoidosis. *Int J Cardiovasc Imaging.* (2018) 34:251–62. doi: 10.1007/s10554-017-1233-9
196. Fields CL, Ossorio MA, Roy TM, Denny DM, Varga DW. Thallium-201 scintigraphy in the diagnosis and management of myocardial sarcoidosis. *South Med J.* (1990) 83:339–42. doi: 10.1097/00007611-199003000-00022
197. White JA, Rajchl M, Butler J, Thompson RT, Prato FS, Wisenberg G. Active cardiac sarcoidosis: first clinical experience of simultaneous positron emission tomography–magnetic resonance imaging for the diagnosis of cardiac disease. *Circulation.* (2013) 127:e639–41. doi: 10.1161/CIRCULATIONAHA.112.001217
198. Ohira H, Tsujino I, Ishimaru S, Oyama N, Takei T, Tsukamoto E, et al. Myocardial imaging with 18F-fluoro-2-deoxyglucose positron emission tomography and magnetic resonance imaging in sarcoidosis. *Eur J Nucl Med Mol Imaging.* (2008) 35:933–41. doi: 10.1007/s00259-007-0650-8
199. Moraes GL, Higgins CB, Ordovas KG. Delayed enhancement magnetic resonance imaging in nonischemic myocardial disease. *J Thorac Imaging.* (2013) 28:84–92. doi: 10.1097/RTI.0b013e3182828f89
200. Maron BJ. Hypertrophic cardiomyopathy: a systematic review. *JAMA.* (2002) 287:1308–20. doi: 10.1001/jama.287.10.1308
201. Maceira AM, Joshi J, Prasad SK, Moon JC, Perugini E, Harding I, et al. Cardiovascular magnetic resonance in cardiac amyloidosis. *Circulation.* (2005) 111:186–93. doi: 10.1161/01.CIR.0000152819.97857.9D
202. Zipse MM, Sauer WH. Cardiac sarcoidosis. *Curr Cardiol Rep.* (2014) 16:514. doi: 10.1007/s11886-014-0514-3
203. Chapelon-Abric C. Cardiac sarcoidosis. *Curr Opin Pulm Med.* (2013) 19:493–502. doi: 10.1097/MCP.0b013e32836436da
204. Valantine HA, Tazelaar HD, Macoviak J, Mullin AV, Hunt SA, Fowler MB, et al. Cardiac sarcoidosis: response to steroids and transplantation. *J Heart Transplant.* (1987) 6:244–50.
205. Ishikawa T, Kondoh H, Nakagawa S, Koiwaya Y, Tanaka K. Steroid therapy in cardiac sarcoidosis. *Chest.* (1984) 85:445–7. doi: 10.1378/chest.85.3.445
206. Oni AA, Hershberger RE, Norman DJ, Ray J, Hovaguimian H, Cobanoglu AM, et al. Recurrence of sarcoidosis in a cardiac allograft: control with augmented corticosteroids. *J Heart Lung Transplant.* (1992) 11(2 Pt 1):367–9.
207. Al-Kofahi K, Korsten P, Ascoli C, Virupannavar S, Mirsaeidi M, Chang I, et al. Management of extrapulmonary sarcoidosis: challenges and solutions. *Ther Clin Risk Manag.* (2016) 12:1623–34. doi: 10.2147/TCRM.S74476
208. Boursiquot JN, Gérard L, Malphettes M, Fieschi C, Galicier L, Boutboul D, et al. Granulomatous disease in CVID: a retrospective analysis of clinical characteristics and treatment efficacy in a cohort of 59 patients. *J Clin Immunol.* (2013) 33:84–95. doi: 10.1007/s10875-012-9778-9
209. Allaoui A, Moudatir M, Echchilal K, Alaoui FZ, Elkabli H. A misleading diagnosis of sarcoidosis in an older woman. *Eur J Case Rep Intern Med.* (2017) 4:000463. doi: 10.12890/2017_000463
210. Ho H-E, Cunningham-Rundles C. Non-infectious complications of common variable immunodeficiency: updated clinical spectrum, sequelae, and insights to pathogenesis. *Front Immnol.* (2020) 11:149. doi: 10.3389/fimmu.2020.00149
211. Cowen JE, Stevenson J, Paravasthu M, Darroch J, Jacob A, Tueger S, et al. Common variable immunodeficiency with granulomatous-lymphocytic interstitial lung disease and preceding neurological involvement: a case-report. *BMC Pulm Med.* (2020) 20:205. doi: 10.1186/s12890-020-01231-6
212. Mannina A, Chung JH, Swigris JJ, Solomon JJ, Huie TJ, Yunt ZX, et al. Clinical predictors of a diagnosis of common variable immunodeficiency-related granulomatous-lymphocytic interstitial lung disease. *Ann Am Thorac Soc.* (2016) 13:1042–9. doi: 10.1513/AnnalsATS.201511-728OC
213. Bates CA, Ellison MC, Lynch D, Cool CD, Brown KK, Routes JM. Granulomatous-lymphocytic lung disease shortens survival in common variable immunodeficiency. *J Allergy Immunol.* (2004) 114:415–21. doi: 10.1016/j.jaci.2004.05.057
214. Oksenhendler E, Gerard L, Fieschi C, Malphettes M, Mouillot G, Jaussaud R, et al. DEFI study group. Infections in 252 patients with common variable immunodeficiency. *Clin Infect Dis.* (2008) 46:1547–54. doi: 10.1086/587669
215. Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, Grimbacher B, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. *Blood.* (2008) 112:277–86. doi: 10.1182/blood-2007-11-124545
216. Bouvry D, Mouthon L, Brillet PY, Kambouchner M, Ducroix JP, Groupe sarcoidose francophone, et al. Granulomatous-associated common variable immunodeficiency disorder: a case-control study versus sarcoidosis. *Eur Respir J.* (2013) 41:115–22. doi: 10.1183/09031936.00189011
217. Abbott JK, Gelfand EW. Common variable immunodeficiency: diagnosis, management, and treatment. *Immunol Allergy Clin North Am.* (2015) 35:637–58. doi: 10.1016/j.iac.2015.07.009
218. Shanks AM, Alluri R, Herriot R, Dempsey O. Misdiagnosis of common variable immune deficiency. *BMJ Case Rep.* (2014) 2014:bcr2013202806. doi: 10.1136/bcr-2013-202806

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.

frontiers in Medicine

CASE REPORT
published: 27 January 2021
doi: 10.3389/fmed.2020.610384

Case Report: Testicular Sarcoidosis: The Diagnostic Role of Contrast-Enhanced Ultrasound and Review of the Literature

Antonio De Cinque[1], Beniamino Corcioni[1], Martina Sofia Rossi[2], Alessandro Franceschelli[2,3], Fulvio Colombo[2,3], Rita Golfieri[1], Matteo Renzulli[1†] and Caterina Gaudiano[1†]*

[1] Department of Radiology, Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, [2] Department of Urology, Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, [3] Andrology-Unit, Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

OPEN ACCESS

Edited by:
Peter Korsten, University Medical Center Göttingen, Germany

Reviewed by:
Gunnar N. Hillerdal, Karolinska University Hospital, Sweden
Viktor Korendovych, University Medical Center Göttingen, Germany

***Correspondence:**
Antonio De Cinque
antdecinque@hotmail.it

† These authors have contributed equally to this work and share last authorship

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: *25 September 2020*
Accepted: *18 December 2020*
Published: *27 January 2021*

Citation:
De Cinque A, Corcioni B, Rossi MS, Franceschelli A, Colombo F, Golfieri R, Renzulli M and Gaudiano C (2021) Case Report: Testicular Sarcoidosis: The Diagnostic Role of Contrast-Enhanced Ultrasound and Review of the Literature. Front. Med. 7:610384. doi: 10.3389/fmed.2020.610384

Sarcoidosis is a multisystemic disease histologically characterized by non-caseating epithelioid granulomas and multinucleated giant cells; the etiology is still uncertain, and likely related to a complex interplay between environmental and genetic factors. The genitourinary system is affected in fewer than 0.2% of all clinically diagnosed cases of sarcoidosis and in 5% of those identified in autopsy studies. In this report, we describe a case of a 42–year-old male with one hypoechoic lesion per testis on B-mode evaluation; contrast-enhanced ultrasound (CEUS) on both lesions was carried out. During the early phase, the masses showed a hypovascular appearance as compared to the surrounding testicular tissue, maintaining the hypo-enhancement in the late phase. Tissue biopsy for pathological evaluation confirmed testicular sarcoid involvement, showing non-caseating granulomas. Allowing visualization of testicular microvascularisation, CEUS may play an important role in excluding malignancy, avoiding unnecessary aggressive treatment for benign conditions, such as sarcoidosis. A review of the literature of reported cases since 2004 of sarcoidosis involving the testis is also included.

Keywords: andrology, urology, sarcoidosis, ultrasonography, contrast media

INTRODUCTION

Sarcoidosis is a multisystemic disease that usually affects patients in the fifth decade of their life with a variable incidence rate depending on countries and ethnic group (1). A recent study from the Mayo Clinic reported an incidence rate of 11 per 100,000 people/year among a cohort mostly composed of white people (2) while another study from the United States estimated an incidence rate of 8.1 per 100,000 people/year in Caucasians, 17.8 in African Americans, 4.3 in Hispanics and 3.2 in Asians (3). In Europe, an incidence of 11.5 per 100,000 people/year has been reported in Sweden (4) and of 5.0 in the UK (5). The incidence rate of sarcoidosis is increasing over time in developed countries, such as Korea, together with an increase in the age of diagnosis probably due to population aging (6).

Sarcoidosis is histologically characterized by non-caseating epithelioid granulomas and multinucleated giant cells. Its etiology is still uncertain, and likely related to a complex relationship between environmental and genetic factors (7–9).

Sarcoidosis is characterized by bilateral chest hilar lymphadenopathy and/or reticulonodular pulmonary infiltrates in the vast majority of cases (>90%). However, this systemic pathology can involve any organ (10): among them the genitourinary system is involved in 5% of cases identified in autoptic studies and in fewer than 0.2% of clinically diagnosed cases. According to a review published in 2004, the male genitourinary organs most frequently involved by sarcoidosis are the epididymis (73%), the testis (47%), the spermatic cord (8%) and the prostate (3%) (11). In particular, the total number of testicular sarcoidosis accounted for 28 cases in 2004. We performed a new review of the literature, finding additional cases involving the testis associated with histologically proven diagnosis.

Testicular sarcoid presentation can vary from testicular swelling to painless or painful unilateral or bilateral masses (12, 13). Especially in cases of painless unilateral mass, the differential diagnosis is complex and difficult, ranging from testicular malignancies to infections, and could result in diagnostic errors leading to inappropriate and unnecessary treatments, such as the orchiectomy (13).

Usually the achievement of a correct diagnosis of sarcoidosis with genitourinary and in particular testicular involvement relies on the association between clinical and imaging findings (14). The most utilized imaging techniques for this issue are Ultrasound (US), Magnetic Resonance Imaging (MRI) and Positron Emission Tomography-Computed Tomography (PET-CT).

New and non-invasive imaging techniques, such as contrast-enhanced US (CEUS), have recently been refined and adopted in many guidelines (15, 16). However, to the best of our knowledge, this is the first report in the English literature to explore in detail the potentiality of CEUS in a case of histologically proven testicular sarcoidosis.

A very rare case of testicular sarcoidosis is herein reported together with a detailed review of the literature highlighting the role of CEUS to confidently achieve the final diagnosis excluding other possible differential diagnoses, such as testicular tumor masses.

CASE REPORT

A 42–year-old Caucasian male was admitted to our Hospital referring a 4-month history of gradually increasing upper left quadrant pain. His medical history included bladder neck sclerosis, cholecystectomy, diabetes insipidus, hypogonadotropic hypogonadism, allergic asthma, and a smoking history until 10 months before this admission. The medical examination did not show any relevant features and, therefore, the subsequent diagnostic work-up continued with abdominal US.

Abdominal US showed multiple hypoechoic splenic and hepatic lesions ranging from 5 to 27 mm and, therefore, the patient underwent total body CT scan for a correct lesions characterization and staging. CT scan demonstrated bilateral hilar lymphadenopathies in the chest, pulmonary perilymphatic micronodules, enlarged retroperitoneal lymph nodes, and confirmed the hepatic and splenic lesions (**Figures 1A,B**). The first diagnostic hypothesis was the lymphoma and a ^{18}F-Fludeoxyglucose (^{18}F-FDG) PET/CT was performed, showing increased ^{18}F-FDG uptake (standardized uptake value (SUV) max=17 at the chest hilar lymphadenopathies level) in all the lesions revealed on CT (**Figure 1C**). Moreover, a focal uptake was detected in left testis (**Figure 1D**). A brain MRI excluded central nervous system involvement. Lymphopenia was the only abnormal blood cell count value. Alpha-fetoprotein (AFP), Human chorionic gonadotropin (HCG), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) were within their normal ranges, while alanine aminotransferase (ALT) (55 U/L), gamma-glutamyl transferase (GGT) (60 U/L), C-reactive protein (CRP) (0.82 mg/dL) and Erythrocyte sedimentation rate (ESR) (20 mm) were mildly elevated. Moreover, Angiotensin Converting Enzyme (ACE) levels were elevated (95 U/L). He had no history or signs of tuberculosis (QuantiFERON®-TB Gold Plus test was negative).

These findings raised the suspicion of lymphoma or sarcoidosis. An endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) was performed on the chest hilar lymphadenopathies, showing non-caseating granulomas, consistent with sarcoidosis. Stains for acid-fast bacilli and polymerase chain reaction (PCR) in specimens were negative. The final diagnosis was sarcoidosis with multiorgan involvement. A testicular US evaluation was carried out using a Canon-Toshiba Aplio 500™ (Otawara, Kanto, Japan) with a high frequency (4–14 MHz) linear transducer. The US demonstrated, using the B-mode evaluation, a hypoechoic lesion of 20 mm with ill-defined margins in the left testis (**Figure 2A**) corresponding to the lesion identified on PET-CT; moreover, differently from the latter technique, the US identified a smaller and well-shaped hypoechoic lesion also in the right testis (6 mm). The Color Doppler demonstrated the presence of vascular flow within the lesions (**Figure 2B**). Therefore, it was decided to perform CEUS, conducted with the administration of 4.8 ml of second-generation contrast media (SonoVue™, Bracco, Milano, Italy) followed by 10 mL of 0.9% saline solution. Both the testicular lesions demonstrated the same pattern on CEUS. In particular, during the arterial phase, the masses showed a hypovascular appearance as compared to the surrounding testicular tissue (**Figure 2C**), maintaining the hypo-enhancement in the late phase. This CEUS pattern was not typical of the most frequent testicular tumors such as seminomas, which usually are arterialized appearing hyperechoic on CEUS (17). Finally, the imaging diagnosis was testicular sarcoidosis, based principally on bilateral involvement of testes on CEUS and on the CEUS pattern (hypoenhancement). Testicular sarcoid involvement was confirmed by surgical biopsy of both testicular masses that demonstrated non-caseating granulomas.

The patient was treated with corticosteroids and then with a second-line therapy (methotrexate), thus achieving a reduction of the SUVmax in all of the sarcoid lesions (SUVmax = 2.6 at the testicular level) at 6- and 12-month PET/CT follow-up.

According to our review (**Table 1**), from 2004 (11) until now we have identified 20 cases (including the present one) of testicular sarcoidosis. Finally, to date, the total number of

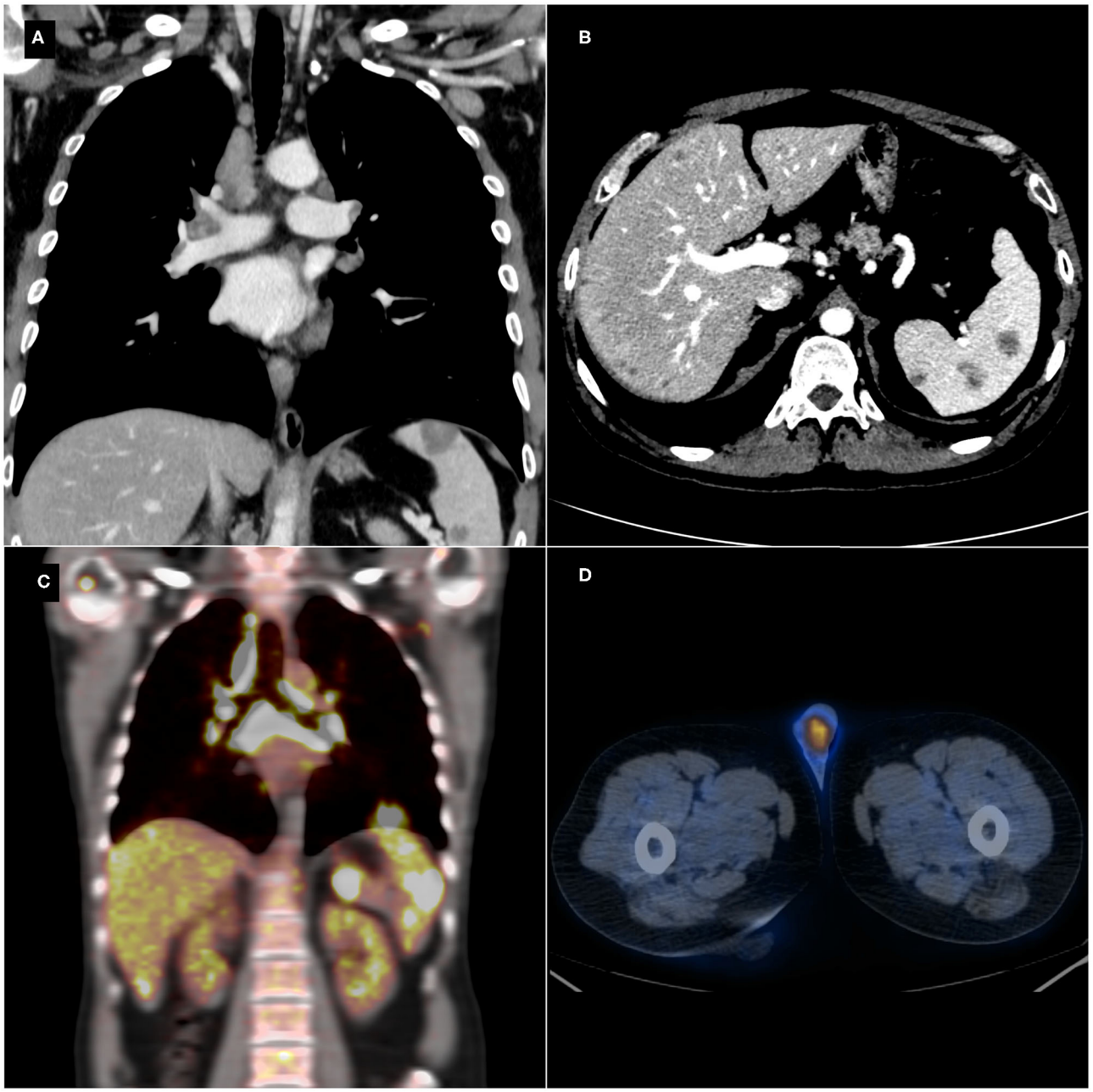


FIGURE 1 | Thoraco-abdominal contrast-enhanced computed tomography (CT) scan showing hilar and mediastinal lymphadenopathies **(A)**, enlarged retroperitoneal lymph nodes, and multiple hepatic and splenic hypodense lesions **(B)**. Positron emission tomography-computed tomography (PET/CT) detecting an increased focal ^{18}F-Fludeoxyglucose (^{18}F-FDG) uptake in the mediastinal and abdominal lesions **(C)** and in the left testis **(D)**.

histologically proven testicular sarcoidosis published in literature account for 48 cases.

