SARCOIDAL GRANULOMATOUS DERMATITIS: Study of clinicopathological and molecular biological features implicated in its pathogenesis and prognosis

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PROGRAMA DE DOCTORADO EN MEDICINA

DEPARTAMENTO DE MEDICINA

Universitat Autònoma de Barcelona

SARCOIDAL GRANULOMATOUS DERMATITIS:

Study of clinicopathological and molecular biological features implicated in its pathogenesis and prognosis

DOCTORAL THESIS

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Barcelona, 2017

Tesis presentada por Tiago Castro Esteves para optar al grado de Doctor por la Universitat Autònoma de Barcelona, realizada bajo la dirección del Prof. Dr. Vicente García-Patos Briones.

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Barcelona, 2017

El director de la Tesis Doctoral, Prof. Dr. Vicente García-Patos Briones, certifica que el trabajo presentado por Tiago Castro Esteves y titulado "SARCOIDAL GRANULOMATOUS DERMATITIS: STUDY OF CLINICOPATHOLOGICAL AND MOLECULAR BIOLOGICAL FEATURES IMPLICATED IN ITS PATHOGENESIS AND PROGNOSIS" cumple las exigencias metodológicas y científicas para ser presentado en el Tribunal legalmente constituido, según las normas vigentes en el Departamento de Medicina de la Universitat Autònoma de Barcelona.

Vicente García-Patos Briones

Tiago Castro Esteves

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ACKNOWLEDGMENTS

ACKOWLEDGMENTS

First of all, I would like to express my gratitude and special appreciation to my Director: Prof. Dr. Vicente Garcia-Patos Briones, for providing everything I need for the development of this PhD thesis, for the immense knowledge, friendship and continuous availability. Through the last years, I really felt a "lucky person" to have him as my advisor.

I extend much appreciation to all Dermatologists at the Hospital Vall d`Hebron for their time, assistance, friendship, availability, and for giving me the opportunity to learn. From their examples, all I have learned is not quantifiable.

To my family, especially to my wife, Rubina Alves, for giving me the love, harmony and tranquility necessary to continue and finishing this thesis. To my daughters (Carolina and Sofia) for the real joy of life. This accomplishment would not have been possible without them.

To everyone who supported and believed in me, especially to my fathers.

Tiago Castro Esteves

LIST OF ABBREVIATIONS

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ACCESS	A case control etiologic study of sarcoidosis
ACE	Angiotensin-converting enzyme
AFB	Acid-fast bacilli
APC	Antigen-presenting cell
B. burgdorferi	*Borrelia burgdorferi*
BLAST	Basic Local Alignment Search Tool
bp	Base-pair
CI	Confidence interval
CL	Cutaneous leishmaniasis
CS	Cutaneous sarcoidosis
CT	Computed tomography
DC	Dendritic cell
EBV	Epstein-Barr virus
EN	Erythema nodosum
GM-CSF	Granulocyte macrophage colony-stimulating factor
H&E	Hematoxylin and eosin stain
HCV	Hepatitis C virus
HHV-6	Human herpes virus-6
HHV-8	Human herpes virus-8
HLA	Human leukocyte antigen

hsp65	65-kDa heat shock protein gene
HTLV	Human T-cell lymphotropic virus
IHC	Immunohistochemical
IL	Interleukin
ITS	Internal transcribed spacer
MCP-1	Monocyte chemotactic protein 1
MHC	Major histocompatibility
MIP-1	Macrophage inflammatory protein 1
mKatG	*Mycobacterium tuberculosis* catalase-peroxidase
MRI	Magnetic resonance imaging
MTBC	*Mycobacterium tuberculosis* complex
NTM	Nontuberculous mycobacteria
OR	Odds ratio
P. acnes	*Propionibacterium acnes*
PPD	Purified protein derivative
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
rpoB	RNA polymerase β-subunit gene
SodA	Superoxide dismutase A
Th1	Type 1 helper T
TNF-α	Tumor necrosis factor alpha
16S rRNA	Ribosomal RNA common to all bacteria
18F-FDG PET	Fluorodeoxyglucose positron emission tomography
67Ga	Gallium 67 scintigraphy

INTRODUCTION

1. SARCOIDAL GRANULOMATOUS DERMATITIS

Granulomatous disorders comprise a large family sharing the histological denominator of granuloma formation. A granuloma is defined as a focal chronic inflammatory infiltrate characterized by collection of activated histiocytes, epithelioid cells, and multinucleate giant cells,[1] usually as a result of the persistence of a non-degradable product and of active cell mediated hypersensitivity.[1]

Granulomatous disorders of the skin frequently present a diagnostic challenge to dermatopathologists. An identical histological pattern may be produced by several disorders, and, conversely, a single condition can produce different histological patterns.[2]

Sarcoidal granulomatous dermatitis is characterized by circumscribed collections of epithelioid histiocytes without necrosis and scant mononuclear cell infiltrate. This histological reaction pattern may be present in a wide variety of systemic and cutaneous diseases, including infections; however it is widely acceptable as the prototype pattern of sarcoidosis. This disorder can present both clinically and histologically with a variety of phenotypes, which may be difficult to diagnose and distinguish from other cutaneous granulomatous disorders.[3]

1.1 Sarcoidosis

In Greek, sarcoidosis means a fleshlike condition (*sarco* means "flesh," *eidos* means "like," and *osis* means "condition").[4] Actually, sarcoidosis is a multisystem disorder of unknown origin, characterized by the accumulation of lymphocytes and mononuclear phagocytes that induce the formation of noncaseating epithelioid granulomas.

1.1.1 Epidemiology

The incidence of sarcoidosis varies widely throughout the world, probably because of differences in environmental exposures, surveillance methods, and predisposing HLA

alleles and other genetic factors.[5] In the United States, the disease prevalence is estimated to be between 10 to 40 cases per 100,000 persons,[6,7] with a much higher annual incidence in African Americans (35.5-64 cases/100,000 population) than in whites (10.9-14 cases/100,000 population).[7-9] Scandinavia has the world's highest prevalence of reported cases (64 cases/100,000 population).[7,10] In other countries, the incidence of sarcoidosis is reported as follows: United Kingdom, 20/100,000; France, 10/100,000; Germany, 9/100,000; Greece, 7/100,000; Spain, 1.4/100,000; Japan, 1.4/100,000 and Korea, 0,125/100,000.[11-14]

Sarcoidosis affects all races and all ages. Most commonly it presents in winter and early spring,[11,15] and usually develops before the age of 50 years.[5,8] The onset of sarcoidosis peaks during the third and fourth decade of life in the general population.[7,8] In black Americans, the peak incidence occurs later in life, in the fourth decade.[5,8] In Japan, the peaks are in the third decade of life,[5,16] while in Scandinavian women, the incidence appears to be bimodal, with one peak at 25 to 29 years of age and another at 65 to 69 years of age.[5,17]