DISCUSSION

Testicular masses may have many differential diagnoses including malignancies and, very rarely, benign processes such as traumatic or infective/inflammatory lesions including sarcoidosis which incidence is increasing (6, 35). US has a pivotal role in investigating testicular lesions since it is extremely accurate in the detection of the masses, even very small ones, due to its high spatial resolution (35). Moreover, the US often represents the sole imaging technique needed prior to surgery, although the recognition of benign entities may be challenging (36). The most common US findings in testicular sarcoidosis are multifocal small hypoechoic

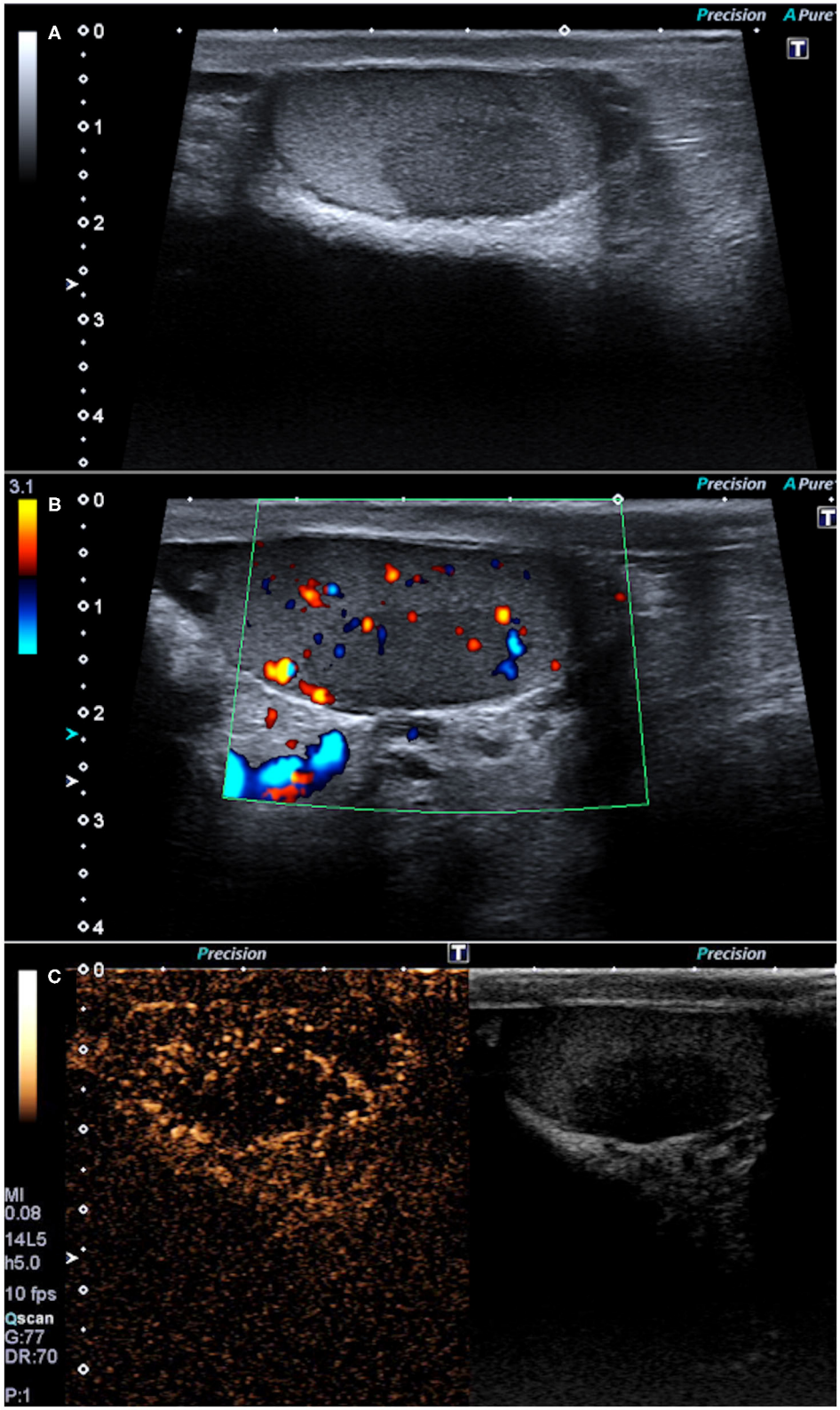


FIGURE 2 | B-mode ultrasonography showing one hypoechoic lesion in the left testis with ill-defined margins **(A)** and some Color-Doppler flow **(B)**. Contrast-enhanced ultrasonography showing the hypovascular appearance of the lesions as compared to the surrounding testicular tissue **(C)**.

TABLE 1 | Histologically proven cases of sarcoidosis involving the testis reported in the English literature from 2004 to 2020 (including the present case).

References	Imaging	Uni- or Bilateral	Orchiectomy
Rees et al. (18)	US	Bilateral	No
Rehman et al. (19)	US	Bilateral	No
Massarweh et al. (12)	US	Bilateral	No
Thuret et al. (20)	US	Unilateral (R)	Yes
Real et al. (21)	US	Bilateral	Yes
Gupta and Senadhi (13)	US	Unilateral (R)	Yes
Kim et al. (22)	US	Unilateral (R)	No
Paknejad et al. (23)	US	Bilateral	No
Kovac et al. (24)	N/A	N/A	No
Esnakula et al. (25)	US	Unilateral (R)	Yes
Joel et al. (26)	US	Unilateral (L)	Yes
Patel et al. (27)	US	Bilateral	No
Knox et al. (28)	N/A	Bilateral	Yes
Chierigo et al. (29)	US	Bilateral	No
Babst et al. (30)	US	Bilateral	No
Konishi et al. (31)	US, CT	Bilateral	No
Hamitouche et al. (32)	US	Bilateral	No
Kimura et al. (33)	US, MRI, Gallium-67 scintigraphy	Bilateral	No
Parida et al. (34)	CT, FDG-PET/CT	Unilateral (L)	Yes
This study	FDG-PET/CT, US, CEUS	Bilateral	No

US, Ultrasound; CT, Computed Tomography; MRI, Magnetic Resonance Imaging; FDG-PET/CT, Fludeoxyglucose-Positron Emission Tomography/Computed Tomography; CEUS, Contrast-enhanced ultrasound; R, right; L, left; N/A, not available.

TABLE 2 | Differential diagnosis of testicular sarcoidosis with the most frequent testicular lesions on B-Mode ultrasound and contrast-enhanced ultrasound.

	B-Mode US	CEUS
Seminoma	Hypoechoic	Fast wash-in and rapid washout
Leydigioma	Hypoechoic	Wash-in and delayed washout
Sarcoidosis	Hypoechoic	Hypo-enhancement
Lymphoma	Hypoechoic	Fast wash-in and rapid washout ("straight vessel pattern")*
Testicular adrenal rest tumors	Hypoechoic	Fast wash-in and delayed washout

**"Straight vessel pattern" (or "nonbranching linear pattern") of increased vascularity represents a parallel arrangement of testicular small vessels that reflects the interstitial growth pattern of lymphoma, which preserves the vascular architecture of the testis.*
US, Ultrasound; CEUS, Contrast-enhanced ultrasound.

lesions which can range from a few millimeters to a few centimeters, with well-circumscribed or ill-defined margins. Bilateral unifocal lesions, as in the present case, are less common (37).

However, a question remains unsolved: what is the correct diagnosis of the left testicular lesion? In fact, the testicular sarcoid involvement is very rare, and an association between testicular cancer and sarcoidosis has been reported (38). Moreover, the ^{18}F-FDG uptake was detected only in the left testis (not in both testes) and the max SUV of the left testicular lesion (SUVmax = 11.6) was different from the sarcoid lesions involving the other organs. All these findings could not allow the exclusion of a testicular tumor.

In our case, on Color Doppler US some areas of vascularity within the lesion were detected. Usually, the color flow detectable in primary tumors of the testis using this imaging technique is higher than that of non-neoplastic lesions. However, some malignant lesions of the testis could lack internal vascularity on Color Doppler, such as burned-out testis tumors, being difficult to characterize with respect to non-neoplastic lesions (39). Therefore, the sole use of Color-Doppler US was not sufficient to exclude the possible diagnosis of testicular tumor lesion. Furthermore, ^{18}F-FDG-PET scan has high sensitivity in detecting lesions having an increased glucose uptake, but it is unable to differentiate an inflammatory process such as sarcoidosis from malignancy (40). Furthermore, in our case, the right testicular lesion did not demonstrate ^{18}F-FDG uptake probably due the small lesion dimension under the resolution power of this technique.

In the present case, the testicular unifocal lesions did not demonstrate hyperenhancement on CEUS and, moreover, the lesions were bilateral. The CEUS hyperenhancement of a testicular lesion has a positive predictive value of 97% for neoplasia and, although its presence alone is not specific enough to establish an unequivocal diagnosis, it is suggestive for a neoplastic testicular lesion, including malignancy (41). Moreover, the most common testicular malignancies in the same age of the patient described in this case are the testicular germ cell tumors that are bilateral in only 2% of cases (42).

The sarcoid testicular masses could pose problems of differential diagnosis with other bilateral testicular lesions that appear hypoechoic on B-mode US (**Table 2**), such as Seminoma, Leydigiomas, lymphomas, and testicular adrenal rest tumors. Seminomas usually demonstrate rapid wash-in coupled with rapid washout on CEUS (17). Leydigiomas show hyperenhancement as compared to the surrounding testicular parenchyma on CEUS, with delayed wash-out (17) and therefore the differential diagnosis with sarcoid testicular masses appear easy to perform. Lymphomas, along with leukemia, show marked CEUS hypervascularization (visible also with Color Doppler), a "nonbranching linear pattern" (known also as "straight vessel pattern") and a rapid filling time (43, 44), differently from sarcoid testicular masses. Testicular adrenal rest tumors demonstrate high hypervascularization as compared to the sarcoid lesions; moreover, these rare lesions arise in younger patients with congenital adrenal hyperplasia (45). Different approach could be have in case of oncological patients, in whom metastases (the most common are from prostate carcinoma, melanoma, colon and kidney cancer) are rare

but could not be excluded especially in cases of advanced malignancy (46).

A possible limitation of our case was the absence of MRI evaluation. However, MRI is not able to identify a specific pattern for sarcoidosis (47), and therefore it was not performed after CEUS in our case.

In conclusion, CEUS could play an important role in the evaluation of testicular lesions, and in particular of benign lesions, such as sarcoidosis. Due to the rising incidence rate of this disease and the reported association with testicular cancer, the need to establish a correct differential diagnosis will likely increase over time. CEUS could allow to achieve a correct diagnosis of testicular lesions, due to its ability in identifying very small testis lesions, such as sarcoidosis, coupled with its high accuracy in excluding malignancies, thus avoiding aggressive and potentially unnecessary maneuvers, such as biopsy for benign conditions.

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

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patient has given his written informed consent to publish the case (including publication of images).

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Arkema EV, Cozier YC. Epidemiology of sarcoidosis: current findings and future directions. *Ther Adv Chronic Dis.* (2018) 9:227–40. doi: 10.1177/2040622318790197
2. Ungprasert P, Crowson CS, Matteson EL. Influence of gender on epidemiology and clinical manifestations of sarcoidosis: a population-based retrospective cohort study 1976-2013. *Lung.* (2017) 195:87–91. doi: 10.1007/s00408-016-9952-6
3. Baughman RP, Field S, Costabel U, Crystal RG, Culver DA, Drent M, et al. Sarcoidosis in America. Analysis based on health care use. *Ann Am Thorac Soc.* (2016) 13:1244–52. doi: 10.1513/AnnalsATS.201511-760OC
4. Arkema EV, Grunewald J, Kullberg S, Eklund A, Askling J. Sarcoidosis incidence and prevalence: a nationwide register-based assessment in Sweden. *Eur Respir J.* (2016) 48:1690–9. doi: 10.1183/13993003.00477-2016
5. Gribbin J, Hubbard RB, Le Jeune I, Smith CJ, West J, Tata LJ. Incidence and mortality of idiopathic pulmonary fibrosis and sarcoidosis in the UK. *Thorax.* (2006) 61:980–5. doi: 10.1136/thx.2006.062836
6. Yoon HY, Kim HM, Kim YJ, Song JW. Prevalence and incidence of sarcoidosis in Korea: a nationwide population-based study. *Respir Res.* (2018) 19:158. doi: 10.1186/s12931-018-0871-3
7. Gerke AK, Hunninghake G. The immunology of sarcoidosis. *Clin Chest Med.* (2008) 29:379–90. doi: 10.1016/j.ccm.2008.03.014
8. Müller-Quernheim J, Schürmann M, Hofmann S, Gaede KI, Fischer A, Prasse A, et al. Genetics of sarcoidosis. *Clin Chest Med.* (2008) 29:391–414, viii. doi: 10.1016/j.ccm.2008.03.007
9. Newman LS, Rose CS, Bresnitz EA, Rossman MD, Barnard J, Frederick M, et al. A case control etiologic study of sarcoidosis: environmental and occupational risk factors. *Am J Respir Crit Care Med.* (2004) 170:1324–30. doi: 10.1164/rccm.200402-249OC
10. Baughman RP, Teirstein AS, Judson MA, Rossman MD, Yeager H Jr, Bresnitz EA, et al. Clinical characteristics of patients in a case control study of sarcoidosis. *Am J Respir Crit Care Med.* (2001) 164:1885–9. doi: 10.1164/ajrccm.164.10.2104046
11. Kodama K, Hasegawa T, Egawa M, Tomosugi N, Mukai A, Namiki M. Bilateral epididymal sarcoidosis presenting without radiographic evidence of intrathoracic lesion: review of sarcoidosis involving the male reproductive tract. *Int J Urol.* (2004) 11:345–8. doi: 10.1111/j.1442-2042.2004.00783.x
12. Massarweh NN, Bhalani VK, Shaw KK, Crawford B, Lang E, Davis R. Testicular presentation of sarcoidosis and organ preservation: case report and review of management strategies. *Urology.* (2006) 67:200. doi: 10.1016/j.urology.2005.08.011
13. Gupta R, Senadhi V. A diagnostic dilemma: metastatic testicular cancer and systemic sarcoidosis - a review of the literature. *Case Rep Oncol.* (2011) 4:118–24. doi: 10.1159/000324184
14. Stewart VR, Sidhu PS. The testis: the unusual, the rare and the bizarre. *Clin Radiol.* (2007) 62:289–302. doi: 10.1016/j.crad.2006.10.005
15. Sidhu PS, Cantisani V, Dietrich CF, Gilja OH, Saftoiu A, Bartels E, et al. The EFSUMB guidelines and recommendations for the clinical practice of contrast-enhanced ultrasound (CEUS) in non-hepatic applications: update 2017 (long version). *Ultraschall Med.* (2018) 39:e2–44. doi: 10.1055/a-0586-1107
16. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol.* (2018) 69:182–236. doi: 10.1016/j.jhep.2018.03.019
17. Isidori AM, Pozza C, Gianfrilli D, Giannetta E, Lemma A, Pofi R, et al. Differential diagnosis of nonpalpable testicular lesions: qualitative and quantitative contrast-enhanced US of benign and malignant testicular tumors. *Radiology.* (2014) 273:606–18. doi: 10.1148/radiol.14132718
18. Rees DA, Dodds AL, Rathbone N, Davies JS, Scanlon MF. Azoospermia in testicular sarcoidosis is an indication for corticosteroid therapy. *Fertil Steril.* (2004) 82:1672–4. doi: 10.1016/j.fertnstert.2004.07.950
19. Rehman J, Rizkala ER, Chughtai B, Khan SA. Hypoechoic testicular mass: a case of testicular and epididymal sarcoidosis. *Urology.* (2005) 66:657. doi: 10.1016/j.urology.2005.03.008
20. Thuret R, Cariou G, Aerts J, Cochand-Priollet B. Testicular sarcoidosis with elevated levels of cancer-associated markers. *J Clin Oncol.* (2008) 26:6007–8. doi: 10.1200/JCO.2008.17.9861
21. Real V, de Loyola GL, Zanoni PE, Real LF. Testicular sarcoidosis: a diagnosis to be considered. *Rev Col Bras Cir.* (2011) 38:145–6. doi: 10.1590/S0100-69912011000200015
22. Kim YB, Chung YG, Kim SJ, Kim SJ, Ahn HS, Joo HJ, et al. Extensive systemic sarcoidosis with testicular involvement mimicking metastatic testicular cancer. *Korean J Urol.* (2011) 52:295–7. doi: 10.4111/kju.2011.52.4.295
23. Paknejad O, Gilani MA, Khoshchehreh M. Testicular masses in a man with a plausible sarcoidosis. *Indian J Urol.* (2011) 27:269–71. doi: 10.4103/0970-1591.82848
24. Kovac JR, Flood D, Mullen JB, Fischer MA. Diagnosis and treatment of azoospermia resulting from testicular sarcoidosis. *J Androl.* (2012) 33:162–6. doi: 10.2164/jandrol.110.012534
25. Esnakula AK, Coleman P, Ahaghotu CA, Naab TJ. Scrotal mass and unilateral lung masses with pleural effusion mimicking metastatic testicular malignancy: an unusual presentation of sarcoidosis. *BMJ Case Rep.* (2013) 2013:bcr2012008658. doi: 10.1136/bcr-2013-008658

26. Joel J, Thomas J, Gill K, Biyani CS. Testicular sarcoidosis masquerading as testicular carcinoma. *Cent European J Urol.* (2014) 67:261–3. doi: 10.5173/ceju.2014.03.art10
27. Patel H, Shaaban H, Kumar A, Modi T, Maroules M. A rare case report of bilateral testicular masses as an initial manifestation of systemic sarcoidosis. *Urol Ann.* (2015) 7:378–9. doi: 10.4103/0974-7796.152046
28. Knox A, Black N, Agbaje I. Pretesticular and testicular effects of systemic sarcoidosis: a case report. *J Reprod Med.* (2017) 62:204–6.
29. Chierigo F, Alnajjar HM, Haider A, Walkden M, Shaikh T, Muneer A. Testicular pain as an atypical presentation of sarcoidosis. *Ann R Coll Surg Engl.* (2019) 101:e99–101. doi: 10.1308/rcsann.2019.0015
30. Babst C, Piller A, Boesch J, Schmid HP. Testicular sarcoidosis. *Urol Case Rep.* (2018) 17:109–10. doi: 10.1016/j.eucr.2018.01.021
31. Konishi S, Hatakeyama S, Yoneyama T, Yoneyama T, Hashimoto Y, Ohyama C. Bilateral scrotal mass mimicking testicular cancer: an unusual presentation of sarcoidosis. *Int J Urol.* (2019) 26:1079–81. doi: 10.1111/iju.14089
32. Hamitouche F, Lacoste M, Korenbaum C, Galland J. Diffuse lesions. *Rev Med Interne.* (2019) 40:476–7. doi: 10.1016/j.revmed.2018.09.005
33. Kimura S, Momozono K, Shimamatsu K, Noguchi M. Testicular sarcoidosis with bilateral scrotal swelling. *IJU Case Rep.* (2019) 3:12–4. doi: 10.1002/iju5.12124
34. Parida GK, Kumar A, Mitra S, Suman A, Muthu GS. Rare case of testicular sarcoidosis detected on FDG PET/CT in a patient with PUO. *Clin Nucl Med.* (2020) 45:e368–9. doi: 10.1097/RLU.0000000000003120
35. Appelbaum L, Gaitini D, Dogra VS. Scrotal ultrasound in adults. *Semin Ultrasound CT MR.* (2013) 34:257–73. doi: 10.1053/j.sult.2013.01.008
36. Bertolotto M, Muça M, Currò F, Bucci S, Rocher L, Cova MA. Multiparametric US for scrotal diseases. *Abdom Radiol.* (2018) 43:899–917. doi: 10.1007/s00261-018-1510-7
37. Eraso CE, Vrachliotis TG, Cunningham JJ. Sonographic findings in testicular sarcoidosis simulating malignant nodule. *J Clin Ultrasound.* (1999) 27:81–3. doi: 10.1002/(SICI)1097-0096(199902)27:2<81::AID-JCU6>3.0.CO;2-N
38. Paparel P, Devonec M, Perrin P, Ruffion A, Decaussin-Petrucci M, Akin O, et al. Association between sarcoidosis and testicular carcinoma: a diagnostic pitfall. *Sarcoidosis Vasc Diffuse Lung Dis.* (2007) 24:95–101.
39. Miacola C, Colamonico O, Bettocchi C, Ricapito V, Palazzo S, Campagna M, et al. Burned-out in a mixed germ cell tumor of the testis: the problem of pT0. Case report. *Arch Ital Urol Androl.* (2014) 86:389–90. doi: 10.4081/aiua.2014.4.389
40. Akaike G, Itani M, Shah H, Ahuja J, Yilmaz Gunes B, Assaker R, et al. PET/CT in the diagnosis andworkup of sarcoidosis: focus on atypical manifestations. *Radiographics.* (2018) 38:1536–49. doi: 10.1148/rg.2018180053
41. Lock G, Schmidt C, Helmich F, Stolle E, Dieckmann KP. Early experience with contrast-enhanced ultrasound in the diagnosis of testicular masses: a feasibility study. *Urology.* (2011) 77:1049–53. doi: 10.1016/j.urology.2010.12.035
42. Zequi Sde C, da Costa WH, Santana TB, Favaretto RL, Sacomani CA, Guimaraes GC. Bilateral testicular germ cell tumours: a systematic review. *BJU Int.* (2012) 110:1102–9. doi: 10.1111/j.1464-410X.2012.11056.x
43. Kachramanoglou C, Rafailidis V, Philippidou M, Bertolotto M, Huang DY, Deganello A, et al. Multiparametric sonography of hematologic malignancies of the testis: grayscale, color doppler, and contrast-enhanced ultrasound and strain elastographic appearances with histologic correlation. *J Ultrasound Med.* (2017) 36:409–20. doi: 10.7863/ultra.16.02013
44. Lock G, Schmidt C, Schröder C, Löning T, Dieckmann KP. Straight vessel pattern and rapid filling time: characteristic findings on contrast-enhanced sonography of testicular lymphoma. *J Ultrasound Med.* (2016) 35:1593–9. doi: 10.7863/ultra.15.05049
45. Engels M, Span PN, van Herwaarden AE, Sweep FCGJ, Stikkelbroeck NMML, Claahsen-van der Grinten HL. Testicular adrenal rest tumors: current insights on prevalence, characteristics, origin, and treatment. *Endocr Rev.* (2019) 40:973–87. doi: 10.1210/er.2018-00258
46. Patel SR, Richardson RL, Kvols L. Metastatic cancer to the testes: a report of 20 cases and review of the literature. *J Urol.* (1989) 142:1003–5. doi: 10.1016/S0022-5347(17)38969-3
47. Handa T, Nagai S, Hamada K, Ito I, Hoshino Y, Shigematsu M, et al. Sarcoidosis with bilateral epididymal and testicular lesions. *Intern Med.* (2003) 42:92–7. doi: 10.2169/internalmedicine.42.92

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.

frontiers in Medicine

MINI REVIEW
published: 28 January 2021
doi: 10.3389/fmed.2021.595522

Role of the Microbiome in Interstitial Lung Diseases

Ozioma S. Chioma[1], Laura E. Hesse[2], Austin Chapman[1] and Wonder P. Drake[1,2]*

[1] Division of Infectious Diseases, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN, United States, [2] Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, TN, United States

There are trillions of microorganisms in the human body, consisting of bacteria, viruses, fungi, and archaea; these collectively make up the microbiome. Recent studies suggest that the microbiome may serve as a biomarker for disease, a therapeutic target, or provide an explanation for pathophysiology in lung diseases. Studies describing the impact of the microorganisms found in the respiratory tract on lung health have been published and are discussed here in the context of interstitial lung diseases. Additionally, epidemiological and experimental evidence highlights the importance of cross-talk between the gut microbiota and the lungs, called the gut–lung axis. The gut-lung axis postulates that alterations in gut microbial communities may have a profound effect on lung disease. Dysbiosis in the microbial community of the gut is linked with changes in immune responses, homeostasis in the airways, and inflammatory conditions in the gastrointestinal tract itself. In this review, we summarize studies describing the role of the microbiome in interstitial lung disease and discuss the implications of these findings on the diagnosis and treatment of these diseases. This paper describes the impact of the microbial communities on the pathogenesis of lung diseases by assessing recent original research and identifying remaining gaps in knowledge.

Keywords: interstitial lung disease (ILD), fibrosis, sarcoidosis, gut microbiome, infection, idiopathic pulmonary fibrosis, lung microbiome

OPEN ACCESS

Edited by:
Nadera J. Sweiss, University of Illinois at Chicago, United States

Reviewed by:
Garifallia Sakellariou, University of Pavia, Italy
Christian Ascoli, University of Illinois at Chicago, United States
Catarina Sousa Guerreiro, University of Lisbon, Portugal

****Correspondence:***
Ozioma S. Chioma
ozioma.s.chioma@vumc.org

Specialty section:
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

Received: *16 August 2020*
Accepted: *06 January 2021*
Published: *28 January 2021*

Citation:
Chioma OS, Hesse LE, Chapman A and Drake WP (2021) Role of the Microbiome in Interstitial Lung Diseases. Front. Med. 8:595522. doi: 10.3389/fmed.2021.595522

INTRODUCTION

The gut microbiota is defined as the diverse microbial communities that inhabit the host's gastrointestinal (GI) tract (1). The GI tract is a nutrient rich environment that supports up to 100 trillion microbes which collectively make up the gut microbiome (2). The gut microbiome profoundly impacts human physiology and nutrition, and is essential for human life (3). Many factors, including diet, age, antibiotics, lifestyle behaviors, and mode of delivery at birth are influential contributors to the composition of the gut microbiome (4). Advanced techniques that identify microbial sequences, including 16S rRNA gene and shotgun metagenomic analysis, have provided new insights into the diversity of microbial organisms present both in the diseased and normal gut (5). The composition of the gut microbiome is stable within individuals and largely shared between healthy individuals (1, 6). Microbial imbalance or dysbiosis in the gut microbiome is associated with illness and disorders, including interstitial lung diseases (ILDs) (7–10).

For many years, the lung was thought to be a sterile environment (11). However, recent advances in microbial sequencing techniques suggest that a variety of microbial organisms dwell in both the upper and lower respiratory tract and that composition of this microbial community is altered in respiratory disease states (12). Characterizing the composition of the lung microbiome during disease and elucidating the contribution of the dysbiotic microbiome to disease progression is an area of active research for many respiratory ailments, including ILDs.

ILDs, otherwise called diffuse parenchymal lung diseases, are a group of disorders characterized by chronic inflammation that result in fibrosis (scarring) of the lung (13). The most common symptom of all ILDs is shortness of breath or dyspnea, often accompanied by a dry cough, chest discomfort, fatigue, and occasionally weight loss. Examples of ILDs include sarcoidosis, asbestosis, hypersensitivity pneumonitis, idiopathic pulmonary fibrosis (IPF), non-specific interstitial pneumonia (NSIP), and acute interstitial pneumonitis (14, 15). Some of the risk factors of ILDs are genetics; exposure to hazardous material, such as asbestos; prior infection with microorganisms, including tuberculosis and hepatitis C; radiation and chemotherapy treatments; smoking; connective tissue diseases; and chronic inflammatory diseases like rheumatoid arthritis (16). In some cases, such as IPF and sarcoidosis, the causes may be unknown. There is a growing body of literature supporting dysbiosis of the microbiome as a contributor to ILD, which will be discussed in this review.

Epidemiological and experimental evidence highlights an important cross-talk between the gut microbiota and the lungs (9). Alterations in the microbial community due to factors such as drugs, disease, or diet is linked with changes in immune responses and disruption of homeostasis in the airways, as well as in the gastrointestinal tract (17–20). The altered inflammatory state resulting from microbial dysbiosis, as well as the microbes themselves, may play an important role in ILD progression. Alterations to the microbiome may serve as biomarkers for disease, explain disease progression or serve as a therapeutic target. In this review, we discuss the current understanding of the involvement of the both the lung and gut microbiome in ILD.

TABLE 1 | Studies conducted on microbiome in interstitial lung diseases.

Disease state	Assessment method(s)	Conclusions	References
Idiopathic pulmonary fibrosis (IPF)	16S rRNA sequencing of BALF; 454 pyrosequencing; quantitative BALF culture	IPF patients have increased bacterial burdens in lungs, enriched for pathogenic genera like *Staphylococcus* and *Streptococcus*	(22–26)
Sarcoidosis	16S rRNA sequencing of BALF, lymph node biopsies, spleen tissue; shotgun metagenomic sequencing of BALF	Alteration of microbiome in sarcoidosis is unclear—results vary by study and specimen type, but possible loss of diversity in sarcoidosis lung microbial community	(28–31)
Idiopathic interstitial pneumonia (IIP)	16S rRNA sequencing of BALF	No change between lung microbiome of health and IIP patients	(29)
Systemic Sclerosis (SSc)	16S rRNA sequencing of SSc patient fecal samples	Dysbiosis of intestinal microbiome in SSc patients compared to healthy controls	(34)
Silicosis	16S rRNA sequencing of silicosis patient fecal samples	Decrease in microbial diversity in intestinal community with enrichment of Proteobacteria	(8)
Hypersensitivity pneumonitis	16S rRNA sequencing of fecal samples using a murine model of HP	*Bacteroidetes* phylum enriched in streptomycin treated animals that develop severe HP	(36)

LUNG MICROBIOME IMPACTS INTERSTITIAL LUNG DISEASE PROGRESSION

Microbial communities present in a specific body niche will have an impact on the physiology of that body site through direct interactions with host cells. Numerous studies have implicated an altered lung microbiome in the pathogenesis of interstitial lung diseases, such as idiopathic pulmonary fibrosis (IPF) and sarcoidosis. The findings of these studies are summarized in **Table 1**, and discussed in the following section.

IPF

The lung microbiome of IPF patients is distinct from healthy individuals (21). In one study, IPF patients had a higher lung bacterial load in their lungs enriched for *Haemophilus*, *Streptococcus*, *Neisseiria*, and *Veillonella* genera compared to healthy individuals (22). Additional analysis of IPF patient bronchoalveolar lavage fluid (BALF) by 16S rRNA sequencing showed that lung bacteria may play a causative role in acute exacerbation of IPF, and a high bacterial load at the time of diagnosis may be a biomarker for rapidly progressive disease (23, 24). A multicenter cohort study was conducted on the microbial signatures associated with progression of IPF, in which the researchers retrospectively sequenced lung microbiota using 454 pyrosequencing in 55 IPF patients' baseline BALF samples and reported that the presence of specific *Streptococcus* or *Staphylococcus* species above a certain threshold was associated with a faster-progressing disease (25). A different investigation using 16S rRNA sequencing identified the most prevalent operational taxonomic units (OTUs) in IPF patients BALF as *Prevotella* and *Staphylococcus* species (26). Overall, these investigations demonstrate that IPF patients have high bacterial loads in their lungs compared to healthy controls, which are enriched for potentially pathogenic genera such as *Staphylococcus* and *Streptococcus*, implicating the lung microbiome in disease progression.

Sarcoidosis

Sarcoidosis is an additional ILD of unknown etiology that may be influenced by lung microbiota composition (27).

In a cross-sectional study to compare the lung microbiota from bronchoalveolar lavage fluid (BALF) of 71 patients with sarcoidosis and 10 healthy controls, the investigators identified *Atopobium* and *Fusobacterium* as increased in abundance in sarcoidosis samples compared with those from healthy controls using 16S rRNA sequencing (28). In contrast, a different study used 16S rRNA gene-based pyrosequencing to characterize microbial communities in upper and lower airways in ILD patients, including idiopathic interstitial pneumonia and sarcoidosis. The microbiota in lower airways of the majority of patients primarily consisted of *Prevotellaceae, Streptococcaceae*, and *Acidaminococcaceae*. However, according to this study, the diagnosis of ILD did not alter the overall lung microbiota compared to healthy controls (29). Metagenomic sequencing to analyze various specimens including lymph node biopsies, BALF, Kveim reagent samples, and fresh granulomatous spleens from 93 sarcoidosis patients and 72 control subjects revealed elevated levels of certain bacterial and fungal orders in single sarcoidosis sample types but did not detect enrichment of the same orders across multiple sample types (30). Another study investigating microbial composition during airway abnormalities using 16S rRNA sequencing found that the microbiome composition in BALF of sarcoidosis patients is similar to that in rheumatoid arthritis (RA) patients and distinct from healthy controls, with reduced presence of *Actynomyces* and *Burkhordelia*, but enrichment of periodontopathic taxa, including *Treponema, Prevotella,* and *Porphyromonas* (31).

Using these studies and others to develop a consensus "ILD respiratory microbiome," in addition to optimizing and standardizing a method of sample collection and detection for respiratory microbiome sequencing, could lead to improvements in the diagnosis of ILDs. Missing from the current ILD lung microbiome literature is a dissection of the mechanism by which the genera enriched in the ILD respiratory microbiome impact lung health. Whether ILD pathophysiology drives microbial community changes or the microbial dysbiosis drives ILD progression is also an outstanding question that is key to understanding and treating ILDs.

GUT MICROBIOME IMPACTS LUNG HEALTH

The respiratory microbiome develops alongside the gut microbiome during early life (32). Epidemiological and experimental evidence highlights an important cross-talk between the gut microbiota and the lungs, called the gut–lung axis. Recent studies suggest that dysbiosis in the gut microbiota is linked with changes in immune responses and homeostasis in the airways in diverse lung pathologies, including ILD, asthma, and pneumonia. **Figure 1** describes the mechanisms by which the gut is known to impact lung physiology.

Systemic Sclerosis

Systemic sclerosis (SSc), also called scleroderma, is an immune-mediated rheumatic disease that is characterized by vasculopathy, fibrosis of the skin and internal organs, such as the gastrointestinal (GI) tract and the lungs, and often progresses to ILD (33). Dysbiotic gut microbiota has been shown in patients with SSc, particularly those with extra-intestinal manifestations, including lung fibrosis (34). In a study that analyzed the link between gut inflammation and ILD in a large SSc population, increased fecal calprotectin levels correlated with ILD progression (35). While this suggests intestinal inflammation and alteration of the gut microbiome are present in ILD, further studies are required to elucidate the role calprotectin and gut dysbiosis may play in the development of ILD in SSc.

Silicosis

Investigation of the gut microbial composition of fecal samples from 18 patients with silicosis, an ILD caused by inhalation of silica, and 21 healthy subjects using 16S rRNA gene sequencing noted striking changes in the microbial composition. Compared with the healthy subjects, the bacterial diversity of the intestinal microbiome was reduced in patients with silicosis. This decrease in diversity was accompanied by an expansion of Proteobacteria (8).

Hypersensitivity Pneumonitis

Agents, such as antibiotics, can induce changes in the gut microbiota which alter disease phenotypes in ILDs. For example, a study conducted to assess the effects of antibiotic treatment on a T_H1/T_H17-mediated ILD, hypersensitivity pneumonitis (HP), found that the severity of HP was unaffected by vancomycin, but increased dramatically after streptomycin treatment (36). Treatment with either antibiotic altered the gut microbiome of the animals, with Bacteroidetes enriched after streptomycin treatment, while vancomycin treatment led to higher levels of Firmicutes in the intestinal communities.

While these studies using SSc and silicosis patient cohorts and a mouse model of HP indicate there is a connection between the gut microbiome and ILDs, future studies that expand the breath of ILDs investigated for their gut microbiota-dependence and better define the alterations that occur in specific disease states are needed. These studies should include both patient cohorts and studies that model and monitor disease progression in mice, including germ-free mice, to strengthen the claim that the gut microbiome impacts ILD progression or development.

Despite the scarcity of data on the influence of the microbiome on ILDs, there are studies on other inflammatory lung conditions that support the importance of a healthy gut microbiome to promote lung health. Two common non-ILD lung diseases, asthma and pneumonia, are reviewed in the next sections to provide additional evidence for a gut-lung axis and suggest the gut microbiome is a major contributor to lung health.

Asthma

Asthma is a chronic lung disease where alterations in the gut microbiome have been shown to impact disease progression (37). Reductions in the genus *Bifidobacteria* and increase in *Clostridia* in the intestine are associated with asthma in early life (38). In murine studies, depletion of certain members of the gut microbiome through antibiotic administration early in life enhances future susceptibility to allergic asthma, increasing

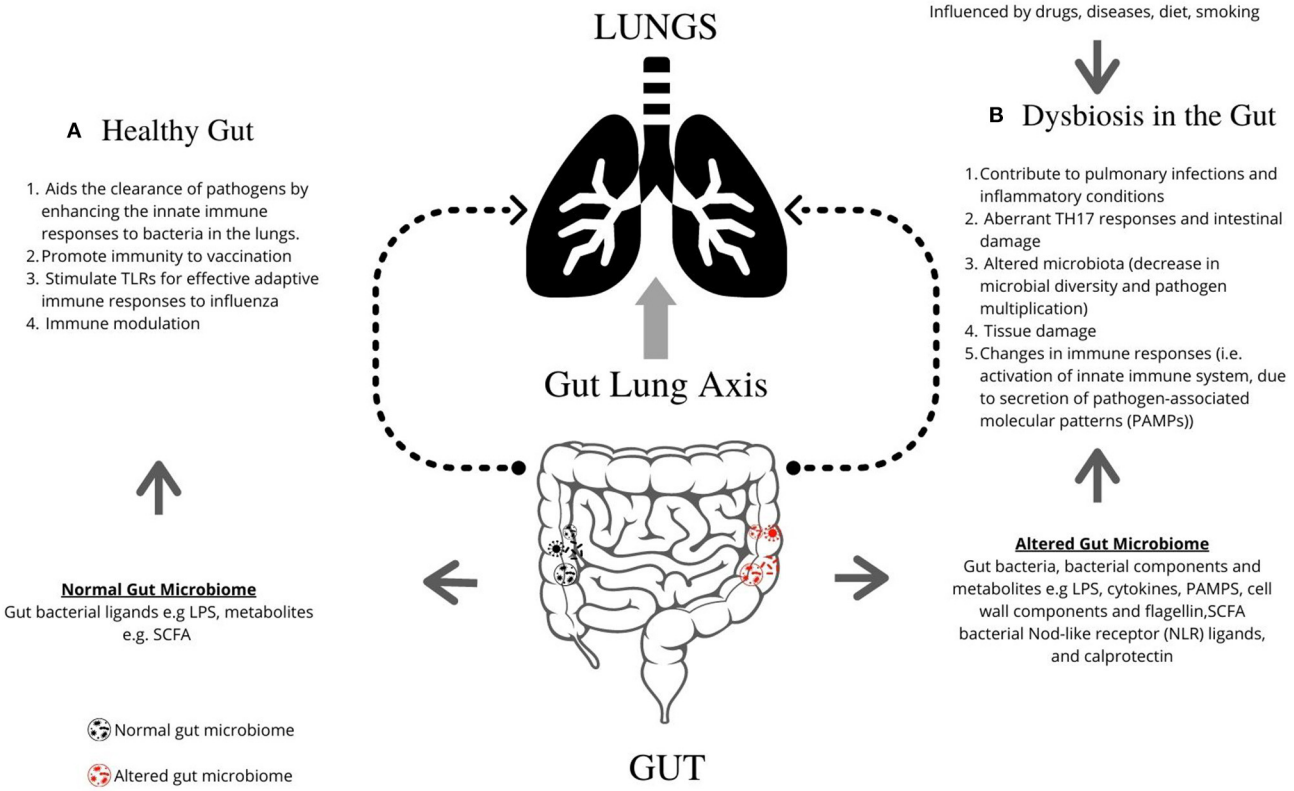


FIGURE 1 | Graphical representation of the role of gut microbiome in regulating lung pathogenesis. The gut microbiome profoundly impacts human physiology and nutrition, and is essential for human life. **(A)** The healthy gut aids the clearance of pathogens by enhancing the innate immune responses to bacteria in the lungs, promotes immunity following vaccination, and can stimulate TLRs for effective adaptive immune responses to influenza. **(B)** Microbial imbalance or dysbiosis in the gut microbiome is influenced by drugs, diet and diseases, and can be associated with illness and disorders, including interstitial lung diseases. Gut bacteria, bacterial components, and metabolites such as LPS, PAMPs, cell wall components, and flagellin contribute lead to changes in lung immunity. Dysbiosis contributes to pulmonary infections and inflammatory conditions, aberrant TH17 responses and intestinal damage, altered microbiota (decrease in microbial diversity and pathogen multiplication), tissue damage, and changes in immune responses [i.e., activation of innate immune system, due to secretion of pathogen-associated molecular patterns (PAMPs)]. TLR, Toll-like receptors; LPS, Lipopolysaccharide; PAMPs, pathogen-associated molecular patterns; SCFA, short-chain fatty acids; NLR, Nod-like receptor.

predisposition to airway diseases and pulmonary viral infections (39). Additionally, neonatal mice administered vancomycin demonstrate an increase in allergic asthma and significant alterations to their gut microbiota as compared to control mice (40).

Pneumonia

A study focused on the role of the gut microbiota in bacterial pneumonia found that the intestinal microbiota act as a protective mediator during pneumococcal pneumonia (41). This study was carried out by intranasal infection of conventional and germ-free mice with *S. pneumoniae*, and identified that the gut microbiota enhanced primary alveolar macrophage function. Subsequent studies demonstrated that this enhanced macrophage function is mediated through GM-CSF signaling (42) and that the presence of filamentous segmented bacteria protects against *Staphylococcus aureus* pneumonia through induction of type 17 immunity (43).

These studies indicate that the gut microbiota is important for lung health. Interestingly, specific community members are associated with protection from asthma or pneumonia, while others exacerbate disease. This suggests that while future work is needed to uncover the role of specific bacterial species, probiotic or microbiota transfer methods may be successful methods of treating lung diseases.

GUT MICROBIOME IMPACTS IMMUNE RESPONSES

Several mechanisms have been proposed to describe the impact of the gut microbiota on lung physiology. A prevailing hypothesis is that changes in gut microbial composition impact host immune responses in the lung. Support for this hypothesis demonstrating that the gut microbiome impacts immunity in the gut, lungs, and systemically, is discussed in the following section.