The disease affects both sexes, with a slight preponderance among females,[18] especially in the second peak.[13,19] The female predominance is particularly high, ranging from 71 to 76%, in patients with cutaneous sarcoidosis.[20-23]

The phenotypic expression of sarcoidosis varies across different races or regions.[8,21] Sarcoidosis is more frequent in individuals of Afro-American origin. In addition, skin involvement in these individuals may be more acute and severe than in other races.[11,24] In contrast to this, cases affecting white persons have a tendency to be asymptomatic with a more favorable prognosis.[11,25]

1.1.2 Pathogenesis

The current concept on the pathogenesis of sarcoidosis is that it is a chronic immunologic response produced by exposure of a genetically susceptible individual to an as yet undefined external eliciting antigen.[26,27]

Clinicians and scientists have entertained a number of possible causes, including infectious agents, genetic mutations, and various environmental exposures, with inconclusive results.

The notion of a single causative agent is not in keeping with the worldwide distribution of disease. Based upon the available evidence, it seems likely that multiple factors, including host genetics and environmental exposures, independently contribute to the pathogenesis of sarcoidosis.

1.1.2.1 Immunologic pathogenesis

The development of noncaseating granulomas constitutes the classic pathologic finding in sarcoidosis. These granulomas are compact and consisted of centrally organized collections of macrophages and epithelioid cells encircled by lymphocytes. Although the immunopathogenesis of sarcoidosis remains unknown, certain unidentified sarcoid antigens potentially trigger a dysregulated type 1 helper T cell immune response that generates the formation and accumulation of granulomas.[7]

The initial step in the formation and maintenance of granulomas is the recognition and phagocytosis of a putative agent by an antigen-presenting cell (APC) such as macrophages or dendritic cells (DC). The activated APC produce high levels of tumor necrosis factor alpha (TNF-α) and secrete several other cytokines such as interleukin (IL)-1, IL-6, IL-12, IL-15, IL-18, macrophage inflammatory protein 1 (MIP-1), monocyte chemotactic protein 1 (MCP-1), and granulocyte macrophage colony-stimulating factor (GM-CSF).[27-30]

The interaction of CD4+ T cells with APCs elicits the amplification of the local cellular immune response.[31] These activated CD4+ cells differentiate into type 1 helper T (Th1)–like cells and secrete predominantly interleukin-2 and interferon-γ and augment macrophage TNF-α production.[5,32] The elaborated cytokines further regulate cell recruitment and accumulation at sites of disease activity.[27,29] Interferon-γ induces transformation of activated macrophages into multinucleated giant cells. TNF-α induces proliferation of monocytes and differentiation of macrophages into the epithelioid cells of granulomas.[27,29]

The efficiency of antigen processing, antigen presentation, and cytokine release is probably under genetic control and influences the susceptibility to and phenotypic expression of sarcoidosis.[5,7,33]

Sarcoidosis also represents an unresolved immunologic paradox; a state of anergy is present, as indicated by the suppression of immune response to tuberculin, despite an

extensive immune response at the sites of granulomatous inflammation.[34,35] Over the years, several theories have been put forward to explain the paradoxical immunity associated with sarcoidosis.[27,36] The expansion of a regulatory T-cell subset (CD25) in active sarcoidosis, may account for this state of anergy by abolishing interleukin-2 production and strongly inhibiting T-cell proliferation.[5,37]

1.1.2.2 Genetic pathogenesis

A genetic origin is supported by the presence of positive familial clusters of sarcoidosis.[11,38] No consistent mode of inheritance has been found, although a recessive pattern may be more common.[39]

Genetic studies have identified several chromosomal regions and specific genes associated with sarcoidosis. Schurmann *et al*.[40,41] indicated in their genetic analysis that the major histocompatibility (MHC) region on chromosome 6p21 was linked to sarcoidosis susceptibility.

Specific human leukocyte antigen (HLA) alleles can have impact on the risk of developing sarcoidosis. HLA DR 11, 12, 14, 15, 17, HLA-DRB*1101 and HLA-DQB1 were found to confer susceptibility to sarcoidosis, whereas others may predict a protection against the disease (e.g. HLA-DR1, DR4, HLA DQ*0202).[27] Furthermore, some HLA alleles can also have impact on the course and prognosis of the disease. It is well known that the presence of the HLA-DRB1*03 allele is strongly linked to a favorable prognosis and disease course with resolution within 2 years.[42]

In conclusion, no single gene alterations can explain the disease susceptibility. Identification of interactions between specific sarcoidosis-susceptibility genes and environmental antigenic stimulus will probably be important in delineating the causes of sarcoidosis.[5]

1.1.2.3 Environmental pathogenesis

Several external infectious and non-infectious agents have been proposed as putative agents responsible for the sarcoid immune response (Table 1).[26,28,43-55]

Some reported non-infectious agents suggested in the origin of sarcoidosis have included metals, moldy environments, combustible wood products, and World Trade Center disaster dust.[26,48,51,52,56]

Given the multiple environmental risk factors reported to date, it seems credible that several ubiquitous environmental triggers may be implicated in the pathogenesis of sarcoidosis.

Infectious			**Environmental and occupational**
Bacteria	**Virus**	**Fungi**	
Mycobacterium tuberculosis complex	Epstein-Barr virus (EBV)	*Cryptococcus*	Organic agents (e.g. pollen, pine)
Nontuberculous mycobacteria	Cytomegalovirus	*Histoplasma*	Wood (dust, burning)
Cell wall deficient mycobacteria	Human herpes virus-6 (HHV-6)	*Sporothrix*	Metal dust
Propionibacterium	Human herpes virus-8 (HHV-8)	*Candida albicans*	Insecticides/pesticides
Rickettsia helvetiva	Coxsackie B virus		Mold/mildew
Chlamydia pneumoniae	Hepatitis C virus (HCV)		Building material
Borrelia burgdoferi	HIV		Industrial organic dusts
Mycoplasma	Human T-cell lymphotropic virus (HTLV)		Carbon nanotubes
Duganella zoogloeoides			
Corynebacterium			

Table 1. *Etiological agents associated with sarcoidosis*

1.1.2.4 Infectious pathogenesis

A growing body of evidence links microbial infection to sarcoidosis etiology (Table 1). However, there is no consensus on the pathogenetic mechanisms by which microorganisms cause sarcoidosis.

Parkes *et al*.[57] were among the first to provide objective evidence for an infectious cause of sarcoidosis. Moreover, apparent transmission of sarcoidosis following organ transplant from affected donors to recipients further supports an infectious cause.[58,59]

Several infectious agents have been investigated as potential antigens of which Propionibacteria and Mycobacteria have emerged as the most probable candidates.

Mycobacteria have long been implicated as etiological agents. Using molecular techniques, investigators have detected mycobacteria in sarcoidosis samples but not in non-sarcoid control tissue.[43] Using more sensitive molecular techniques combining enzyme-linked immunospot assay and real-time PCR, Allen *et al*.[60] demonstrated the presence of mycobacterial virulence factor superoxide dismutase A (SodA) in patients with sarcoidosis. Additionally, Song *et al.*[61] confirmed *Mycobacterium tuberculosis* catalase-peroxidase (mKatG) as one of the antigens in sarcoidosis tissues using *in situ* hybridization. These investigations suggest the presence of mycobacterial proteins preferentially in sarcoidosis patients.[61] However, it is important to note that the mere presence of mycobacterial DNA, RNA or proteins does not confirm a cause-and-effect link. The research challenge is made more daunting by the inability to find mycobacterial DNA in many sarcoidosis tissues, the difficulty of isolating and growing the organism in culture and the fact that severe mycobacterial diseases do not develop if there is use of immunosuppressive drugs in sarcoidosis subjects.[62-64] However, Ezzie *et al.*[65] suggested that the discovery of some mycobacterial components (DNA, RNA or proteins) in sarcoidosis tissues, along with the finding of the existence of an antibody-antigen reaction to these specific components, provides strong evidence that some cases of sarcoidosis, particularly in its chronic and relapsing forms, may be caused by a delayed hypersensitivity immune response to infectious agents.[65]

Propionibacterium acnes (*P. acnes*) is the only bacterium to be isolated from lymph node biopsy samples taken from sarcoidosis patients,[66,67] though some authors have suggested

that *P. acnes* is not specific for sarcoidosis because is a normal inhabitant of peripheral lung tissue and mediastinal lymph nodes.[68]

A large study conducted by Japanese and European researchers confirmed the importance of *P. acnes* in the etiology of sarcoidosis.[69] The authors found a significant quantitative difference of the presence of the *P. acnes* genome in sarcoidosis patients compared to control subjects.[69] In that study, DNA was extracted from histological sections, which according to the authors reduced the risk of contamination.[69] Additionally, the genomes of *P. acnes* were abundant in sarcoidosis patients alone, supporting the role of *P. acnes* in sarcoidosis pathogenesis.[69]

Multiple research groups have suggested a variety of other pathogens (Table 1) including fungi, viruses, spirochetes, and other bacteria as potential causes of sarcoidosis but lack wider confirmation. In all of these cases, there is a lack of clinical or microbiologic evidence that these infectious agents play a direct role in pathogenesis of sarcoidosis.

Future studies should attempt to clarify the available evidence and should continue the search of an infectious etiology for sarcoidosis.

1.1.3 Clinical

Sarcoidal granulomas can involve any organ, but in more than 90% of patients, clinical sarcoidosis is manifested as hilar lymphadenopathy, pulmonary involvement, skin and ocular signs and symptoms, or some combination of these findings.[5]

1.1.3.1 Cutaneous involvement

Cutaneous involvement in sarcoidosis occurs in about one-quarter of the patients and is generally observed at the onset of the disease process although it may occur coincident with or after systemic involvement.[70,71] Recognition of cutaneous lesions is important because they provide a clue about the diagnosis and provide an easily accessible source of tissue for histopathological examination.

The cutaneous manifestations are variable, sometimes being very obvious and at other times perplexing.[72] They mimic a wide array of dermatological conditions posing a diagnostic challenge to dermatologists worldwide. Hence, the disease is aptly referred to as the 'great imitator'.[11,70,72,73]

Cutaneous sarcoidosis lesions are classified as specific when the histologic examination shows typical sarcoidal granulomas. Nonspecific sarcoidosis skin lesions show a nondiagnostic inflammatory reaction pattern on histologic evaluation. Nonspecific skin lesions are often associated with an acute presentation of sarcoidosis and, in general, portend a good prognosis.[74] Specific skin lesions tend to be more problematic than nonspecific lesions and are associated with worse outcomes.[74] Common specific sarcoidosis skin lesions are manifested as maculopapules, nodules, plaques, subcutaneous nodules, infiltrative scars and lupus pernio. Most common nonspecific lesions are erythema nodosum, erythema multiforme, calcifications and prurigo.[49,75]

Maculopapular and Papular forms

Maculopapular lesions are the most common specific cutaneous manifestations of sarcoidosis.[74,76,77] They are usually asymptomatic red-brown to purple papules and measure less than 10 mm in diameter (Fig. 1).[78] Normally, these lesions favor the face, neck, trunk and extremities, however they can appear anywhere in the body including mucous membranes.[76,79] Maculopapular eruptions may be transient or may enlarge and coalesce to form plaques or annular lesions.[72,74,76]

Papular sarcoidosis consist of 1 to 5 mm, smooth-surfaced, reddish-brown papules that occurs primarily on the face and neck with a predilection for eyelids and nasolabial folds.[76]