Immunity in the Gut

The gut microbiota is now recognized as an influential player in host immune responses (44, 45). Murine studies show that the gut microbiome, particularly commensal bacteria, are important in shaping the host immune system (46–48). The T_h17: T_{reg} balance in the lamina propria is determined by gut microbiota composition and specific members of the gut bacterial community may influence intestinal immunity, tolerance, and susceptibility to inflammatory bowel diseases (49). For instance, the segmented filamentous bacteria (SFB), can induce the appearance of $CD4^+$ T helper cells that produce T_h17 cells in the lamina propria. Colonization of the small intestine with commensal SFB resulted in enhanced resistance to the intestinal pathogen *Citrobacter rodentium,* and is linked with an increase in expression of genes associated with antimicrobial defenses and inflammation (50).

Immunity in the Lungs

The gut microbiota can aid the clearance of pathogens by enhancing the innate immune responses to pathogens in the lungs. In a study designed to investigate the role of commensal microflora in the gut on the host defense in pneumonia through toll-like receptors (TLRs), the authors found that antibiotic pretreatment to deplete commensal gut flora prior to *Escherichia coli* pneumonia challenge led to increased bacterial burdens in both the blood and the lungs. They also noted cytokine suppression (TNF-α, IL-6, IL-1β) as well as suppressed nuclear factor κB activity in the intestine. They concluded that the gut microbiota is critical in inducing TLR4 expression and nuclear factor κB activation of intestinal and lung innate immune defense against *E. coli* pneumonia (51). Bacterial Nod-like receptor (NLR) ligands including a NOD1 ligand, $MurNAcTri_{DAP}$, and a NOD2 ligand, muramyl dipeptide, from the gastrointestinal tract have also been shown to rescue host defenses in the lung (52). Additionally, gut microbiota promote lung immunity following vaccination (53). Cell wall components and flagellin of gut bacteria have been shown to stimulate TLRs for effective adaptive immune responses to influenza (54).

Microbes influence host immunity in many ways, including the ability of *Bacteroides* to expand Treg cell populations or skew the TH1/TH2 phenotype in either direction, and suppress host inflammatory responses through production of short chain fatty acids (SCFAs) (55). SCFAs are produced by many enteric bacterial species and act through free fatty acid (FFA) receptors or epigenetic regulation of immune cells to promote broad anti-inflammatory effects (56). SCFA administration is linked to reduced pulmonary pathology following both and viral and bacterial infection in mice (57–59). Overall, these studies suggest that recognition of the gut microbiota, as well as specific bacterial metabolites, primes the immune response to counter microbial challenges in the lung.

Systemic Immune Responses

Studies have shown that dysbiosis in the gut microbiome may result in the development of systemic inflammatory conditions, such as rheumatoid arthritis and atherosclerosis (60–62). In the case of sarcoidosis, an altered composition of the gut microbiota may activate the innate immune system, promoting the formation of granulomas through the secretion of proinflammatory cytokines, such as IL-6, IL-12, IL-18, and TNF-α (63).

Due to the relationship between host immunity and enteric microorganisms, various strategies have been put in place to target the gut microbiota as a way of managing or preventing chronic inflammatory diseases. These strategies include the use of antimicrobials to deplete or supplement microbial load, changes in diet, or supplementation with live microorganisms. Microbial transplantation has become a useful tool in manipulating the gut microbiome for management or prevention of certain illnesses. In recent times, fecal microbial transplantation (FMT) strategies have been used in a range of infections with encouraging results (64–66). The mechanisms underlying the success of FMT is still an area of active research.

LUNG MICROBIOME IMPACTS GUT MICROBIOME COMPOSITION AND HEALTH

In addition to the gut microbiome impacting lung health, there is evidence that changes in lung microbial communities impact gut physiology. This suggests that the gut-lung axis is bidirectional and should be considered during investigations of the impact of gut dysbiosis on lung disease.

Viral

Perturbations to the lung microbial community, such as the introduction of a respiratory virus, influence the gut microbial communities. For example, murine models of pulmonary influenza virus infection increase *Enterobacteriaceae* while reducing *Lactobacilli* and *Lactococci* in the intestinal microbial community (67). In a study designed to access the occurrence of gastroenteritis-like symptoms using a mouse model of respiratory influenza infection, researchers found that influenza infection altered the intestinal microbiota composition, which was mediated by IFN-gamma production from lung-derived $CCR9^+$ $CD4^+$ T cells recruited into the small intestine. This resulted in aberrant T_h17 responses and intestinal damage (68). Perturbations to the gut microbiome as a result of respiratory viruses have also been demonstrated in human studies. A recent study using 16S rRNA sequencing of patient fecal samples described reduced diversity in the gut flora of patients hospitalized with COVID-19 and H1N1 influenza. Interestingly, COVID-19 and H1N1 influenza patients had different bacterial genera that predominated their dysbiotic gut communities (69).

Bacterial

Pulmonary microbial dysbiosis following intratracheal lipopolysaccharide (LPS) administration in mice is accompanied by disturbances in their gut microbiota secondary to movement of bacteria from their lung into the bloodstream. This causes an increase in the bacterial load in the intestines, thereby disrupting the gut microbial community (70).

Limited, but compelling evidence suggests that the lung microbiota may be influencing the composition of the gut microbiome and overall gut health. These studies address an additional, novel aspect of the gut-lung axis and may help explain the concordance of respiratory and GI symptoms during infection with pulmonary pathogens, such as *Legionella pneumophila* and SARS-CoV-2 (71, 72). Additional studies focused on a variety of states of lung dysbiosis, including ILDs, are necessary to elucidate the impact the lung microbiota has on the gut.

CONCLUSIONS

The impact of the microbiome on human physiology is substantial. Studies evaluating the composition of the lung and the gut microbiome in patients with interstitial lung diseases suggests that dysbiosis in the communities of either body site are correlated with disease. Whether this is true in all types of ILD, and whether the altered community contributes to ILD progression remains to be elucidated. Patient data and mouse models in other lung diseases, such as asthma and pneumonia, suggest that the gut microbiome has a profound influence on lung health. Specific members of the gut and lung communities can either ameliorate or exacerbate disease, indicating further research is needed to define the impact of individual microbes on disease states, including ILDs. Enhancement of the immune response is likely the mechanism by which the gut microbiome is capable of impacting lung homeostasis, as considerable evidence has shown that recognition of gut flora is key to regulating immune responses. Targeting the gut microbiome is an attractive target for therapeutic intervention in lung diseases, but more research is needed to identify the specific alterations that would be beneficial. The literature reviewed here provides support for a link between the microbiome and ILDs.

AUTHOR CONTRIBUTIONS

OC wrote the manuscript. WD, LH, and AC modified the manuscript and made final corrections. All authors contributed to and approved the final version of the manuscript for publication.

FUNDING

This work was supported by Interdisciplinary Training in Rheumatic Diseases T32 Training Grant (5T32AR059039-10) and Foundation of Sarcoidosis Research Fellowship Grant (FSR RFP 17-9041) to OC, K24 HL127301 to WD.

ACKNOWLEDGMENTS

We thank all the authors for their contributions.

REFERENCES

1. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature.* (2010) 464:59–65. doi: 10.1038/nature08821
2. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell.* (2006) 124:837–48. doi: 10.1016/j.cell.2006.02.017
3. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. *Science.* (2005) 307:1915–20. doi: 10.1126/science.1104816
4. Hasan N, Yang H. Factors affecting the composition of the gut microbiota, and its modulation. *PeerJ.* (2019) 2019:1–31. doi: 10.7717/peerj.7502
5. Ranjan R, Rani A, Metwally A, McGee HS, Perkins DL. Analysis of the microbiome: advantages of whole genome shotgun versus 16S amplicon sequencing. *Biochem Biophys Res Commun.* (2016) 469:967–77. doi: 10.1016/j.bbrc.2015.12.083
6. Zoetendal EG, Akkermans ADL, De Vos WM. Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. *Appl Environ Microbiol.* (1998) 64:3854–9. doi: 10.1128/AEM.64.10.3854-3859.1998
7. Shukla SD, Budden KF, Neal R, Hansbro PM. Microbiome effects on immunity, health and disease in the lung. *Clin Transl Immunol.* (2017) 6:e133–12. doi: 10.1038/cti.2017.6
8. Zhou Y, Chen L, Sun G, Li Y, Huang R. Alterations in the gut microbiota of patients with silica-induced pulmonary fibrosis. *J Occup Med Toxicol.* (2019) 14:1–11. doi: 10.1186/s12995-019-0225-1
9. Budden KF, Gellatly SL, Wood DLA, Cooper MA, Morrison M, Hugenholtz P, et al. Emerging pathogenic links between microbiota and the gut-lung axis. *Nat Rev Microbiol.* (2017) 15:55–63. doi: 10.1038/nrmicro.2016.142
10. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Human gut microbes associated with obesity. *Nature.* (2006) 444:1022–23. doi: 10.1038/4441022a
11. Moffatt MF, Cookson WOCM. The lung microbiome in health and disease. *Clin Med.* (2017) 17:525–9. doi: 10.7861/clinmedicine.17-6-525
12. Hilty M, Burke C, Pedro H, Cardenas P, Bush A, Bossley C, et al. Disordered microbial communities in asthmatic airways. *PLoS one.* (2010) 5:e8578. doi: 10.1371/journal.pone.0008578
13. Travis WD, Costabel U, Hansell DM, King TE, Lynch DA, Nicholson AG, et al. An official American thoracic society/european respiratory society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med.* (2013) 188:733–48. doi: 10.1164/rccm.201308-1483ST
14. Riario Sforza GG, Marinou A. Hypersensitivity pneumonitis: a complex lung disease. *Clin Mol Allergy.* (2017) 15:1–8. doi: 10.1186/s12948-017-0062-7
15. Farioli A, Violante FS, La Vecchia C, Negri E, Pelucchi C, Spatari G, et al. Temporal patterns of exposure to asbestos and risk of asbestosis. *J Occup Environ Med.* (2018) 60:536–41. doi: 10.1097/JOM.0000000000001260
16. Choi W Il, Dauti S, Kim HJ, Park SH, Park JS, Lee CW. Risk factors for interstitial lung disease: a 9-year Nationwide population-based study. *BMC Pulm Med.* (2018) 18:1–7. doi: 10.1186/s12890-018-0660-2
17. Willing BP, Russell SL, Finlay BB. Shifting the balance: antibiotic effects on host-microbiota mutualism. *Nat Rev Microbiol.* (2011) 9:233–43. doi: 10.1038/nrmicro2536
18. Rutten EPA, Lenaerts K, Buurman WA, Wouters EFM. Disturbed intestinal integrity in patients with COPD : effects of activities of daily living. *Chest.* (2014) 145:245–52. doi: 10.1378/chest.13-0584
19. Rapozo DCM, Bernardazzi C, De Souza HSP. Diet and microbiota in inflammatory bowel disease: the gut in disharmony. *World J Gastroenterol.* (2017) 23:2124–40. doi: 10.3748/wjg.v23.i12.2124
20. Dang AT, Marsland BJ. Microbes, metabolites, and the gut–lung axis. *Mucosal Immunol.* (2019) 12:843–50. doi: 10.1038/s41385-019-0160-6
21. Molyneaux PL, Maher TM. The role of infection in the pathogenesis of idiopathic pulmonary fibrosis. *Eur Respir Rev.* (2013) 22:376–81. doi: 10.1183/09059180.00000713

22. Richter AG, Stockley RA, Harper L, Thickett DR. Pulmonary infection in Wegener granulomatosis and idiopathic pulmonary fibrosis. *Thorax.* (2009) 64:692–7. doi: 10.1136/thx.2008.110445
23. Molyneaux PL, Cox MJ, Willis-Owen SAG, Mallia P, Russell KE, Russell AM, et al. The role of bacteria in the pathogenesis and progression of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* (2014) 190:906–13. doi: 10.1164/rccm.201403-0541OC
24. Molyneaux PL, Cox MJ, Wells AU, Kim HC, Ji W, Cookson WOC, et al. Changes in the respiratory microbiome during acute exacerbations of idiopathic pulmonary fibrosis. *Respir Res.* (2017) 18:10–15. doi: 10.1186/s12931-017-0511-3
25. Han MLK, Zhou Y, Murray S, Tayob N, Noth I, Lama VN, et al. Lung microbiome and disease progression in idiopathic pulmonary fibrosis: an analysis of the COMET study. *Lancet Respir Med.* (2014) 2:548–56. doi: 10.1016/S2213-2600(14)70069-4
26. Huang Y, Ma SF, Espindola MS, Vij R, Oldham JM, Huffnagle GB, et al. Microbes are associated with host innate immune response in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* (2017) 196:208–19. doi: 10.1164/rccm.201607-1525OC
27. Baughman RP, Culver DA, Judson MA. A concise review of pulmonary sarcoidosis. *Am J Respir Crit Care Med.* (2011) 183:573–81. doi: 10.1164/rccm.201006-0865CI
28. Zimmermann A, Knecht H, Häsler R, Zissel G, Gaede KI, Hofmann S, et al. Atopobium and Fusobacterium as novel candidates for sarcoidosis-associated microbiota. *Eur Respir J.* (2017) 50:1–10. doi: 10.1183/13993003.00746-2016
29. Garzoni C, Brugger SD, Qi W, Wasmer S, Cusini A, Dumont P, et al. Microbial communities in the respiratory tract of patients with interstitial lung disease. *Thorax.* (2013) 68:1150–6. doi: 10.1136/thoraxjnl-2012-202917
30. Clarke EL, Lauder AP, Hofstaedter CE, Hwang Y, Fitzgerald AS, Imai I, et al. Microbial lineages in sarcoidosis a metagenomic analysis tailored for low-microbial content samples. *Am J Respir Crit Care Med.* (2018) 197:225–34. doi: 10.1164/rccm.201705-0891OC
31. Scher JU, Joshua V, Artacho A, Abdollahi-Roodsaz S, Öckinger J, Kullberg S et al. The lung microbiota in early rheumatoid arthritis and autoimmunity. *Microbiome.* (2016) 4:60. doi: 10.1186/s40168-016-0206-x
32. Madan JC, Koestle DC, Stanton BA, Davidson L, Moulton LA, Housman ML, et al. Serial analysis of the gut and respiratory microbiome in cystic fibrosis in infancy: interaction between intestinal and respiratory tracts and impact of nutritional exposures. *MBio.* (2012) 3:e00251-12. doi: 10.1128/mBio.00251-12
33. Denton CP, Khanna D. Systemic sclerosis. *Lancet.* (2017) 390:1685–99. doi: 10.1016/S0140-6736(17)30933-9
34. Andréasson K, Alrawi Z, Persson A, Jönsson G, Marsal J. Intestinal dysbiosis is common in systemic sclerosis and associated with gastrointestinal and extraintestinal features of disease. *Arthritis Res Ther.* (2016) 18:1–8. doi: 10.1186/s13075-016-1182-z
35. Caimmi C, Bertoldo E, Venturini A, Caramaschi P, Frulloni L, Ciccocioppo R, et al. Relationship between increased fecal calprotectin levels and interstitial lung disease in systemic sclerosis. *J Rheumatol.* (2019) 46:274–8. doi: 10.3899/jrheum.171445
36. Russell SL, Gold MJ, Reynolds LA, Willing BP, Dimitriu P, Thorson L, et al. Perinatal antibiotic-induced shifts in gut microbiota have differential effects on inflammatory lung diseases. *J Allergy Clin Immunol.* (2015) 135:100–9.e5. doi: 10.1016/j.jaci.2014.06.027
37. Huang YJ, Boushey HA. The microbiome in asthma. *J Allergy Clin Immunol.* (2015) 135:25–30. doi: 10.1016/j.jaci.2014.11.011
38. Kalliomäki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol.* (2001) 107:129–34. doi: 10.1067/mai.2001.111237
39. Russell SL, Gold MJ, Willing BP, Thorson L, McNagny KM, Finlay BB. Perinatal antibiotic treatment affects murine microbiota, immune responses and allergic asthma. *Gut Microbes.* (2013) 4:158–64. doi: 10.4161/gmic.23567
40. Russell SL, Gold MJ, Hartmann M, Willing BP, Thorson L, Wlodarska M, et al. Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. *EMBO Rep.* (2012) 13:440–7. doi: 10.1038/embor.2012.32
41. Schuijt TJ, Lankelma JM, Scicluna BP, de Sousa e Melo F, Roelofs JJTH, de Boer JD, et al. The gut microbiota plays a protective role in the host defence against pneumococcal pneumonia. *Gut.* (2016) 65:575–83. doi: 10.1136/gutjnl-2015-309728
42. Brown RL, Sequeira RP, Clarke TB. The microbiota protects against respiratory infection via GM-CSF signaling. *Nat Commun.* (2017) 8:1512. doi: 10.1038/s41467-017-01803-x
43. Gauguet S, D'Ortona S, Ahnger-Pier K, Duan B, Surana NK, Lu R, et al. Intestinal microbiota of mice influences resistance to *Staphylococcus aureus* pneumonia. *Infect Immun.* (2015) 83:4003–14. doi: 10.1128/IAI.00037-15
44. Rakoff-Nahoun S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. *Cell.* (2004) 118:229–41. doi: 10.1016/j.cell.2004.07.002
45. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell.* (2014) 157:121–41. doi: 10.1016/j.cell.2014.03.011
46. Östman S, Rask C, Wold AE, Hultkrantz S, Telemo E. Impaired regulatory T cell function in germ-free mice. *Eur J Immunol.* (2006) 36:2336–46. doi: 10.1002/eji.200535244
47. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y et al. Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science.* (2011) 331:337–41. doi: 10.1126/science.1198469
48. Worbs T, Bode U, Yan S, Hoffmann MW, Hintzen G, Bernhardt G, et al. Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *J Exp Med.* (2006) 203:519–27. doi: 10.1084/jem.20052016
49. Ivanov II, Frutos RDL, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, et al. Specific microbiota direct the differentiation of IL-17-Producing T-Helper cells in the mucosa of the small intestine. *Cell Host Microbe.* (2008) 4:337–49. doi: 10.1016/j.chom.2008.09.009
50. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell.* (2009) 139:485–98. doi: 10.1016/j.cell.2009.09.033
51. Chen LW, Chen PH, Hsu CM. Commensal microflora contribute to host defense against *Escherichia coli* pneumonia through toll-like receptors. *Shock.* (2011) 36:67–75. doi: 10.1097/SHK.0b013e3182184ee7
52. Clarke TB. Early innate immunity to bacterial infection in the lung is regulated systemically by the commensal microbiota via Nod-like receptor ligands. *Infect Immun.* (2014) 82:4596–606. doi: 10.1128/IAI.02212-14
53. Oh JZ, Ravindran R, Chassaing B, Carvalho FA, Maddur MS, Bower M, et al. TLR5-mediated sensing of gut microbiota is necessary for antibody responses to seasonal influenza vaccination. *Immunity.* (2014) 41:478–92. doi: 10.1016/j.immuni.2014.08.009
54. Ichinohe T, Pang IK, Kumamoto Y, Peaper DR, Ho JH, Murray TS, et al. Microbiota regulates immune defense against respiratory tract influenza a virus infection. *Proc Natl Acad Sci USA.* (2011) 108:5354–9. doi: 10.1073/pnas.1019378108
55. Samuelson DR, Welsh DA, Shellito JE. Regulation of lung immunity and host defense by the intestinal microbiota. *Front Microbiol.* (2015) 6:1085. doi: 10.3389/fmicb.2015.01085
56. Li M, van Esch BCAM, Wagenaar GTM, Garssen J, Folkerts G, Henricks PAJ. Pro- and anti-inflammatory effects of short chain fatty acids on immune and endothelial cells. *Eur J Pharmacol.* (2018) 831:52–9. doi: 10.1016/j.ejphar.2018.05.003
57. Kishino E, Takemura N, Masaki H, Ito T, Nakazawa M. Dietary lactosucrose suppresses influenza A (H1N1) virus infection in mice. *Biosci Microbiota Food Heal.* (2015) 34:67–76. doi: 10.12938/bmfh.2015-005
58. Vieira AT, Rocha VM, Tavares L, Garcia CC, Teixeira MM, Oliveira SC, et al. Control of *Klebsiella pneumoniae* pulmonary infection and immunomodulation by oral treatment with the commensal probiotic *Bifidobacterium longum* 51A. *Microbes Infect.* (2016) 18:180–9. doi: 10.1016/j.micinf.2015.10.008
59. Bernard H, Desseyn JL, Bartke N, Kleinjans L, Stahl B, Belzer C, et al. Dietary pectin-derived acidic oligosaccharides improve the pulmonary bacterial clearance of *Pseudomonas aeruginosa* lung infection in mice by modulating intestinal microbiota and immunity. *J Infect Dis.* (2015) 211:156–65. doi: 10.1093/infdis/jiu391
60. Tang WHW, Kitai T, Hazen SL. Gut microbiota in cardiovascular health and disease. *Circ Res.* (2017) 120:1183–96. doi: 10.1161/CIRCRESAHA.117.309715