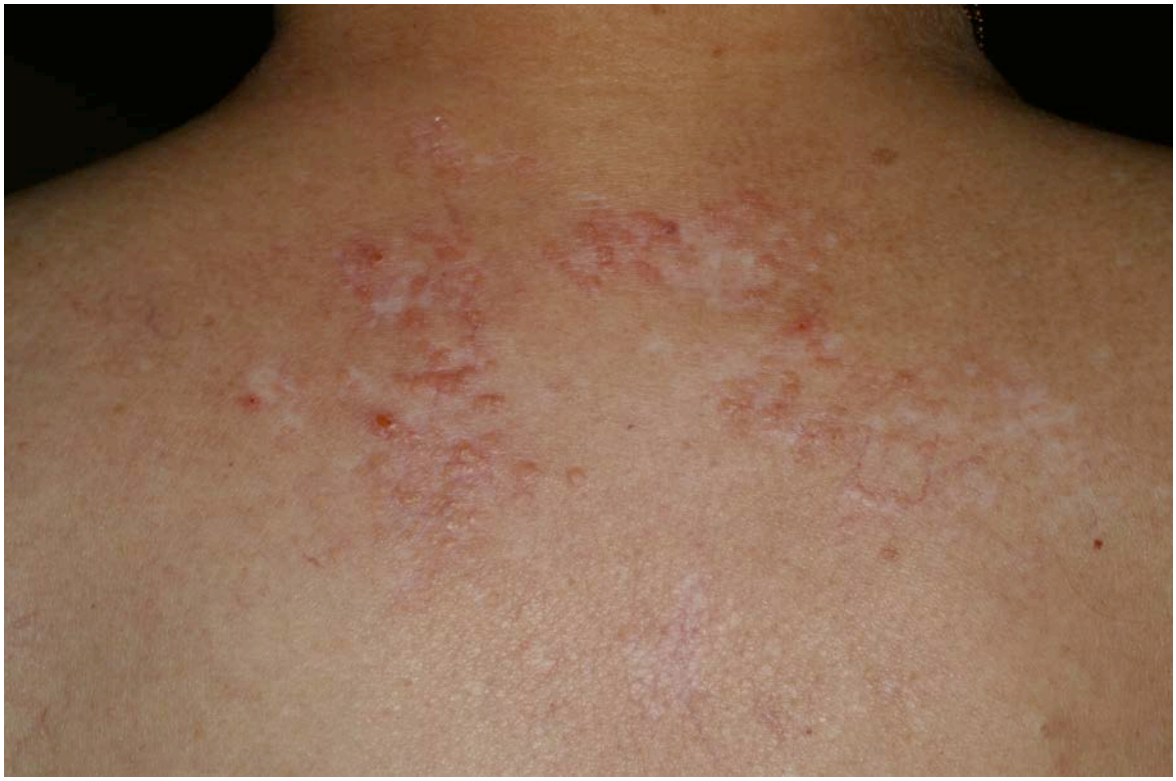


Fig. 1. *Maculopapular lesions on back, the most common type of cutaneous lesions in sarcoidosis.*

Plaque form

Skin plaques consist of one or multiple smooth-surfaced round to oval lesions, varying in color from red-brown to flesh colored. These lesions are usually greater than 5 mm, symmetrically distributed and generally elevated with induration. The plaques occur on the face (nose and cheeks), scalp, back, buttocks, and extensor surfaces of the extremities (Fig. 2).[76]

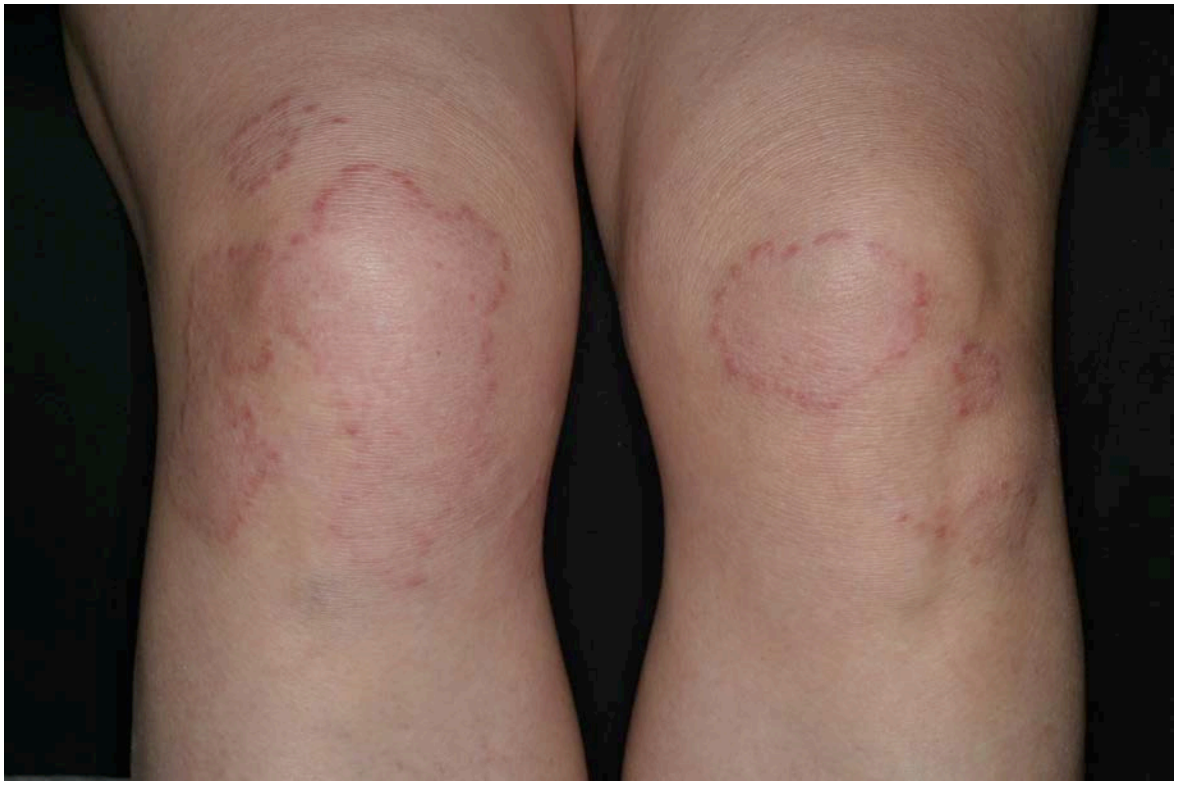


Fig. 2. *Plaque-type lesions symmetrically distributed on the knees*

The annular variant is a form of sarcoidosis in which the plaques seem to clear in the center, giving an annular appearance.[81] These lesions predominate on the forehead, face and neck and may heal with permanent scarring and alopecia (Fig. 3).[82]

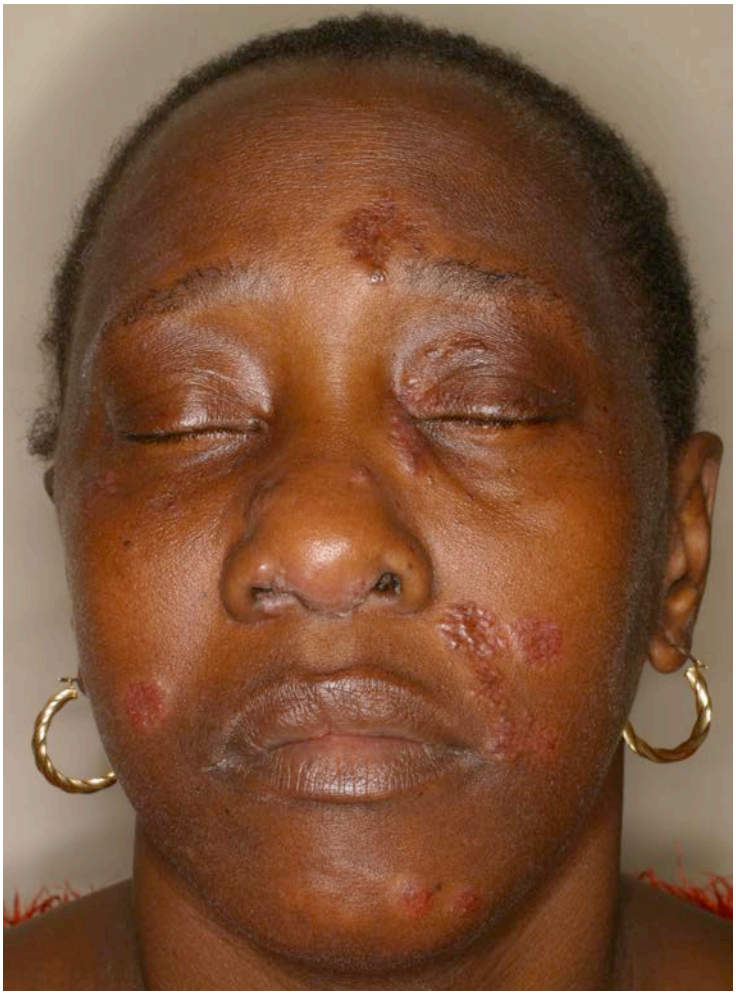


Fig. 3. *Plaque lesions in an African patient. Several of these red-brown plaques, on the face, have an annular configuration.*

Nodular forms

Nodular lesions are larger than 5 mm, usually single or relatively few, and remain circumscribed. Red or yellowish-red at first, becoming violaceous or purplish-brown later, they are soft or firm, round and occur more frequently on extremities (proximal parts of the limbs) or the trunk, but may also affect the face (Fig. 4).[83]

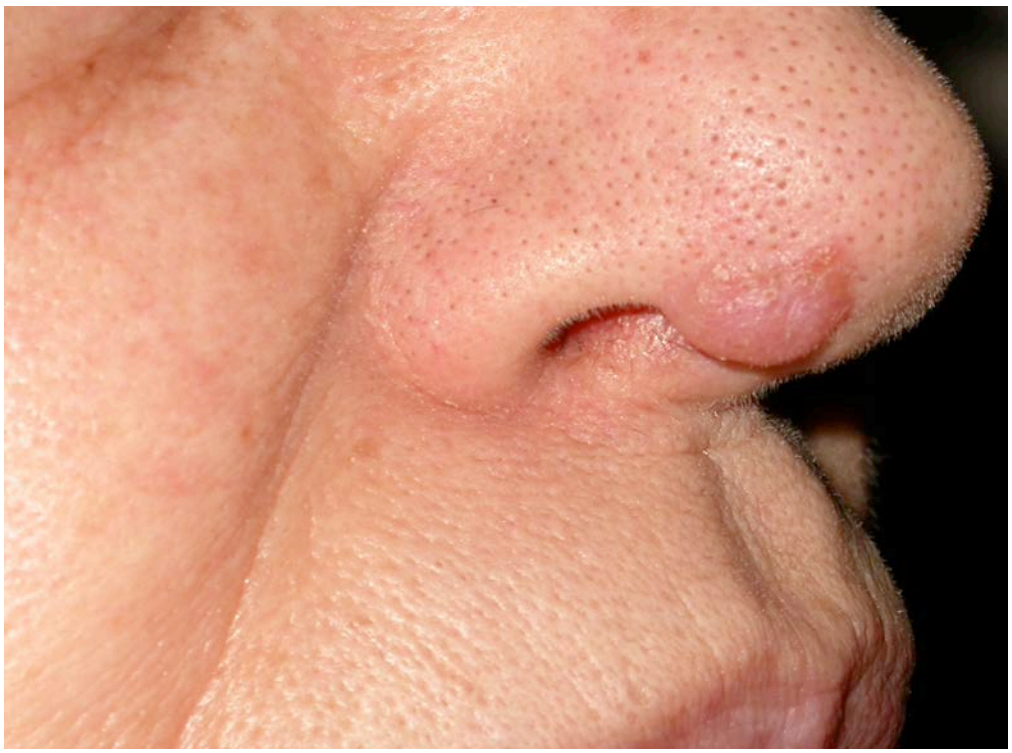


Fig. 4. *Sarcoidosis: single nodular lesion on the nose*

Lupus pernio

This is the most characteristic skin lesion of sarcoidosis, more commonly seen in African American women.[74,78] It is characterized by chronic, indolent, indurated, red-purple to violaceous plaque-like and nodular skin lesions affecting the nose (Fig. 5), cheeks, ear lobes, lips, forehead and, in some cases, the hands and feet.[74,78] Lesions of lupus pernio can be disfiguring; they may involve the upper respiratory tract and cause nasal ulceration, airway obstruction, and perforation of the nasal septum.[81,84] Lupus pernio is the hallmark of chronic fibrotic disease; it usually follows a chronic course and may coexist with pulmonary fibrosis.[82,85]