61. Picchianti-Diamanti A, Rosado MM, D'Amelio R. Infectious agents and inflammation: the role of microbiota in autoimmune arthritis. *Front Microbiol.* (2018) 8:2696. doi: 10.3389/fmicb.2017.02696
62. Horta-Baas G, Romero-Figueroa MDS, Montiel-Jarquín AJ, Pizano-Zárate ML, García-Mena J, Ramírez-Durán N. Intestinal dysbiosis and rheumatoid arthritis: a link between gut microbiota and the pathogenesis of rheumatoid arthritis. *J Immunol Res.* (2017) 2017:4835189. doi: 10.1155/2017/4835189
63. Inaoka PT, Shono M, Kamada M, Espinoza JL. Host-microbe interactions in the pathogenesis and clinical course of sarcoidosis. *J Biomed Sci.* (2019) 26:45. doi: 10.1186/s12929-019-0537-6
64. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N Engl J Med.* (2013) 368:407–15. doi: 10.1056/NEJMoa1205037
65. Kang DW, Adams JB, Gregory AC, Borody T, Chittick L, Fasano A, et al. Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome.* (2017) 5:10. doi: 10.1186/s40168-016-0225-7
66. Kootte RS, Levin E, Salojärvi J, Smits LP, Hartstra AV, Udayappan SD, et al. Improvement of insulin sensitivity after lean donor feces in metabolic syndrome is driven by baseline intestinal microbiota composition. *Cell Metab.* (2017) 26:611–9.e6. doi: 10.1016/j.cmet.2017.09.008
67. Groves HT, Cuthbertson L, James P, Moffatt MF, Cox MJ, Tregoning JS. Respiratory disease following viral lung infection alters the murine gut microbiota. *Front Immunol.* (2018) 9:182. doi: 10.3389/fimmu.2018.00182
68. Wang J, Li F, Wei H, Lian ZX, Sun R, Tian Z. Respiratory influenza virus infection induces intestinal immune injury via microbiotamediated Th17 cell-dependent inflammation. *J Exp Med.* (2014) 211:2397–410. doi: 10.1084/jem.20140625
69. Gu S, Chen Y, Wu Z, Chen Y, Gao H, Lv L, et al. Alterations of the gut microbiota in patients with COVID-19 or H1N1 influenza. *Clin Infect Dis.* (2020) 71:2669–78. doi: 10.1093/cid/ciaa709
70. Sze MA, Tsuruta M, Yang SWJ, Oh Y, Man SFP, Hogg JC, et al. Changes in the bacterial microbiota in gut, blood, and lungs following acute LPS instillation into mice lungs. *PLoS ONE.* (2014) 9:e111228. doi: 10.1371/journal.pone.0111228
71. Ueda A, Oki M, Yanagi H, Ozawa H, Takagi A. Clinical characteristics of Legionella pneumonia diagnosed with Legionella urinary antigen test. *Tokai J Exp Clin Med.* (2016) 41:8–13.
72. Villapol S. Gastrointestinal symptoms associated with COVID-19: impact on the gut microbiome. *Transl Res.* (2020) 226:57–69. doi: 10.1016/j.trsl.2020.08.004

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.

frontiers in Medicine

ORIGINAL RESEARCH
published: 24 February 2021
doi: 10.3389/fmed.2021.595077

Unsupervised Clustering Reveals Sarcoidosis Phenotypes Marked by a Reduction in Lymphocytes Relate to Increased Inflammatory Activity on 18FDG-PET/CT

Christen Vagts[1†], Christian Ascoli[1†], Dustin R. Fraidenburg[1], Robert P. Baughman[2], Yue Huang[1], Russell Edafetanure-Ibeh[1], Samreen Ahmed[3], Benjamin Levin[4,5], Yang Lu[6], David L. Perkins[7], Patricia W. Finn[1] and Nadera J. Sweiss[1,3]*

[1] Division of Pulmonary, Critical Care, Sleep, and Allergy, Department of Medicine, University of Illinois at Chicago, Chicago, IL, United States, [2] Department of Internal Medicine, University of Cincinnati Medical Center, Cincinnati, OH, United States, [3] Division of Rheumatology, Department of Medicine, University of Illinois at Chicago, Chicago, IL, United States, [4] Division of Cardiology, Department of Medicine, College of Medicine, University of Illinois at Chicago, Chicago, IL, United States, [5] Jesse Brown VA Medical Center, Chicago, IL, United States, [6] Division of Diagnostic Imaging, Department of Nuclear Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, United States, [7] Division of Nephrology, Department of Medicine, University of Illinois at Chicago, Chicago, IL, United States

Introduction: Sarcoidosis is a T-helper cell mediated disease characterized by granulomatous inflammation. We posited that unsupervised clustering of various features in sarcoidosis would establish phenotypes associated with inflammatory activity measured by 18FDG-PET/CT. Our goal was to identify unique features capable of distinguishing clusters and subsequently examine the relationship with FDG avidity to substantiate their potential use as markers for sarcoidosis inflammation.

Methods: We performed a retrospective study of a diverse, but primarily African American, cohort of 58 subjects with biopsy proven sarcoidosis followed at the University of Illinois Bernie Mac Sarcoidosis Center and Center for Lung Health who underwent 18FDG-PET/CT scan. Demographic, therapeutic, radiographic, and laboratory data were utilized in unsupervised cluster analysis to identify sarcoidosis phenotypes. The association between clusters, their defining features, and quantitative measurements on 18FDG-PET/CT was determined. The relevance of these features as markers of 18FDG-PET/CT inflammatory activity was also investigated.

Results: Clustering determined three distinct phenotypes: (1) a predominantly African American cluster with chronic, quiescent disease, (2) a predominantly African American cluster with elevated conventional inflammatory markers, advanced pulmonary disease and extrathoracic involvement, and (3) a predominantly Caucasian cluster characterized by reduced lymphocyte counts and acute disease. In contrast to the chronic quiescent cluster, Clusters 2 and 3 were defined by significantly greater FDG avidity on 18FDG-PET/CT. Despite similarly increased inflammatory activity on 18FDG-PET/CT, Clusters 2, and 3 differed with regards to extrathoracic FDG avidity and circulating lymphocyte

OPEN ACCESS

Edited by:
Lorenzo Cavagna, Fondazione Ospedale San Matteo (IRCCS), Italy

Reviewed by:
Fabrizio Luppi, University of Milano Bicocca, Italy
Paola Rottoli, University of Siena, Italy
Alain Meyer, Hôpitaux Universitaires de Strasbourg, France

***Correspondence:**
Nadera J. Sweiss
nsweiss@uic.edu

†These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

Received: *15 August 2020*
Accepted: *22 January 2021*
Published: *24 February 2021*

Citation:
Vagts C, Ascoli C, Fraidenburg DR, Baughman RP, Huang Y, Edafetanure-Ibeh R, Ahmed S, Levin B, Lu Y, Perkins DL, Finn PW and Sweiss NJ (2021) Unsupervised Clustering Reveals Sarcoidosis Phenotypes Marked by a Reduction in Lymphocytes Relate to Increased Inflammatory Activity on 18FDG-PET/CT. Front. Med. 8:595077. doi: 10.3389/fmed.2021.595077

profiles, specifically CD4+ T-cells. Notably, absolute lymphocyte counts and CD4+ T-cell counts were found to predict 18FDG-PET/CT inflammatory activity by receiver operating curve analysis with a 69.2 and 73.42% area under the curve, respectively.

Conclusions: Utilizing cluster analysis, three distinct phenotypes of sarcoidosis were identified with significant variation in race, disease chronicity, and serologic markers of inflammation. These phenotypes displayed varying levels of circulating inflammatory cells. Additionally, reduction in lymphocytes, specifically CD4+ T-cells, was significantly related to activity on 18FDG-PET/CT. Though future studies are warranted, these findings suggest that peripheral lymphocyte counts may be considered a determinant of sarcoidosis phenotypes and an indicator of active inflammation on 18FDG-PET/CT.

Keywords: sarcoidosis, lymphopenia, 18FDG-PET/CT, immunopathogenesis, cluster analysis, phenotype

INTRODUCTION

Sarcoidosis is a heterogeneous multisystem disease characterized by granulomatous inflammation. While sarcoidosis affects a variety of races, African American women have the highest prevalence of disease and new cases most commonly are diagnosed in the 4th and 5th decades of life (1). Sarcoidosis has been characterized as a T-helper cell mediated disease (2). The ACCESS trial identified genetic risk factors for sarcoidosis development in various races, and some genes were linked to immune dysregulation and decreased lymphocytes in a Caucasian subset of this cohort (3, 4). Moreover, despite the lack of sarcoidosis specific thresholds, absolute lymphocyte counts $\leq$1.5 kcells/μL have been associated with disease activity and progression (5, 6). Peripheral depletion of CD4+, CD8+, and CD19+ T-cells has also been shown to be a characteristic of patients with severe sarcoidosis (7). Furthermore, lymphocyte gene expression is decreased in patients with severe disease and CD4+ T-cell exhaustion has been described as a manifestation of progressive disease (8, 9). Thus, further characterization of lymphopenia, as it relates to disease pathogenesis and inflammation, merits investigation within specific phenotypes of sarcoidosis.

Given the heterogeneous nature of sarcoidosis, establishing disease phenotypes is critical to identify common pathways that may further shed light on disease etiology and pathogenesis. Existing phenotypes in sarcoidosis have focused on subject and disease characteristics, to include radiographic staging, organ involvement, disease acuity, and need for treatment (10–12). Even though useful clinical tools have been proposed, the classification criteria for various clinical phenotypes of sarcoidosis are not well-standardized and criteria for research purposes are lacking (13). Cluster analysis techniques have been utilized in diseases that are difficult to classify, including other autoimmune and pulmonary diseases, and have yielded novel insights (14–18). To this end, a recent report utilized cluster analysis of a large predominantly Caucasian sarcoidosis cohort and identified 6 subsets of subjects that better predicted disease progression than classical analysis (19). A second study utilized a supervised hierarchical cluster analysis to identify phenotypes of sarcoidosis based on 18FDG-PET/CT (PET scan) uptake. Cluster and PET scan analyses provided a meticulous evaluation of organ involvement and a more precise evaluation of the extent of disease compared to traditional assessment (20). Cluster analysis has therefore proven helpful at identifying similarities of disease beyond conventional classification schemes which may have implications for treatment and prognostication.

PET scan is an emerging tool for assessing inflammation in sarcoidosis as well as other inflammatory and autoimmune disorders (21, 22). Uptake on PET scan, commonly defined as SUV >2.5, has been consistently associated with disease activity as well as pulmonary function in patients with sarcoidosis (23–25). The sensitivity of PET scan in identifying active inflammation ranges 89–100% and is overall higher when compared to traditional biomarkers (24). PET scan use has been evaluated in both acute and chronic disease, is superior at detecting ongoing inflammation in persistent disease, and shows promise in guiding treatment and prognosis (26–28). We posited the usefulness of PET scan as a tool in the absence of a biomarker specific enough to monitor inflammation in sarcoidosis (29). ATS guidelines support use of PET scan to assess extracardiac sarcoidosis, albeit with low quality of evidence, suggesting further research is needed to develop a disease specific biomarker with adequate sensitivity to serve as a more cost-effective assessment strategy (30).

Our goal was to identify unique phenotypes of sarcoidosis using an unsupervised cluster analysis in subjects with biopsy proven sarcoidosis in order to establish the relationship between these phenotypes and inflammatory activity, as measured by serologic markers of inflammation and avidity on 18FDG-PET/CT scan. Our postulate was that a parsimonious set of demographic features along with clinically relevant therapeutic, radiographic, and laboratory features would identify subgroups of subjects with sarcoidosis at risk of active disease characterized by high levels of inflammation.

METHODS

Subject Selection

Study approval was obtained through the University of Illinois at Chicago (UIC) institutional review board. Adult subjects 18 years of age and older followed in the Bernie Mac Sarcoidosis

Translational Advanced Research (STAR) Center at UIC with a history and tissue biopsy consistent with sarcoidosis, in accordance with ATS/ERS/WASOG criteria, were included (31). All subjects underwent a skull to thigh PET scan to evaluate clinically suspected metabolically active intrathoracic sarcoidosis, between December 2014 and June 2019. Subjects who had comorbid inflammatory disease that may result in increased metabolic activity on PET scan, including malignancy, connective tissue or autoimmune disease, or active infection were excluded. Subjects who did not have sufficient laboratory data available within 180 days prior or 30 days after the PET scan was completed were also excluded.

Data Collection

The electronic medical record was retrospectively reviewed to collect variables regarding subject demographics as well as clinically relevant therapeutic, radiographic, and laboratory data. In addition to sex and self-reported race, subject demographics included age, body mass index (BMI), and smoking status at time of PET scan. Time from diagnosis to PET scan was measured from the time of biopsy. Treatment at the time of PET scan was also abstracted from medical records and categorized into the following regimens (A) naïve or local treatment, (B) systemic corticosteroids, (C) non-steroidal immune modulator, (D) combination therapy with steroids, (E) combination therapy without steroids. Laboratory values obtained within 180 days prior to or 30 days after PET scan included complete blood counts, lymphocyte subsets, serologic markers of inflammation (ESR, CRP), and markers of extrathoracic organ involvement (complete metabolic panels and vitamin D levels). ACE levels at any point prior to the PET scan were also included (**Supplementary Data Sheet 1**).

PET Scan Interpretation

All 18FDG-PET/CT examinations at UIC were performed on a GE Discovery 690 FDG PET/CT scanner (GE Medical Systems, Milwaukee, WI). Dedicated PET scans from the skull base to the upper thighs were obtained 60–90 min after intravenous injection of 0.370–0.481 GBq of FDG. Image acquisition was performed using non-cardiac-gated technique with PET parameters as follows: 2 min/bed for the non-cardiac fields and 10 min/bed for fields covering the heart. Image acquisition was performed using non-cardiac-gated technique. CT scan was used for attenuation correction and parameters were as follows: 120 kV, 120 mAs, pitch 0.813, 16 × 1.5-mm collimation, slice thickness of 3 mm with an increment of 1.5 mm.

Utilizing the LIFEx radiomics software, assessment of individual PET scans in DICOM format was performed to standardize and extract functional parameter measurements in order to quantitate the burden of 18FDG-avid areas per subject with the "total metabolic tumor volume (MTV) protocol" (32). First, the maximum standardized uptake volume (SUV_{Max}) as well as the mean-SUV uptake in the liver were measured and together used to calculate the $SUV_{Background}$-to-SUV_{Max} ratio (SUV ratio). Liver mean-SUV uptake was calculated using a volume-of-interest of $3cm^3$ from the subject's right hepatic lobe. SUV ratio, shown to be a reliable measure for prognostication and treatment response, and not SUV_{Max}, was utilized to minimize observer variability and standardize PET scan acquisition and reconstruction protocols (33–36). To maximize specificity of identifying a positive PET scan, lesions with a SUV ratio ≥ 2 were considered positive for inflammatory activity related to sarcoidosis, whereas those with a SUV ratio < 2 were regarded as negative. This approach is similar to the use of PET scan to assess chemotherapy response in various lymphomas (35). Total Metabolic Volume (TMV), a measure of the volume of increased FDG activity in milliliters, and Total Lesion Glycolysis (TLG), the product of mean-SUV uptake with the volume of uptake, were then measured as they may better reflect overall inflammatory activity and measurement partitioned into intra- and extrathoracic compartments. Lesions included in these calculations required a minimum SUV ratio of 2, as previously noted, as well as and an SUV >40% of the SUV_{Max}. These thresholds were extrapolated for use in this study as they have been shown to improve accuracy of the metabolic tumor volume measurement in various malignancies (37–39). Activity captured by the LIFEx software was then corroborated with the original radiographic interpretation of the PET scan.

Statistical Analysis

All statistical analyses were performed with the R Statistical Environment (version 3.5.0) (40). A total of 22 subject phenotypic variables comprised of demographic, therapeutic, radiographic, and laboratory data abstracted from the electronic medical records were input as clustering features into the Modha-Spangler algorithm for mixed categorical (**Table 1**) and continuous (**Figure 1**) data in the *kamila* R-package to establish unsupervised sarcoidosis clusters. This algorithm seeks to effectively balance the contribution of continuous and categorical variables in an unsupervised fashion. In doing so, it adaptively selects the relative weight that simultaneously minimizes the within-cluster dispersion and maximizes the between-cluster dispersion for both the continuous and categorical variables (41). The Modha-Spangler framework was utilized to optimize the k-medoids algorithm PAM (partitioning around the medoids) using Gower's distance with default parameters and an optimal weight of 0.8182 by a brute-force search strategy (42). Determination of the ideal number of clusters by average silhouette width was performed utilizing the *pamk* function with criterion specifying a "krange" of 3–6 clusters given cohort heterogeneity and sample size (43).

Clustering features utilized in the algorithm were tested for significant differences between clusters with χ^2-test of independence for categorical data or via Kruskal-Wallis one-way analysis of variance for continuous data ($p \leq 0.05$ were considered statistically significant). *Post-hoc* analysis with Benjamini-Hochberg (BH) adjustment to account for the multiplicity problem that occurs with multiple comparisons was performed using Fisher's exact test for χ^2-tests or Dunn's test for Kruskal-Wallis tests and an adjusted $p < 0.1$ was pre-specified as significant. Correlations for continuous data were performed utilizing Spearman's rank correlation coefficient and considered significant if $p \leq 0.05$. To further investigate the probability of

TABLE 1 | Baseline categorical parameters utilized as clustering features of the UIC-Sarcoidosis cohort ($n = 58$).

Categorical parameters in clustering		Frequency (%)
Race	Caucasian	23 (39.66)
	African American	35 (60.34)
Sex	Male	21 (36.21)
	Female	36 (63.79)
Age	30–39 years	12 (20.69)
	40–49 years	18 (31.03)
	50–59 years	22 (37.93)
	60–69 years	6 (10.35)
Body Mass Index	Normal	11 (18.97)
	Overweight	10 (17.24)
	Class 1 Obesity	11 (18.97)
	Class 2 Obesity	16 (27.59)
	Class 3 Obesity	10 (17.24)
Smoking history	Never	28 (48.28)
	Former	24 (41.38)
	Current	6 (10.34)
Time from diagnosis to 18FDG-PET/CT	Quartile 1	17 (29.31)
	Quartile 2	14 (24.14)
	Quartile 3	13 (22.41)
	Quartile 4	14 (24.14)
Lung parenchyma on CT Chest (at 18FDG-PET/CT)	Normal	21 (36.84)
	Lung Nodules	16 (28.07)
	Consolidation/GGO	8 (14.04)
	Advanced	12 (21.05)
Treatment	A	15 (25.86)
	B	15 (25.86)
	C	6 (12.07)
	D	17 (29.31)
	E	4 (6.89)
Angiotensin converting enzyme level (Ever Elevated)	No	38 (65.52)
	Yes	20 (34.49)

Prevalence of individual parameters is depicted as frequencies and percentages. 18FDG-PET/CT, positron emission tomography with 2-deoxy-2-fluorine-18-fluoro-D-glucose with integrated computed tomography; CT, computed tomography; GGO, ground glass opacities. Time from diagnosis to 18FDG-PET/CT ranged from 0 to 27 years and quartile distribution was as follows: 1 = 0–3 years; 2 = 4–6 years; 3 = 6–10 years; 4 ≥ 11 years. Treatment regimens at time of 18FDG-PET/CT: A = naïve or local therapy; B = systemic corticosteroid alone; C = non-steroidal immune modulator alone; D = combination therapy with corticosteroid; E = combination therapy without corticosteroid.

PET-positivity based on cluster membership a logistic regression model was constructed and odds ratios calculated.

Receiver operator characteristic (ROC) analysis with 1,000 stratified bootstrap replicates and 95% confidence intervals was performed on the cohort using the *pROC* R-package to calculate the predictive accuracy of specific cell counts with regards to determining sarcoidosis inflammatory activity on PET scan. Threshold values for specific cell counts were determined utilizing the *pROC* package's *coords* function, and the optimal numerical threshold for each cell type was calculated by maximizing sensitivities and specificities as determined by Youden's J statistic (Youden's index) (44).