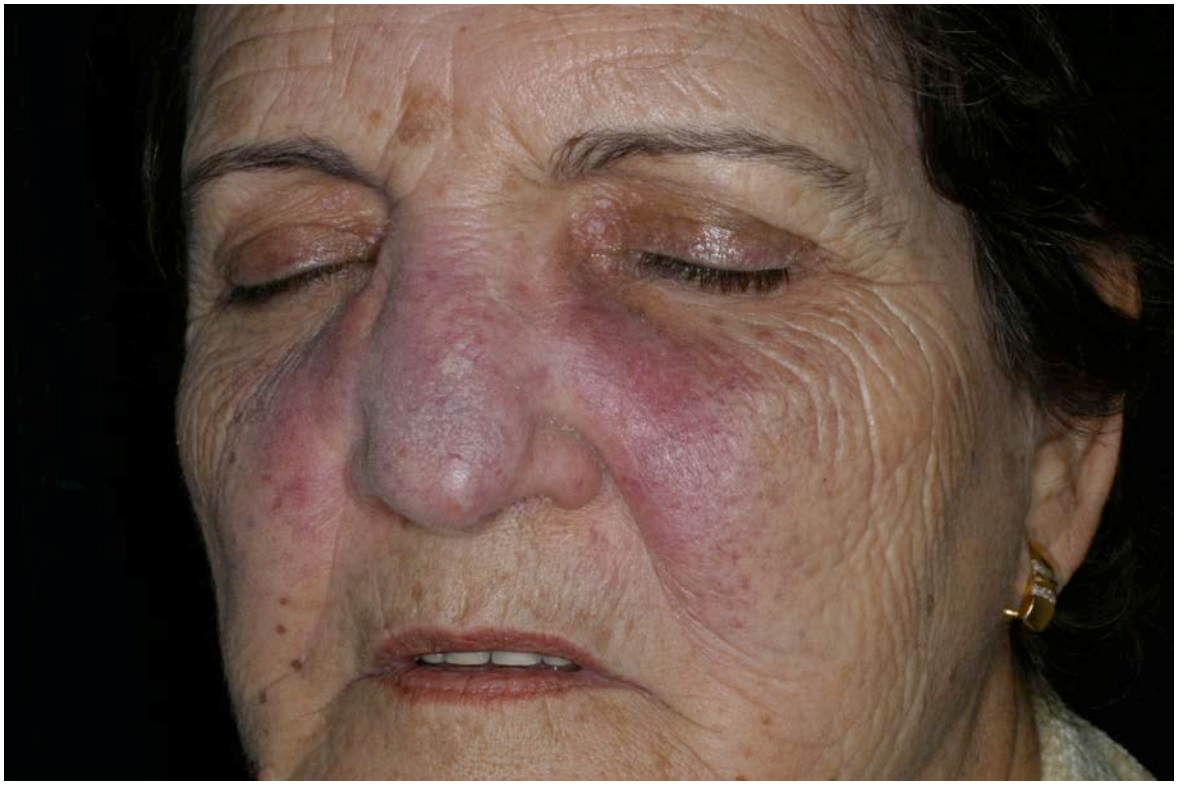


Fig. 5. *Lupus pernio: large dusky violaceous infiltrated plaques distributed symmetrically on the nose and cheeks*

Subcutaneous sarcoidosis

An unusual manifestation of sarcoidosis, also known as *Darier-Roussy sarcoidosis*, is characterized by insidious, firm, mobile, round to oval, flesh-colored nodules, without epidermal involvement (Fig. 6).[74,76,86] The number of nodules can range from 1 to 100 and are primarily distributed on the extremities with the forearms most commonly affected.[74,76,86] Subcutaneous sarcoidosis nodules are usually painless or only slightly tender to palpation and varied in size from 0.5 to 2 cm.[74,76,86]

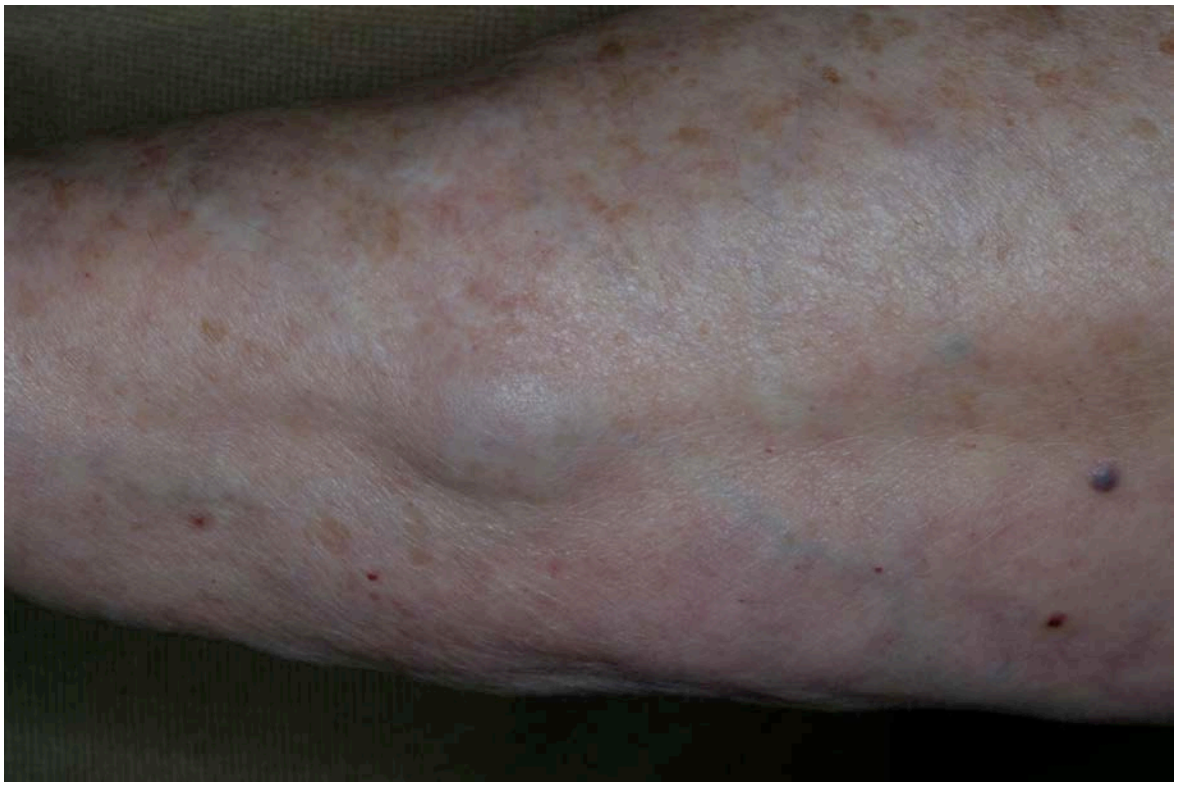


Fig. 6. *Skin-colored, subcutaneous nodules on the upper extremity*

Scar Sarcoidosis

Scar sarcoidosis is characterized by the infiltration of noncaseating sarcoidal granulomas in preexisting scar tissue (e.g. surgical scars, tattoos, skin piercings, and other sites of trauma). Scars become erythematous or violaceous and increase in thickness over a period

of days to weeks.[74,81] Lesions are usually asymptomatic and reflect an exacerbation of the chronic disease.[81]

Unusual and atypical forms

Some documented uncommon atypical skin lesions of sarcoidosis include ulcerative lesions (Fig. 7),[87] psoriasiform lesions,[87] hypopigmented macules,[88,89] faint erythema,[90] verrucous lesions,[87] ichthyosiform lesions,[91,92] pustular folliculitis,[93] lichenoid eruptions,[94] erythroderma,[92,93] cicatricial alopecia,[95] mutilating lesions,[87,96] lower extremity swelling,[97] nodular finger tip lesions,[98] scalp nodules,[99] erythema annular centrifugum,[100] palmar erythema,[101] rosacea-like syndrome,[102] morpheaform plaques,[103] sunlight-induced papules,[76] angiolupoid variant,[104] discoid lupus-like plaques,[105] umbilicated lesions,[77] genital lesions,[106,107] lichen sclerosus-like lesions,[108] and nail changes.[72]

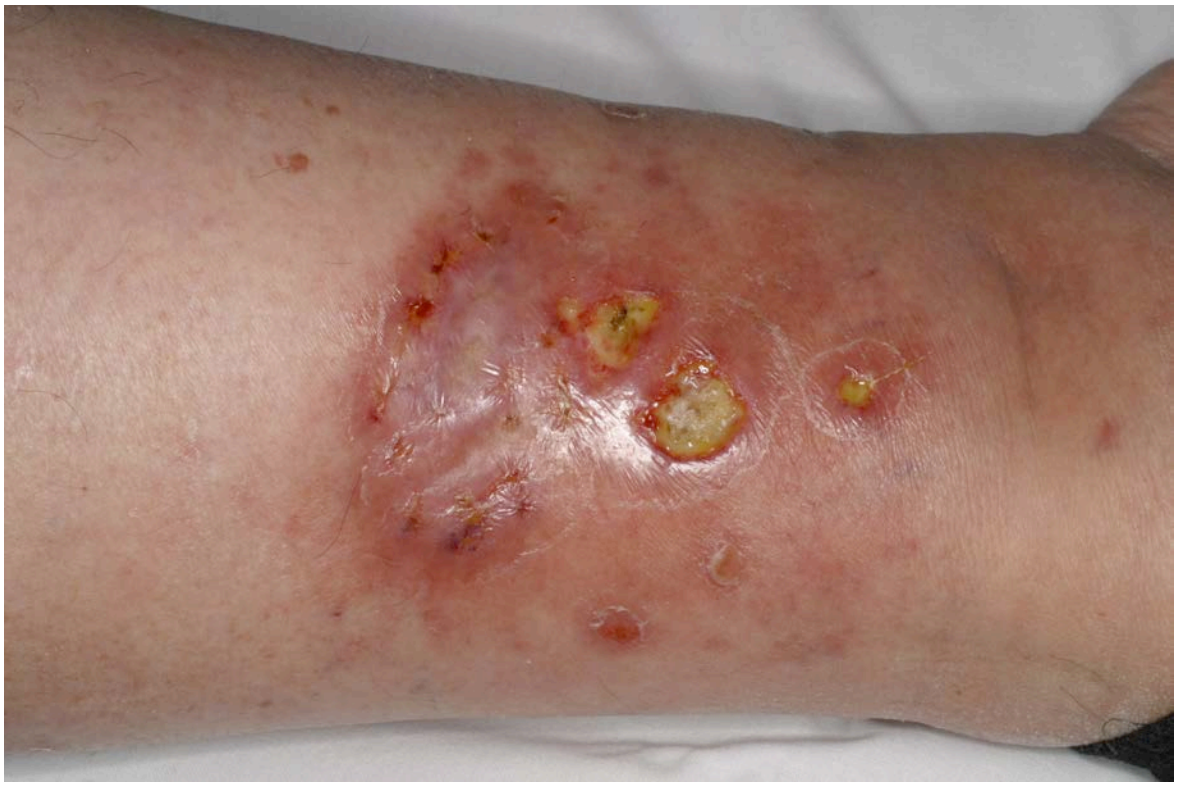


Fig. 7. *Ulcerated cutaneous sarcoidosis on the lower extremities, an uncommon variant of sarcoidosis*

Erythema nodosum

Erythema nodosum (EN) is the most common nonspecific skin lesion and develops in up to 25% of sarcoidosis patients.[109] The lesions consist of tender, erythematous subcutaneous nodules on the anterior legs.[71,78]

The association of EN with bilateral hilar lymphadenopathy with or without pulmonary infiltrates, arthritis, fever, and iritis, represents an acute and benign presentation of systemic sarcoidosis known as *Löfgren syndrome*.[110,111] The presence of this syndrome implies a favorable prognosis with >80% complete resolution within 2 years.[112]

1.1.3.2 Systemic involvement

Multiple organs may be involved, including lungs, mediastinal and peripheral lymph nodes, liver, spleen, skin, and eyes; central nervous system, heart, upper respiratory tract, bones, and joints are less frequently but usually more severely involved (Table 2).[5,113]

In patients with sarcoidosis, one third can present systemic symptoms such as fatigue, night sweats, weight loss, malaise and fever.[28] Other symptoms can be associated with the specific organ system involved.

Systemic Organ Involvement	Frequency	Clinical Findings
Pulmonary disease	90%	Bilateral hilar lymphadenopathy, parenchymal infiltrates, fibrosis, restrictive ventilator dysfunction, obstructive airway disease, pulmonary hypertension
Ocular sarcoidosis	10-90%	Anterior uveitis, intermediate uveitis, posterior uveitis, optic neuropathy, conjunctival nodules, scleral plaques, lacrimal gland enlargement, iritis

Hepatic and Splenic sarcoidosis	5-50%	Elevated serum aminotransferase and alkaline phosphatase levels, cholestatic hepatitis with constitutional symptoms, portal hypertension, cirrhosis
Neurologic sarcoidosis	5-15%	Cranial neuropathy, parenchymal brain lesions, cognitive manifestations, meningeal disease, peripheral neuropathy, spinal lesions, seizure, myopathy
Cardiac sarcoidosis	5%	Atrioventricular block, bundle branch block, ventricular tachycardia, congestive heart failure, sudden death
Hypercalcemia and Renal sarcoidosis	5-10%	Hypercalciuria (40-62%), hypercalcemia (5-10%), kidney stones, interstitial nephritis, renal failure
Musculoskeletal involvement	<40%	Bone cysts, osteolytic lesions, chronic myopathy, muscle nodules, tumorlike lesions, arthralgias, arthritis, tenosynovitis
Upper respiratory track involvement	5-20%	Nasal congestion, palatal obstruction, epistaxis, enlargement of the parotid gland
Hematologic manifestations	<30%	Peripheral lymphopenia, anemia, pancytopenia

Table 2. *Clinical polymorphisms of systemic sarcoidosis. Sarcoidosis granulomas may infiltrate virtually all organs including the breasts, uterus, fallopian tubes, ovaries, testicles, epididymis, prostate, pituitary gland and thyroid gland.*[5,11,114]

1.1.4 Diagnosis

There is no diagnostic test for sarcoidosis. The diagnosis of sarcoidosis is established on the basis of compatible clinical, laboratory, and radiological findings, supported by histological evidence in one or more organs of noncaseating granulomas, with all other causes of granulomas rules out; therefore it is a diagnosis of exclusion.

A biopsy specimen from the involved organ most easily accessed (e.g. skin, peritracheal nodes, salivary glands, or conjunctiva) should be performed in all patients, except those with Lofgren`s syndrome. Tissue culture may be necessary to exclude infectious granulomatous causes. Bronchoscopy with transbronchial lymph node biopsy is often required in patients without cutaneous involvement.