RESULTS

There were 58 subjects identified who had a PET scan and laboratory values within the time constraints. Biopsies were predominantly obtained from lung (44.8%), lymph node (32.8%), and liver (8.6%) tissue. Thirty-five (60.34%) subjects were African American and 36 (63.79%) were women; however, the proportion of men and women within African American subjects was comparable to that of Caucasians in our cohort (χ^2-test $p = 0.3503$). Most subjects were between 50 and 59 years of age (37.93%), though 31.03% were between 40 and 49 years and 20.69% were between age 30 and 39. There were no subjects under age 30. Time from diagnosis to PET scan, ranging from 0 to 27 years, was utilized to assess disease chronicity and distributed into quartiles. In total, 29.31% of subjects were included in quartile 1 (0–3 years), 24.14% in quartile 2 (3–6 years), 22.41% in quartile 3 (7–10 years), and 24.14% in quartile 4 (≥11 years). The most common treatment regimen was combination therapy with corticosteroid (regimen D; 17 of 58, 29.31%), though 25.86% of subjects were on corticosteroids alone (regimen B) and another 25.86% of subjects were treatment naïve (regimen A). Though there was variability in treatment regimens between subjects, individual doses and medications remained stable within the study period for each subject. Other variables, to include BMI, smoking history, lung parenchyma characteristics, and treatment are outlined in **Table 1**. Laboratory values utilized in clustering are summarized in **Figure 1**. Absolute neutrophil values in the cohort were normal while the median absolute lymphocyte count was 1.5 kcells/μL (interquartile range from 1.0 to 2.1 kcells/μL) which coincides with previously described lymphopenia defining thresholds in the general population and sarcoidosis (5, 6, 45, 46). Absolute neutrophil counts were found to be affected by treatment regimen (KW-test $p = 0.0255$); however, upon adjustment for multiple comparisons this difference was found to be specific for subjects on regimen B when compared to those on regimen A (median 5.5 vs. 3.1 kcells/μL, respectively; Dunn's test $p = 0.0362$). In contrast, subjects who were treatment naïve had a comparable prevalence of lymphocyte reduction (≤1.5 kcells/μL) to those on treatment (53.33 vs. 53.49%, respectively; χ^2-test $p = 0.9917$). Multivariate regression analysis was performed to further assess the relationship between inflammatory cells and variables considered likely to alter their quantity (sex, race, age, BMI, smoking history, chronicity of disease, and treatment regimen) and did not identify significant associations ($p > 0.05$). Otherwise, serologic markers of inflammation were mostly within the standard reference ranges with few outliers.

Using 22 independent variables as described in **Table 1**, **Figure 1**, 3 clusters were identified (**Figure 2**). Characteristics of each cluster are listed in **Table 2**. Significant features that defined clusters included race, disease acuity, treatment, lung parenchyma, various laboratory values reflecting extrathoracic

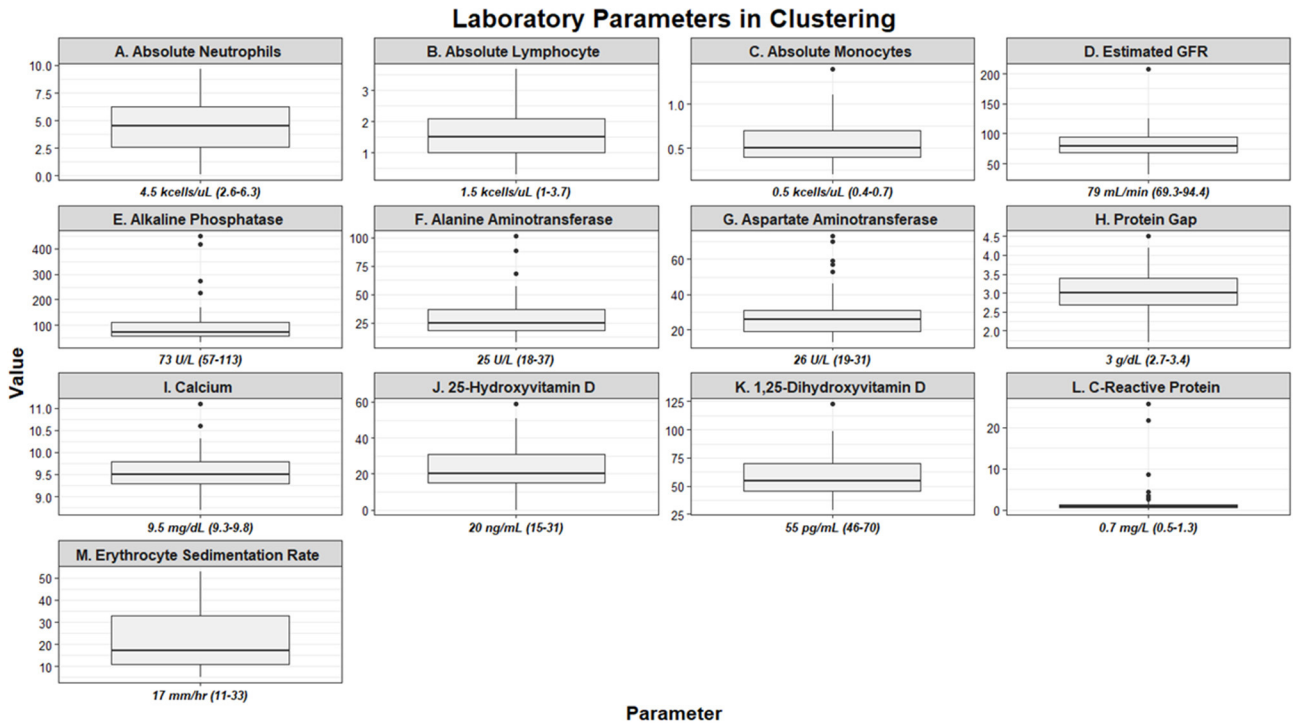


FIGURE 1 | Box and whisker plots demonstrating baseline laboratory parameters utilized as clustering features of the UIC-Sarcoidosis cohort ($n = 58$). Values are depicted as median and interquartile ranges for each individual parameter.

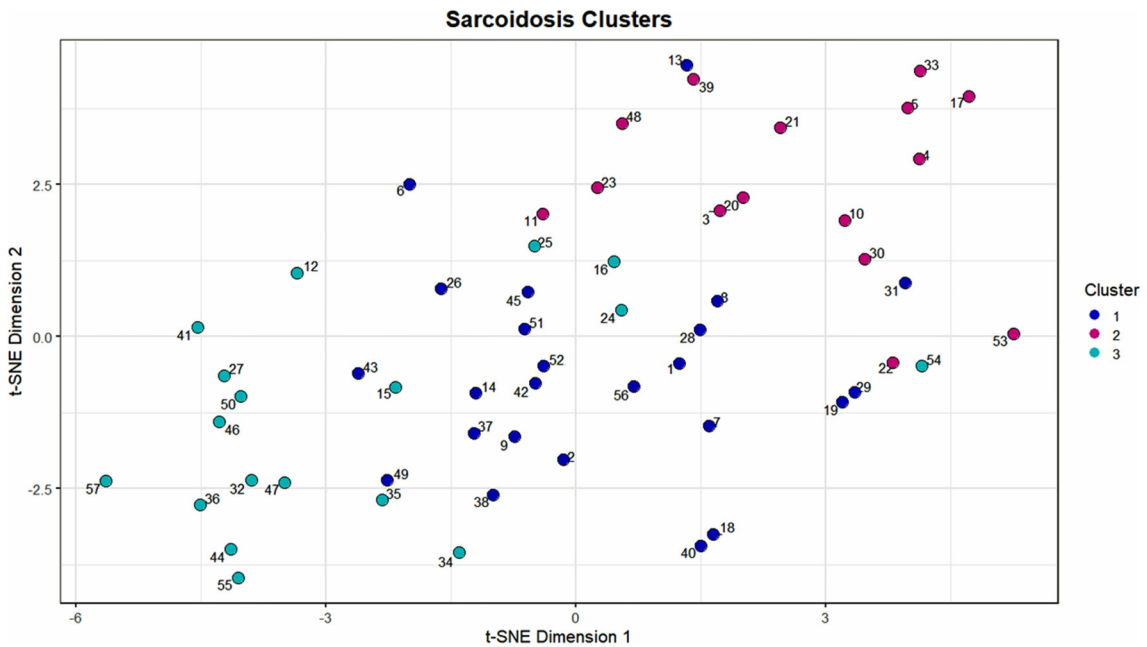


FIGURE 2 | Scatterplot visualization of the UIC-Sarcoidosis cohort over 2 dimensions utilizing the t-stochastic neighbor embedding (t-SNE) dimension reduction algorithm. The first and second dimensions are represented by the x-axis and y-axis, respectively. Points on the scatterplot represent individual subjects in the UIC-Sarcoidosis cohort and the distance between points is indicative of dissimilarity between subjects. Colors represent clusters identified by the Modha-Spangler algorithm utilizing partitioning around medoids with Gower's distance for base clustering of mixed data. Overall, Cluster 1 was identified as the largest cluster and comprised 41.38% of the cohort (24/58 subjects). Cluster 2 and 3 represented 25.86% (15/58) and 32.76% (19/58) of the cohort, respectively.

TABLE 2 | Characteristics of UIC-Sarcoidosis cohort clusters.

Cluster characteristics		Cluster 1	Cluster 2	Cluster 3	*P*-value
Race	Caucasian	7	2	14	0.0007 (β, γ)
	African American	17	13	5	
Sex	Male	6	4	11	0.056 (t)
	Female	18	11	8	
Age	30–39 years	3	2	7	NS
	40–49 years	7	5	6	
	50–59 years	12	7	3	
	60–69 years	2	1	3	
Body Mass Index	Normal	3	2	6	NS
	Overweight	5	3	2	
	Class 1 Obesity	2	6	3	
	Class 2 Obesity	10	2	4	
	Class 3 Obesity	4	2	4	
Smoking history	Never	12	7	9	NS
	Former	11	5	8	
	Current	1	3	2	
Time from diagnosis to 18FDG-PET/CT	Quartile 1	1	4	12	0.0004 (α, β)
	Quartile 2	9	2	3	
	Quartile 3	4	6	3	
	Quartile 4	10	3	1	
Lung parenchyma on CT Chest (at 18FDG-PET/CT)	Normal	14	2	6	0.0010 (α, β, γ)
	Lung nodules	4	5	7	
	Consolidation/GGO	1	1	6	
	Advanced	5	7	0	
Treatment	A	9	2	4	0.0434 (α, β)
	B	2	4	9	
	C	5	0	2	
	D	7	7	3	
	E	1	2	1	
Angiotensin converting enzyme level (Ever Elevated)	No	18	4	16	0.0010 (α, γ)
	Yes	6	11	3	
Estimated GFR (mL/min)	Median (25th-75th Percentile)	87.45 (78.0–96.4)	75.7 (68.3–82.6)	74.7 (67.4–92.9)	0.0853 (t)
Alkaline phosphatase (U/L)		76 (66.0–108.5)	117 (65.0–196.0)	58 (48.5–65.5)	0.0010 (β,γ)
Alanine aminotransferase (U/L)		23.5 (17.0–31.0)	31 (21.5–54.5)	23 (18.0–35.0)	0.0683 (t)
Aspartate aminotransferase (U/L)		26.5 (20.8–30.0)	30 (25.0–51.5)	19 (15.5–25.0)	0.0021 (β,γ)
Protein Gap (g/dL)		3 (2.7–3.5)	3.3 (3.1–3.9)	3 (2.4–3.1)	0.0131 (α,γ)
Calcium (mg/dL)		9.5 (9.3–9.8)	9.5 (9.3–9.7)	9.7 (9.4–9.8)	NS
25-Hydroxyvitamin D (ng/mL)		25 (17.8–37.5)	15 (11.5–22.0)	20 (16.0–31.0)	0.0213 (α,γ)
1, 25-Dihydroxyvitamin D (pg/mL)		57.1 (48.7–72.2)	54.8 (46.5–61.8)	52.5 (39.2–77.4)	NS
C-Reactive protein (mg/L)		0.7 (0.5–1.0)	1.1 (0.6–2.4)	06 (0.5–0.95)	NS
Erythrocyte sedimentation rate (mm/hr)		15.5 (11.8–22.8)	40 (31.0–44.5)	14 (7.5–16.0)	<0.0001 (α,β,γ)

Numeric values for categorical variables denote frequencies and percentages. Data for continuous variables are shown as median and interquartile ranges. Comparison of features between clusters was performed utilizing χ^2-test of independence for categorical and Kruskal-Wallis test for continuous variables. A $p < 0.05$ was considered significant. Post-hoc analysis with Benjamini-Hochberg (BH) adjustment for multiple comparisons was performed with Fisher's exact test for χ^2-tests and Dunn's test for Kruskal-Wallis tests. α, β, γ denote BH-adjusted $p < 0.1$; α, comparison between Cluster 1 and Cluster 2; β, comparison between Cluster 1 and Cluster 3 and γ, comparison between Cluster 2 and Cluster 3. Quartiles and treatment regimens as per ***Table 1****. NS, non-significant; t, trend.*

organ involvement, and serologic markers of inflammation. Cluster 1 and 2 consisted mostly of African American subjects (70.83 and 86.67%, respectively) while only 26.32% of subjects in Cluster 3 were African American (KW-test $p = 0.0007$). Subjects in Clusters 1 and 2 were mostly women (75.00 and 73.33%, respectively) while most subjects in Cluster 3 were men (57.9%); however, despite differences only a trend toward significance was noted (KW-test $p = 0.0560$). Cluster 1 had a considerably high number of subjects with PET scans performed $\geq$ 11 years from diagnosis and less subjects with

acute disease (4.00%), while 78.94% of subjects in Cluster 3 were considered to have acute disease (KW-test $p = 0.0004$). Cluster 1 also consisted of more subjects with normal lung parenchyma on CT portion of the PET scan (77.78%) while Cluster 2 had more advanced disease (46.67%), reflected by moderate-severe emphysema and extensive fibrosis, and Cluster 3 had more lung nodules (36.85%) and either consolidation or ground glass opacities (36.84%). Cluster 1 included more treatment-naïve subjects whereas Cluster 3 had the most subjects on corticosteroid monotherapy (regimen B). Rate of any corticosteroid use (monotherapy or in combination) was highest in Cluster 2, and although a trend indicating possible difference in corticosteroid use was noted, statistical significance was not reached (χ^2-test $p = 0.0633$). Cluster 2 also had more variation in laboratory values suggestive of extrathoracic organ involvement than Clusters 1 and 3, as evidenced by more elevated alkaline phosphatase (median 117 U/L, range 65.0–196.0 U/L; KW-test $p = 0.0010$), aspartate aminotransferase (median 30 U/L, range 25.0–51.5 U/L; KW-test $p = 0.0021$), and protein gap (median 3.3 g/dL, range 3.1–3.9 g/dL; KW-test $p = 0.0131$). There was no significant difference in kidney function between clusters, though there was a trend toward lower GFR in Clusters 2 and 3 than Cluster 1(KW-test $p = 0.0853$). Cluster 2 also had more subjects with abnormal serologic markers of inflammation, which included more subjects with a historically elevated ACE level (73.3%; χ^2-test $p = 0.0010$) and a decreased 25-OH Vitamin D (median value 15.0 ng/mL; KW-test $p = 0.0213$). Variations in circulating inflammatory cells were evaluated across clusters with significant differences found in absolute neutrophil and absolute lymphocyte counts (**Figure 3**). In general, Cluster 1 had less abnormalities in inflammatory cell counts. Subjects in Cluster 3 had significantly higher absolute neutrophils than Cluster 1 but were not statistically different from Cluster 2 (KW-test $p = 0.0181$, median values 5.5, 3.8, and 5.2 kcells/μL, respectively). Subjects in Cluster 3 also had significantly lower absolute lymphocytes than Cluster 1 but were comparable to Cluster 2 (KW-test $p = 0.0253$, median values 1.0, 1.7, and 1.4 kcells/μL, respectively). In total, 68.42% (13/19) of subjects in Cluster 3 were found to have absolute lymphocyte counts ≤1.5 kcells/μL and among these 76.92% (10/13) had more evident reductions with counts ≤1.0 kcells/μL. Whereas, in Cluster 2, a smaller proportion of subjects (10/15) had absolute lymphocyte counts ≤1.5 kcells/μL of which only 30% (3/10) were found to have counts ≤1.0 kcells/μL. Ultimately, comparison of clusters allowed the identification of 3 phenotypic clusters: (1) a more chronic, quiescent cluster, (2) an inflammatory extrathoracic cluster with advanced pulmonary disease, and (3) a cluster with acute disease and markedly reduced lymphocyte counts.

With the significant differences observed in absolute lymphocyte counts between clusters, associations between cluster and the CD4+ T-cell lymphocyte subset were further

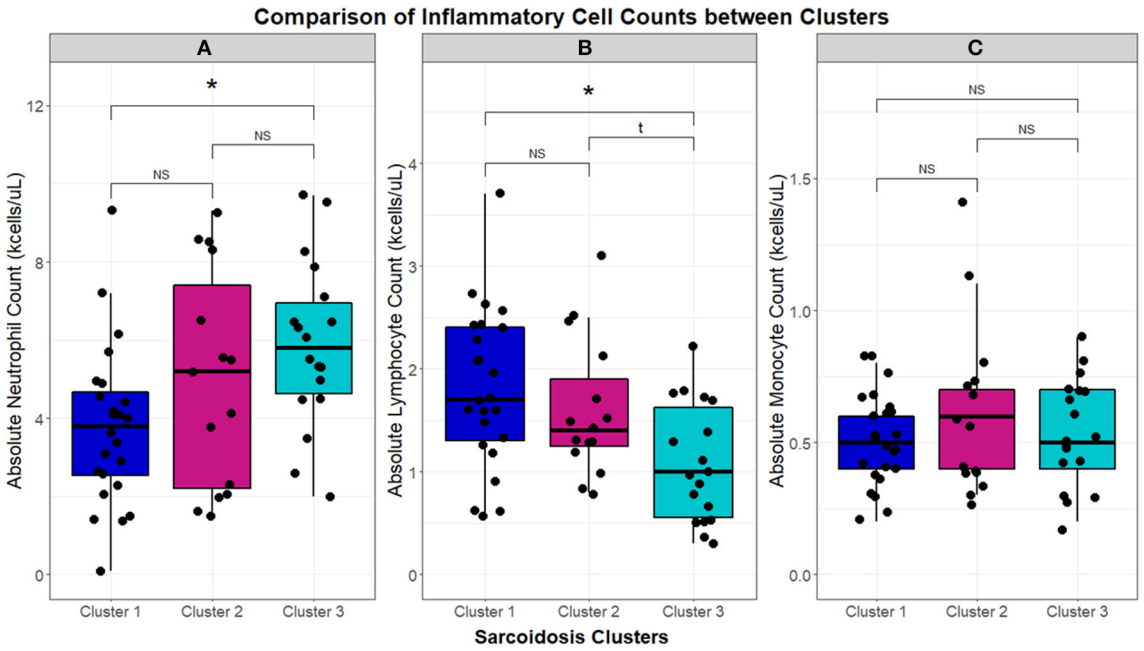


FIGURE 3 | Box and whisker plots demonstrating median and interquartile ranges (kcells/μL) of circulating inflammatory cells in the UIC-Sarcoidosis cohort clusters. Comparisons between clusters with Kruskal-Wallis tests were performed and, if significant ($p < 0.05$), were followed by *post-hoc* analysis with Dunn's test and Benjamini-Hochberg (BH) adjustment for multiple comparisons. Kruskal-Wallis test significance is displayed in the corresponding panel and significance of Dunn's test, denoted with an asterisk (*), is displayed above the brackets linking respective box and whisker plots. Specifically, neutrophils **(A)** were found significantly elevated in Cluster 3 compared to Cluster 1 (BH-adjusted $p = 0.0141$). Conversely, lymphocytes **(B)** were found significantly reduced in Cluster 3 compared to Cluster 1 (BH-adjusted $p = 0.0207$). Neutrophil and lymphocyte counts between Cluster 1 and Cluster 2 and between Cluster 2 and Cluster 3 were comparable. Monocytes **(C)** did not show any significant differences between clusters. NS, non-significant.

evaluated (**Figure 4**). CD4+ T-cells correlated with absolute lymphocytes across the UIC-Sarcoidosis cohort (Spearman's rho = 0.8329, $p < 0.0001$). Between the clusters however, subjects in Cluster 3 had significantly lower CD4+ T-cells than subjects in both the chronic cluster (Cluster 1) and inflammatory extrathoracic-advanced cluster (Cluster 2), (KW-Test $p = 0.0008$, median values 421, 818, and 483 cells/μL, respectively). Interestingly, CD4+ T-cell counts in both Clusters 2 and 3 were found to be decreased in relation to reference ranges in the healthy population and sarcoidosis (7, 47, 48). CD4+ T-cells did not vary significantly between the chronic and advanced phenotypes.

With phenotypes supporting variable chronicity, disease location, and levels of inflammation, we proceeded to evaluate the association of these phenotypes with PET scan activity. **Figure 5** describes the differences in SUV ratio between clusters. Notably, the inflammatory extrathoracic-advanced phenotype (Cluster 2) and acute-markedly reduced lymphocyte phenotype (Cluster 3) had significantly higher SUV ratios than Cluster 1. With a median SUV ratio below 2, Cluster 1 was mostly comprised of subjects with negative PET scans. Conversely, Cluster 2 and 3 both had median SUV ratios ≥2 suggesting more subjects had positive PET scans (odds ratio 6.00 and 6.50, respectively). SUV ratios did not vary between Clusters 2 and 3.

As both the inflammatory extrathoracic-advanced phenotype (Cluster 2) and the acute-markedly reduced lymphocyte phenotype (Cluster 3) had high rates of PET avidity, we further investigated the relationship between inflammatory cells and PET positivity in the entire cohort. Receiver operator characteristic (ROC) analysis demonstrating the sensitivity and specificity of absolute neutrophils, absolute lymphocytes, and CD4+ T-cells as predictors of sarcoidosis inflammatory activity on PET scan are shown in **Figure 6**. Absolute lymphocytes and CD4+ T-cells predict PET positivity with an AUC of 69.20% and 73.42%. There was no difference between their AUC ($p = 0.36$) and both represent strong associations with increased PET avidity and had significantly greater AUC than the absolute neutrophils ($p = 0.0223$ for lymphocytes and $p = 0.0029$ for CD4+ T-cells). Optimal cell count thresholds for the UIC-Sarcoidosis cohort, obtained with "Youden's index," suggest that absolute lymphocyte counts ≤1.25 kcells/μL and CD4+ T-cell counts ≤524.5 cells/μL are associated with PET scan positivity with median sensitivity of 51.72 and 68.97% and a median specificity of 82.76 and 72.41%, respectively.

To further quantify the inflammatory activity on PET scans, we next assessed TMV and TLG, to compare FDG more stringently between phenotypes (**Figure 7**). The chronic-quiescent phenotype (Cluster 1) had near normal TMV and TLG values suggesting minimal, if any, FDG avidity and were therefore essentially negative PET scans. This was consistent with the SUV ratio < 2 and is therefore not surprising. These values were also significantly lower than the other groups. The inflammatory extrathoracic-advanced cluster (Cluster 2) and the acute-markedly reduced lymphocyte cluster (Cluster 3) had significantly higher TMV and TLG suggesting more sarcoidosis related inflammatory activity (KW-test $p = 0.01397$). PET avidity

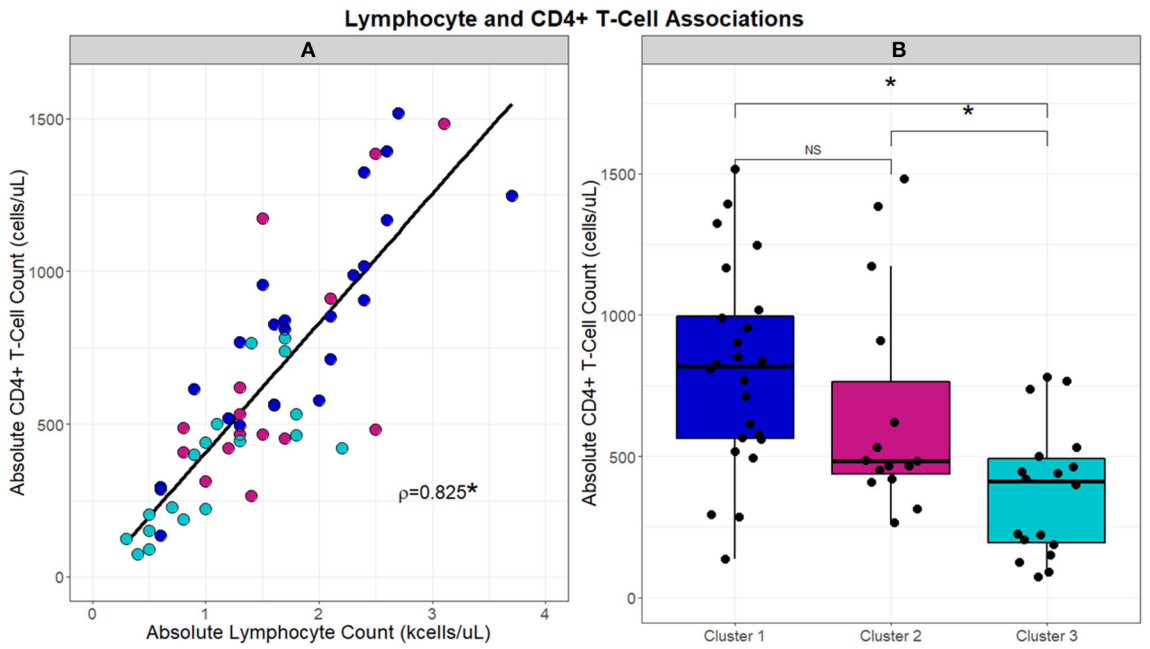


FIGURE 4 | Scatterplot in **(A)** demonstrates the strong direct relationship (Spearman's correlation coefficient; rho = 0.8329, $p < 0.001$) that is observed between absolute lymphocyte counts (kcells/μL) and absolute CD4+ T-cells (cells/μL). Box and whisker plots in **(B)** highlight the differences in absolute CD4+ T-cells (cells/μL) that are observed between clusters. A significant association was identified by Kruskal-Wallis test between clusters and absolute CD4+ T-cells ($p = 0.0008$). *Post-hoc* analysis with Dunn's test demonstrated significantly reduced absolute CD4+ T-cell counts, denoted with an asterisk (*), in Cluster 3 compared to Clusters 1 and 2 (BH-adjusted $p = 0.0005$ and 0.0929, respectively). No difference (NS) in absolute CD4+ T-cell counts was observed between Clusters 1 and 2.

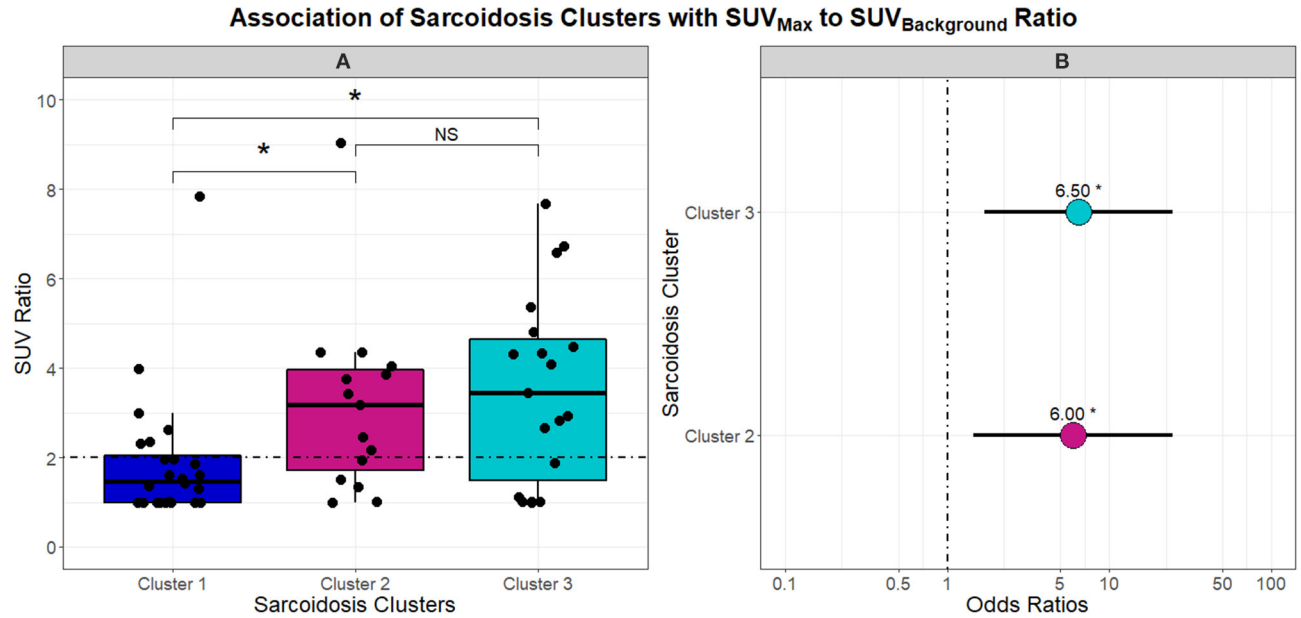


FIGURE 5 | Box and whisker plots in **(A)** demonstrate the association between UIC-Sarcoidosis cohort clusters and the $SUV_{Background}$-to-SUV_{Max} ratio (SUV ratio). An SUV ratio of 2 was pre-specified as a cutoff value to indicate increased inflammatory activity on 18FDG-PET/CT (PET-positivity) and is depicted in **(A)** as the dashed black horizontal line. A significant association between clusters and SUV ratio was found by Kruskal-Wallis test ($p = 0.0052$). *Post-hoc* analysis with Dunn's test demonstrated significantly elevated inflammatory activity, denoted with an asterisk (*), in Clusters 2 and 3 compared to Cluster 1 (BH-adjusted $p = 0.0332$ and 0.0136, respectively). No difference (NS) was observed between Cluster 2 and Cluster 3 with regards to inflammatory activity. To assess the probability of PET-positivity based on clusters, a logistic regression model was constructed and the dot plot in **(B)** highlights the odds ratios derived from the model. Cluster 1 was considered the reference given that it demonstrated significantly decreased 18FDG-PET/CT activity compared to the other two clusters. Ultimately, Cluster 2 and Cluster 3 were found to have a 6 and 6.5-fold greater risk of PET positivity ($\beta = 1.7918$ and 1.8718 and $p = 0.0132$ and 0.0061; respectively).

was further classified into intra and extra-thoracic activity to confirm true extrathoracic involvement in Cluster 2 as suggested by laboratory values. There was no significant difference between clusters in regards to intrathoracic metabolic volume (MV) and lesion glycolysis (LG) (**Figures 7C,D**, respectively); however, Cluster 2 had significantly higher extrathoracic MV (**Figure 7E**) and LG (**Figure 7F**) than both Cluster 1 and Cluster 3 (BH-*adj p* = 0.059 and BH-*adj p* = 0.055, respectively). When comparing the phenotypes identified via cluster analysis with PET avidity, Cluster 1 is consistently quiescent while Cluster 2 and 3 are both hyperinflammatory; Cluster 2 is also confirmed to have more extrathoracic disease.

DISCUSSION

We have identified phenotypes of sarcoidosis in a diverse tertiary sarcoidosis referral center using unsupervised cluster analysis. We also examined the association between these phenotypes and the extent of sarcoidosis related inflammation. Our unbiased approach yielded 3 distinct clusters, (A) a predominant African American group of treatment naïve subjects with chronic sarcoidosis and minimal inflammatory activity on laboratory and PET scan values; (B) a predominant African American group of subjects with varying disease acuity requiring treatment, with advanced pulmonary parenchymal changes on CT and inflammation more extrathoracically located as evidenced by laboratory and PET scan values; and (C) a predominant Caucasian group of subjects with acute disease requiring treatment, with significant PET avidity that correlates with more significant absolute lymphocyte and CD4+ T-cell reduction. Dissimilarities observed between clusters (summarized in **Table 3**) attest to the variable immunogenicity and acuity of sarcoidosis. These findings underscore disparities in disease severity associated with race and to a degree, sex, that have previously been described (1). Prior phenotypic studies support the use of cluster analysis as an approach to identify clinical patterns and dissimilarities among large cohorts (16).

With significantly lower 18-FDG avidity, Cluster 1 identifies subjects with likely inactive sarcoidosis. Conversely, Clusters 2 and 3 are characterized by more subjects with positive PET scans and substantially greater TMV and TLG, consistent with more inflammatory activity and therefore active disease. Using the AUC of the ROC as an integrated measure of test performance, CD4+ T-cell count best predicted increased PET scan activity, though was not statistically superior to the absolute lymphocyte count threshold of 1.25 kcells/μL in our cohort. Notwithstanding, our independent analysis determined that a CD4+ T-cell count of $\leq$ 524.5 cells/μL is related to PET positivity and note that this was comparable to threshold levels previously utilized to assess major organ involvement in sarcoidosis (7). Despite the need for external validation, we conclude that absolute CD4+

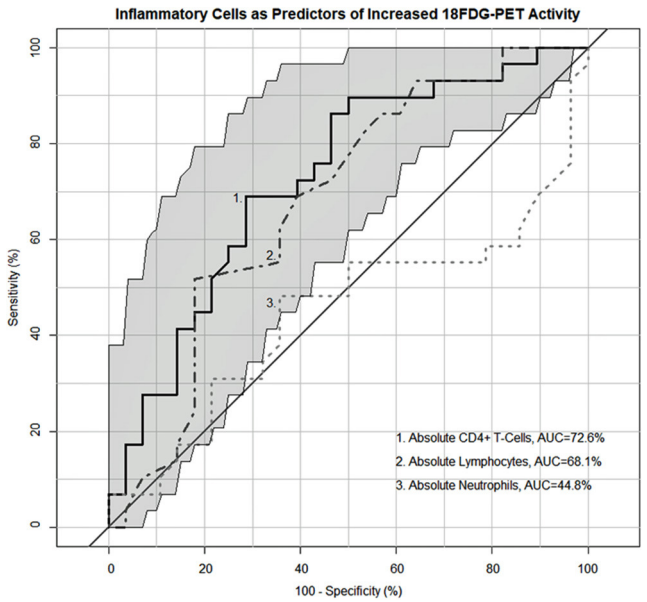


FIGURE 6 | Receiver operating characteristic (ROC) curves demonstrating the sensitivity and specificity of inflammatory cells as predictors of PET-positivity defined as an SUV ratio > 2. Neutrophils (kcells/μL) demonstrated an area under the curve (AUC) of 45.78% whereas lymphocytes (kcells/μL) and CD4+ T-cells (cells/μL) demonstrated a 69.2 and 73.42% AUC, respectively. No difference was identified between the lymphocyte and CD4+ T-cell AUC utilizing the "bootstrap" method with 1,000 replicates ($p = 0.36$). Both lymphocytes and CD4+ T-cells demonstrated a significantly greater AUC compared to neutrophils ($p = 0.0223$ and 0.0029, respectively). Optimal cell count thresholds obtained with "Youden's index" suggest that lymphocyte counts ≤ 1.25 kcells/μL in the UIC-Sarcoidosis cohort are most indicative of PET-positivity with a median sensitivity of 51.72% (95%CI: 34.48–68.97) and a median specificity of 82.76% (95% CI: 68.97–93.1). Similarly, CD4+ T-cell counts ≤ 524.5 cells/μL were most indicative of PET-positivity with a median sensitivity of 68.97% (95%CI: 51.72–82.76) and a median specificity of 72.41% (95% CI: 55.17–89.66).

T-cell counts, or absolute lymphocyte counts in lieu of CD4+ T-cell enumeration, may serve as a predictor of sarcoidosis inflammatory activity.

Notably, in our cohort, while the quiescent phenotype was more treatment naïve, all phenotypes had similar rates of corticosteroid use, in combination with other immunomodulators or as monotherapy, and consequently were not considered to significantly influence the degree of lymphocyte reduction across clusters. As sarcoidosis is predominantly a T-helper cell mediated disease, mitigation of inflammation with use of corticosteroids, antimalarials, TNF-α antagonists, among others, has been a mainstay of treatment (49). Phenotype identification has the potential to guide therapy as has been previously shown in successful treatment of patients with sarcoidosis and CD4+ T-cell lymphopenia with the TNF-α antagonist, infliximab (50). Furthermore, use of PET scan has been described as an effective way to assess treatment response (26, 27). Thus, absolute lymphocyte counts and corresponding trends may be considered a useful parameter for monitoring therapy in addition to a surrogate for active inflammation in sarcoidosis.

A further comparison between Clusters 2 and 3 shows both clusters contain subjects with a reduction in the absolute lymphocyte count. However, the degree of reduction was only significant in the Caucasian/acute cluster and further characterized by a corresponding reduction in CD4+ T-cell counts. While we suspect immune dysregulation inherent to sarcoidosis drives peripheral lymphocyte depletion in this cluster, the effect seems to be present primarily in Caucasians, which is consistent with prior gene studies performed on the ACCESS cohort which was notably also predominantly Caucasian (4). Conversely, African Americans belonging to Cluster 3 did not have statistically lower absolute lymphocyte or CD4+ T-cell counts when compared to African Americans in other clusters. However, our cohort was comprised of only a small number of African Americans with acute disease and is a factor that limits full examination of this aspect and should be investigated in subsequent studies. Cluster 2 was characterized by more extrathoracic PET avidity, which is evident in the abnormalities in liver function tests and inflammatory markers. This group also had relatively more normal lymphocyte counts in comparison to Cluster 3, but overall still exhibited decreased absolute lymphocyte and CD4+ T-cell counts. As Cluster 2 was predominantly African American, there were too few Caucasians in this cluster to effectively assess features that distinguish Caucasians in this cluster from Caucasians in other clusters. Nonetheless, our findings suggest that race may influence immunotypes and underscores the importance of health disparities research in sarcoidosis.

Other than differences in lymphocytes, disease chronicity and radiographic findings on CT were also cluster defining features. Subjects in Cluster 1 had more chronic disease and more normal findings on CT, suggesting this cluster represents patients with resolved sarcoidosis. While Cluster 1 was predominantly African American, these findings were consistent when comparing Caucasians across clusters. Additionally, subjects in Cluster 2 had a higher frequency of advanced pulmonary parenchymal disease which may also be reflective of chronic active disease while subjects in Cluster 3 had more acute disease. Taken together these findings imply interdependence between disease chronicity and immunogenicity.

Despite our significant findings, there are several limitations to this study that should be the focus of future works. First, our study was limited by its retrospective design which did not allow full clinical assessment, such as specific organ involvement, at the time of PET scan and precluded the use of predictive tools such as the WASOG organ assessment instrument in the clustering algorithm which may have contributed to improved cluster determination and increased cluster uniformity (13). As with clinical data, acquisition of laboratory data relating to non-CD4+ T-cells was limited by the retrospective design and would have allowed better characterization of each proposed immunotype (7, 51–54). Additionally, although our cohort is representative of the predominantly African American population we serve and strengthened by strict inclusion criteria, sample size and heterogeneity limited our analysis. Consequently, determination of between cluster dissimilarities, particularly in relation to race and disease chronicity, was not possible. Notably, extrathoracic

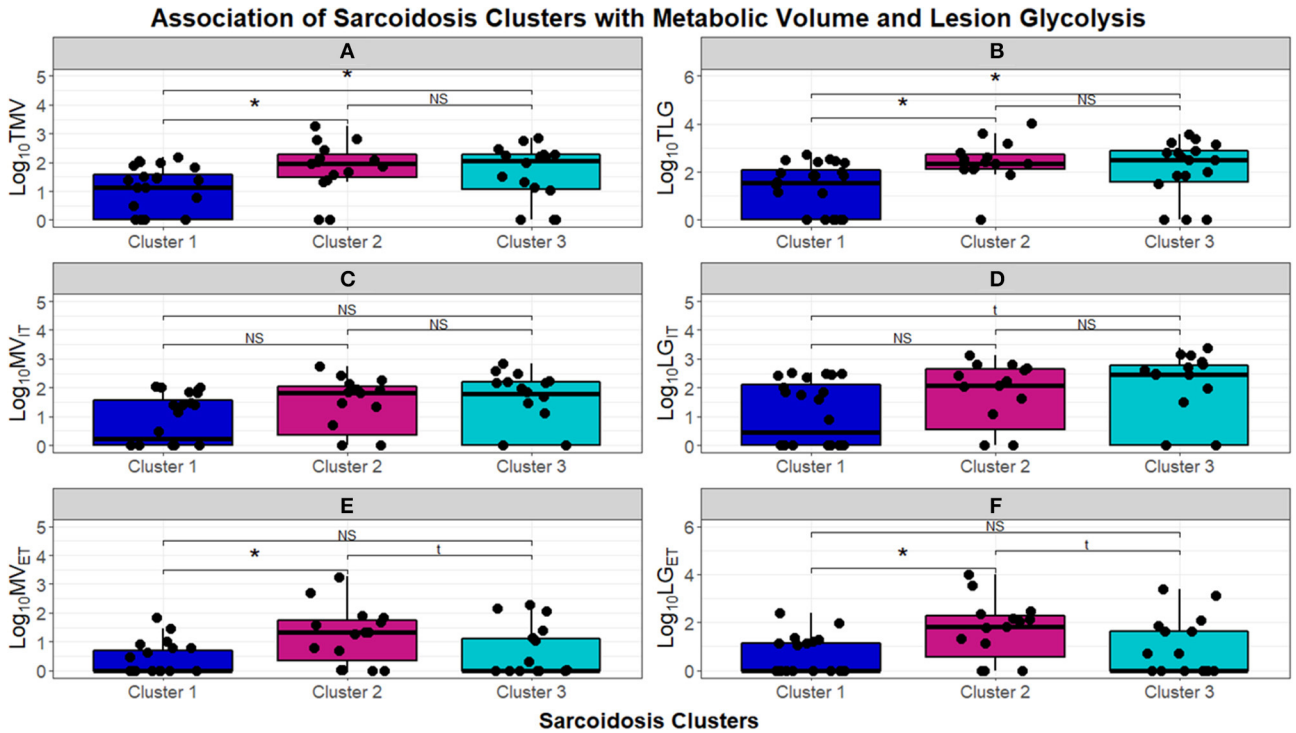


FIGURE 7 | Box and whisker plots demonstrating $\log_{10}$ normalized median and interquartile ranges of radiomic measurements performed to assess burden of inflammatory activity in the UIC-Sarcoidosis cohort clusters. **(A,B)** Depict total metabolic volume (TMV) and total lesion glycolysis (TLG). **(C–F)** Depict intrathoracic metabolic volume MV_{IT}, intrathoracic lesion glycolysis LG_{IT}, extrathoracic metabolic volume MV_{ET}, and extrathoracic lesion glycolysis LG_{ET}, respectively. Comparisons between clusters were performed with Kruskal-Wallis tests and, if significant ($p < 0.05$), were followed by *post-hoc* analysis with Dunn's test. Dunn's test was considered significant if BH-adjusted $p < 0.1$. Kruskal-Wallis test p-values are displayed in their respective panels; brackets above box and whisker plots denote significance of Dunn's test with an asterisk (*). NS, non-significant. Results of significant Dunn's tests: **(A)**- Cluster 1 vs. 2 (BH-adjusted $p = 0.0237$) and Cluster 1 vs. 3 (BH-adjusted $p = 0.0410$); **(B)**- Cluster 1 vs. 2 (BH-adjusted $p = 0.0170$) and Cluster 1 vs. 3 (BH-adjusted $p = 0.0293$); **(E)**- Cluster 1 vs. 2 (BH-adjusted $p = 0.0096$) and Cluster 2 vs. 3 (BH-adjusted $p = 0.0409$); **(F)**- Cluster 1 vs. 2 (BH-adjusted $p = 0.0058$) and Cluster 2 vs. 3 (BH-adjusted $p = 0.0381$).