Dermatologists play an important role in the diagnosis and initial evaluation of sarcoidosis because the skin is involved in one quarter of all cases and is often the initially affected organ.[114] Nonetheless, all patients with cutaneous sarcoidosis should be evaluated for systemic involvement because extracutaneous disease eventually develop in the majority of patients at the time of diagnosis or a few years later.[74,80] Table 3 provides the guidelines for initial screening and follow-up of patients with suspected sarcoidosis.[5,11,71,114]

Table 3. Clinical initial evaluation for all suspected cases of sarcoidosis[5,11,71,114]
Complete history with emphasis on occupational or environmental exposure and family history
Physical examination may suggest additional organ involvement (e.g. lung, abdomen, skin, cardiac, and neurologic examination, palpation of lymph nodes)
Biopsy of affected organ with special stains and cultures
Chest radiographs
Pulmonary function tests
Electrocardiogram
Ophthalmologic evaluation: slit-lamp and fundoscopic eye examination

Complete blood cell count, urine analysis and measurement of serum calcium, creatinine, erythrocyte sedimentation rate, blood urea nitrogen, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase levels
Determination of serum level of angiotensin-converting enzyme (lacks sensitivity and specificity, however serum levels higher than 50% of the upper limit of normal are highly suggestive of the disease
Kviem-Siltzbach test (useful in patients whose lesions are not easily accessible by biopsy) and Tuberculin skin test (suggested if clinically indicated)
Other tests may be used to indicate organ involvement: • High-resolution computed tomography: indicated when the chest radiography is atypical for sarcoidosis; • Magnetic resonance imaging (MRI): cardiac and neurologic involvement; • Fluorodeoxyglucose positron emission tomography (18F-FDG PET): useful in identifying sites for diagnostic biopsy, especially in patients without apparent lung involvement; • Gallium 67 scintigraphy (67Ga)

1.1.5 Prognosis

The course of the disease depends on gender, age, ethnicity and the affected organs. Generally, spontaneous resolution is seen in up to 60 % of patients with sarcoidosis, and an additional 10% to 20% of patients have remissions with corticosteroid use.[11,115] Remission occurs for more than half of patients within 3 years; however the disease follows a chronic and progressive course in 10-30% of patients,[42] leading to clinically significant organ impairment. Recurrent disease may occur in up to 5% of the patients after one or more years of remission.[5]

Concerning ethnicity, the course of the disease among African Americans is often more severe and chronic, while the prognosis in Asian population is significantly better than in Caucasian patients.[42]

Less than 5% of patients will eventually die of the disease.[28] Death from sarcoidosis is usually the result of pulmonary, cardiac or neurologic involvement. Causes of death from sarcoidosis are pneumonia, pulmonary fibrosis with respiratory failure, cardiac arrhythmias, and sudden cardiac death.[11]

It has always been considered that specific skin lesions in sarcoidosis had no prognostic significance, did not show any correlation with the extent of systemic involvement, and did not indicate a more serious form of sarcoidosis.[74,116,117] The only exception is the EN which has been shown to have prognostic significance in sarcoidosis[74] and is usually associated with transient disease with a high rate of spontaneous remissions (>80%).[11,112,118]

However, currently it is known that the risk of severe systemic involvement may vary according to the type of skin lesion. Generally, patients with lupus pernio and plaques have more frequent pulmonary parenchymal involvement with worse chest radiograph stages, greater persistence of cutaneous lesions, and greater chronicity of disease compared with patients presenting macules, papules, or subcutaneous nodules.[83]

The wide information currently available is based on retrospective studies of patients with known systemic disease. More studies, especially prospective studies, following patients with only cutaneous involvement, are needed to establish the association between the type of skin lesions and the development of systemic disease.

1.2 Differential diagnosis

1.2.1 Non-infectious

1.2.1.1 Foreign body granulomas

Foreign bodies that can elicit a sarcoidal granulomatous dermatitis include silica,[119] zirconium,[120] beryllium,[119] sea-urchin spines[121] and tattoo pigment.[122] These may be differentiated from each other and from other sarcoidal granulomas by polarized light and other clinical features.[119] However, polarizable foreign bodies do not exclude sarcoidosis.[119] Moreover, it is histologically impossible to clearly differentiate a sarcoid foreign body reaction from “true” sarcoidosis associated with foreign material.[123]

1.2.1.2 Cutaneous Crohn's disease

The commonest cause of granulomatous inflammation in the gastrointestinal tract is Crohn's disease.[124] The cutaneous lesions of Crohn's disease occur most commonly but not exclusively on the perianal, and less frequently, perioral skin.[119] Rarely, skin involvement distant from perirectal area occurs, such as in retroauricular sites or extremities.[119] The lesions are histologically identical to the intestinal lesions. Cutaneous lesions of Crohn's disease may have striking similarity to sarcoidosis because sarcoidal granulomas are frequently present. Nevertheless, the presence of denser lymphocytic collections, frequently observed in Crohn's disease, may help in differentiation.[119]

1.2.1.3 Granulomatous Rosacea

Granulomatous rosacea may show perifollicular and perivascular epithelioid granulomas. These granulomas are thought to arise as a foreign body reaction to keratin from disrupted hair follicles.[119]

Granulomatous rosacea needs to be distinguished from micronodular sarcoidosis, particularly since both conditions may be associated with iritis, conjunctivitis and sarcoidal granulomas in their biopsies.[124] The presence of *Demodex* mites or eosinophilic remnants of *Demodex* in the center of histiocyte collections and the presence of plasma cells are histological features more common in rosacea. Moreover, sarcoidosis can generally be excluded by the physical examination, chest X-ray, and laboratory tests.

1.2.1.4 Orofacial granulomatosis (Melkersson-Rosenthal syndrome)

This is a rare granulomatous disorder of the mouth and adjacent tissues, involving the oral mucosa, gum, lips, tongue, pharynx, eyelids, and skin of the face. It was described by Melkersson in 1928 as facial palsy associated with swelling of the lip (granulomatous cheilitis). Rosenthal expanded the syndrome to include lingua plicata (scrotal tongue) and genetic factors.[119]

Histopathology shows sarcoid granulomas without a significant inflammatory component and a perivascular mononuclear infiltrate composed of lymphocytes and plasma cells.[119] Histologic differentiation from sarcoidosis and other similar granulomatous processes may

be difficult, but the characteristic clinical features and unusual anatomic location of granulomatous cheilitis usually allow differentiation between the diseases.[119]

1.2.1.5 Lichen striatus

Lichen striatus is a condition that presents as papules along Blaschko's lines in children and adolescents, being rare in adults. It does not become widespread, and the importance of recognizing it is largely to avoid the diagnosis of the many conditions it can mimic.[125]

Sarcoidal granulomas can occur, but usually in association with an irregular lymphocytic infiltrate in the papillary dermis and psoriasiform epidermal hyperplasia. The finding of lymphocytes around eccrine coils can be very helpful in confirming the diagnosis.[125]

1.2.1.6 Lichen nitidus

Lichen nitidus is a condition that is relatively common in dark skinned patients, resulting in tiny shiny flat-topped papules, often on the dorsal hands or genitalia. Its small papules