TABLE 3 | Summary of the most relevant characteristics that comprise the UIC-Sarcoidosis cohort clusters.

Summary of cluster characteristics	Cluster 1	Cluster 2	Cluster 3
Race	African American	African American	Caucasian
Sex(t)	Female	Female	Male
Disease Acuity	Chronic	Variable	Acute
Lung Parenchyma	Normal	Mixed (Nodules & Advanced)	Mixed (Nodules & Consolidation/GGO)
Treatment	Naïve	Combination Corticosteroid & Immune modulator	Corticosteroid Monotherapy
Markers of Inflammation	Normal	Elevated	Normal
Absolute Lymphocytes (kcells/uL)(*)	Normal	Reduced	Reduced
Absolute CD4+ T-cells (cells/uL)(*)	Normal	Reduced	Reduced
18FDG-PET/CT	Normal	Positive	Positive

(t) Indicates that a trend toward significance was observed between clusters (Kruskal-Wallis p = 0.0560). (*) Reduced peripheral lymphocyte counts and reduced absolute CD4+ T-cell counts within the UIC-Sarcoidosis cohort clusters are defined as median values ≤ 1.5 kcells/uL and ≤ 500 cells/uL, respectively.

disease assessment across the cohort was limited as FDG uptake on PET scan was considered excretional in the case of the kidneys or artifact in organs such as the heart and brain. Further limitations include lack of standardization of PET scan in terms of optimal protocols for sarcoidosis and lack of follow up PET scan due to radiation risk or insurance coverage.

In summary, we have identified a novel classification scheme using readily available demographic and clinically relevant data to identify three distinct sarcoidosis phenotypes with significant variation in race, disease chronicity, and inflammation. Whether the different immunotypes are reflective of disease acuity, race, comorbid conditions, or prior treatment remains to be resolved

and follow-up studies should attempt to assess these differences in more homogeneous cohorts. However, peripheral reductions in lymphocytes, specifically CD4+ T-cells, were significantly related to inflammation identified on 18FDG-PET/CT and therefore sarcoidosis activity. While this finding is significant, a definitive threshold for clinically relevant lymphopenia has not been well-established in sarcoidosis. Though future prospective studies with larger cohorts are warranted, reductions in peripheral lymphocytes may be considered a determinant of sarcoidosis phenotypes and an indicator of active inflammation on 18FDG-PET/CT. Our study opens new doors for research that will help implement new classification criteria for the diagnosis and treatment of sarcoidosis. Multicenter validation studies will help to determine if this classification scheme can be applied broadly as well as clarify the clinical and immunologic implications of these findings.

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 the University of Illinois at Chicago Office for the Protection of Research Subjects. Subjects provided written informed consent prior to participation in this study.

AUTHOR CONTRIBUTIONS

NJS, CA, CV, and DRF conceived and designed the study. Subject enrollment was performed by NJS, CA, DRF, RE-I, SA, and BL. Medical record abstraction was performed by CV, CA, RE-I, SA, and YH. Interpretation of imaging studies was performed by CA, CV, and YL. CA, CV, and DRF analyzed the data. CA, CV, RPB, DLP, PWF, and NJS wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

Support for this study was provided by NIH grant numbers: R01 HL138628-01A1S1 and T32 HL144909; the Bernie Mac Foundation; and a donation by the University of Illinois at Chicago Vice-Chancellor of Health Affairs, Robert Barish, MD MBA.

ACKNOWLEDGMENTS

The authors would like to thank Martha Menchaca, MD Ph.D. for acquiring 18FDG-PET-CT images and transferring to DICOM format.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.595077/full#supplementary-material

REFERENCES

1. Baughman RP, Field S, Costabel U, Crystal RG, Culver DA, Drent M, et al. Sarcoidosis in America. analysis based on health care use. *Ann Am Thorac Soc.* (2016) 13:1244–52. doi: 10.1513/AnnalsATS.201511-760OC
2. Grunewald J, Grutters JC, Arkema EV, Saketkoo LA, Moller DR, Muller-Quernheim J. Sarcoidosis. *Nat Rev Dis Primers.* (2019) 5:45. doi: 10.1038/s41572-019-0107-y
3. Baughman RP, Teirstein AS, Judson MA, Rossman MD, Yeager H Jr, Bresnitz EA, et al. Clinical characteristics of patients in a case control study of sarcoidosis. *Am J Respir Crit Care Med.* (2001) 164:1885–9. doi: 10.1164/ajrccm.164.10.2104046
4. Ascoli C, Huang Y, Schott C, Turturice BA, Metwally A, Perkins DL, et al. A circulating microRNA signature serves as a diagnostic and prognostic indicator in sarcoidosis. *Am J Respir Cell Mol Biol.* (2018) 58:40–54. doi: 10.1165/rcmb.2017-0207OC
5. Morell F, Levy G, Orriols R, Ferrer J, De Gracia J, Sampol G. Delayed cutaneous hypersensitivity tests and lymphopenia as activity markers in sarcoidosis. *Chest.* (2002) 121:1239–44. doi: 10.1378/chest.121.4.1239
6. Selroos O, Koivunen E. Prognostic significance of lymphopenia in sarcoidosis. *Acta Med Scand.* (1979) 206:259–62. doi: 10.1111/j.0954-6820.1979.tb13507.x
7. Sweiss NJ, Salloum R, Gandhi S, Alegre ML, Sawaqed R, Badaracco M, et al. Significant CD4, CD8, and CD19 lymphopenia in peripheral blood of sarcoidosis patients correlates with severe disease manifestations. *PLoS ONE.* (2010) 5:e9088. doi: 10.1371/journal.pone.0009088
8. Hawkins C, Shaginurova G, Shelton DA, Herazo-Maya JD, Oswald-Richter KA, Rotsinger JE, et al. Local and systemic CD4(+) T Cell exhaustion reverses with clinical resolution of pulmonary sarcoidosis. *J Immunol Res.* (2017) 2017:3642832. doi: 10.1155/2017/3642832
9. Schott CA, Ascoli C, Huang Y, Perkins DL, Finn PW. Declining pulmonary function in interstitial lung disease linked to lymphocyte dysfunction. *Am J Respir Crit Care Med.* (2020) 201:610–3. doi: 10.1164/rccm.201910-1909LE
10. Baughman RP, Scholand MB, Rahaghi FF. Clinical phenotyping: role in treatment decisions in sarcoidosis. *Eur Respir Rev.* (2020) 29:190145. doi: 10.1183/16000617.0145-2019
11. Prasse A, Katic C, Germann M, Buchwald A, Zissel G, Muller-Quernheim J. Phenotyping sarcoidosis from a pulmonary perspective. *Am J Respir Crit Care Med.* (2008) 177:330–6. doi: 10.1164/rccm.200705-742OC
12. Schupp JC, Freitag-Wolf S, Bargagli E, Mihailovic-Vucinic V, Rottoli P, Grubanovic A, et al. Phenotypes of organ involvement in sarcoidosis. *Eur Respir J.* (2018) 51:1700991. doi: 10.1183/13993003.00991-2017
13. Judson MA, Costabel U, Drent M, Wells A, Maier L, Koth L, et al. The WASOG sarcoidosis organ assessment instrument: an update of a previous clinical tool. *Sarcoidosis Vasc Diffuse Lung Dis.* (2014) 31:19–27.
14. Adegunsoye A, Oldham JM, Chung JH, Montner SM, Lee C, Witt LJ, et al. Phenotypic clusters predict outcomes in a longitudinal interstitial lung disease cohort. *Chest.* (2018) 153:349–60. doi: 10.1016/j.chest.2017.09.026
15. Guo Q, Lu X, Gao Y, Zhang J, Yan B, Su D, et al. Cluster analysis: a new approach for identification of underlying risk factors for coronary artery disease in essential hypertensive patients. *Sci Rep.* (2017) 7:43965. doi: 10.1038/srep43965
16. Just J, Gouvis-Echraghi R, Rouve S, Wanin S, Moreau D, Annesi-Maesano I. Two novel, severe asthma phenotypes identified during childhood using a clustering approach. *Eur Respir J.* (2012) 40:55–60. doi: 10.1183/09031936.00123411
17. Molano-Gonzalez N, Rojas M, Monsalve DM, Pacheco Y, Acosta-Ampudia Y, Rodriguez Y, et al. Cluster analysis of autoimmune rheumatic diseases based on autoantibodies. new insights for polyautoimmunity. *J Autoimmun.* (2019) 98:24–32. doi: 10.1016/j.jaut.2018.11.002
18. Kim S, Lim MN, Hong Y, Han SS, Lee SJ, Kim WJ. A cluster analysis of chronic obstructive pulmonary disease in dusty areas cohort identified three subgroups. *BMC Pulm Med.* (2017) 17:209. doi: 10.1186/s12890-017-0553-9

19. Rubio-Rivas M, Corbella X. Clinical phenotypes and prediction of chronicity in sarcoidosis using cluster analysis in a prospective cohort of 694 patients. *Eur J Intern Med.* (2020) 77:59–65. doi: 10.1016/j.ejim.2020.04.024
20. Papiris SA, Georgakopoulos A, Papaioannou AI, Pianou N, Kallergi M, Kelekis NL, et al. Emerging phenotypes of sarcoidosis based on 18F-FDG PET/CT: a hierarchical cluster analysis. *Expert Rev Respir Med.* (2020) 14:229–38. doi: 10.1080/17476348.2020.1684902
21. Adams H, Keijsers RG, Korenromp IH, Grutters JC. FDG PET for gauging of sarcoid disease activity. *Semin Respir Crit Care Med.* (2014) 35:352–61. doi: 10.1055/s-0034-1376866
22. Lu Y, Grant C, Xie K, Sweiss NJ. Suppression of Myocardial 18F-FDG uptake through prolonged high-fat, high-protein, and very-low-carbohydrate diet before FDG-PET/CT for evaluation of patients with suspected cardiac sarcoidosis. *Clin Nucl Med.* (2017) 42:88–94. doi: 10.1097/RLU.0000000000001465
23. Keijsers RG, Verzijlbergen EJ, van den Bosch JM, Zanen P, van de Garde EM, Oyen WJ, et al. 18F-FDG PET as a predictor of pulmonary function in sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* (2011) 28:123–9.
24. Keijsers RGM, Grutters JC. In which patients with sarcoidosis is FDG PET/CT indicated? *J Clin Med.* (2020) 9:890. doi: 10.3390/jcm9030890
25. Schimmelpennink MC, Vorselaars ADM, Veltkamp M, Keijsers RGM. Quantification of pulmonary disease activity in sarcoidosis measured with (18)F-FDG PET/CT: SUVmax versus total lung glycolysis. *EJNMMI Res.* (2019) 9:54. doi: 10.1186/s13550-019-0505-x
26. Chen H, Jin R, Wang Y, Li L, Li K, He Y. The utility of (18)F-FDG PET/CT for monitoring response and predicting prognosis after glucocorticoids therapy for sarcoidosis. *Biomed Res Int.* (2018) 2018:1823710. doi: 10.1155/2018/1823710
27. Guleria R, Jyothidasan A, Madan K, Mohan A, Kumar R, Bhalla AS, et al. Utility of FDG-PET-CT scanning in assessing the extent of disease activity and response to treatment in sarcoidosis. *Lung India.* (2014) 31:323–30. doi: 10.4103/0970-2113.142092
28. Mostard RL, Voo S, van Kroonenburgh MJ, Verschakelen JA, Wijnen PA, Nelemans PJ, et al. Inflammatory activity assessment by F18 FDG-PET/CT in persistent symptomatic sarcoidosis. *Respir Med.* (2011) 105:1917–24. doi: 10.1016/j.rmed.2011.08.012
29. Chopra A, Kalkanis A, Judson MA. Biomarkers in sarcoidosis. *Expert Rev Clin Immunol.* (2016) 12:1191–208. doi: 10.1080/1744666X.2016.1196135
30. Crouser ED, Maier LA, Wilson KC, Bonham CA, Morgenthau AS, Patterson KC, et al. Diagnosis and detection of sarcoidosis. an official american thoracic society clinical practice guideline. *Am J Respir Crit Care Med.* (2020) 201:e26–51. doi: 10.1164/rccm.202002-0251ST
31. Hunninghake GW, Costabel U, Ando M, Baughman R, Cordier JF, du Bois R, et al. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. *Sarcoidosis Vasc Diffuse Lung Dis.* (1999) 16:149–73.
32. Nioche C, Orlhac F, Boughdad S, Reuze S, Goya-Outi J, Robert C, et al. *LIFEx:* a freeware for radiomic feature calculation in multimodality imaging to accelerate advances in the characterization of tumor heterogeneity. *Cancer Res.* (2018) 78:4786–9. doi: 10.1158/0008-5472.CAN-18-0125
33. He JP, Hao Y, Li M, Wang J, Guo FJ. Tumor-to-background ratio to predict response to chemotherapy of osteosarcoma better than standard uptake values. *Orthop Surg.* (2014) 6:145–53. doi: 10.1111/os.12102
34. Lee SJ, Choi YY, Kim C, Chung MS. Correlations between tumor to background ratio on breast-specific gamma imaging and prognostic factors in breast cancer. *J Korean Med Sci.* (2017) 32:1031–7. doi: 10.3346/jkms.2017.32.6.1031
35. Barrington SF, Kluge R. FDG PET for therapy monitoring in Hodgkin and non-Hodgkin lymphomas. *Eur J Nucl Med Mol Imaging.* (2017) 44(Suppl. 1):97–110. doi: 10.1007/s00259-017-3690-8
36. Sunderland JJ, Christian PE. Quantitative PET/CT scanner performance characterization based upon the society of nuclear medicine and molecular imaging clinical trials network oncology clinical simulator phantom. *J Nucl Med.* (2015) 56:145–52. doi: 10.2967/jnumed.114.148056
37. Meignan M, Cottereau AS, Versari A, Chartier L, Dupuis J, Boussetta S, et al. Baseline metabolic tumor volume predicts outcome in high-tumor-burden follicular lymphoma: a pooled analysis of three multicenter studies. *J Clin Oncol.* (2016) 34:3618–26. doi: 10.1200/JCO.2016.66.9440
38. Erdi YE, Mawlawi O, Larson SM, Imbriaco M, Yeung H, Finn R, et al. Segmentation of lung lesion volume by adaptive positron emission tomography image thresholding. *Cancer.* (1997) 80(12 Suppl):2505–9. doi: 10.1002/(SICI)1097-0142(19971215)80:12+<2505::AID-CNCR24>3.0.CO;2-F
39. Erdi YE, Wessels BW, Loew MH, Erdi AK. Threshold estimation in single photon emission computed tomography and planar imaging for clinical radioimmunotherapy. *Cancer Res.* (1995) 55(23 Suppl):5823s–6s.
40. Team RC, *R: A Language and Environment for Statistical Computing.* Vienna: R Foundation for Statistical Computing (2018).
41. Foss A, Markatou M, Ray B, Heching A. A semiparametric method for clustering mixed data. *Machine Learning.* (2016) 105:419–58. doi: 10.1007/s10994-016-5575-7
42. Foss A, Markatou M. kamila: clustering mixed-type data in R and hadoop. *J Statist Software.* (2018) 83:1–45. doi: 10.18637/jss.v083.i13
43. Hennig C. *fpc: Flexible Procedures for Clustering* (2020).
44. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics.* (2011) 12:77. doi: 10.1186/1471-2105-12-77
45. Zidar DA, Al-Kindi SG, Liu Y, Krieger NI, Perzynski AT, Osnard M, et al. Association of lymphopenia with risk of mortality among adults in the US general population. *JAMA Netw Open.* (2019) 2:e1916526. doi: 10.1001/jamanetworkopen.2019.16526
46. Berezné A, Bono W, Guillevin L, Mouthon L. [Diagnosis of lymphocytopenia]. *Presse Med.* (2006) 35:895–902. doi: 10.1016/S0755-4982(06)74709-1
47. Valiathan R, Deeb K, Diamante M, Ashman M, Sachdeva N, Asthana D. Reference ranges of lymphocyte subsets in healthy adults and adolescents with special mention of T cell maturation subsets in adults of South Florida. *Immunobiology.* (2014) 219:487–96. doi: 10.1016/j.imbio.2014.02.010
48. McQuillan GM, Kruszon-Moran D. HIV infection in the United States household population aged 18-49 years: results from 1999-2006. *NCHS Data Brief.* (2008) 1–8. doi: 10.1037/e407182008-001
49. Celada LJ, Drake WP. Targeting CD4(+) T cells for the treatment of sarcoidosis: a promising strategy? *Immunotherapy.* (2015) 7:57–66. doi: 10.2217/imt.14.103
50. Crouser ED, Lozanski G, Fox CC, Hauswirth DW, Raveendran R, Julian MW. The CD4+ lymphopenic sarcoidosis phenotype is highly responsive to anti-tumor necrosis factor-{alpha} therapy. *Chest.* (2010) 137:1432–5. doi: 10.1378/chest.09-2576
51. Kamphuis LS, van Zelm MC, Lam KH, Rimmelzwaan GF, Baarsma GS, Dik WA, et al. Perigranuloma localization and abnormal maturation of B cells: emerging key players in sarcoidosis? *Am J Respir Crit Care Med.* (2013) 187:406–16. doi: 10.1164/rccm.201206-1024OC
52. Tøndell A, Rø AD, Børset M, Moen T, Sue-Chu M. Activated CD8+ T cells and natural killer T cells in bronchoalveolar lavage fluid in hypersensitivity pneumonitis and sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* (2015) 31:316–24.
53. Snyder-Cappione JE, Nixon DF, Chi JC, Nguyen ML, Kirby CK, Milush JM, et al. Invariant natural killer T (iNKT) cell exhaustion in sarcoidosis. *Eur J Immunol.* (2013) 43:2194–205. doi: 10.1002/eji.201243185
54. Crouser ED. Role of imbalance between Th17 and regulatory T-cells in sarcoidosis. *Curr Opin Pulm Med.* (2018) 24:521–6. doi: 10.1097/MCP.0000000000000498

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.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership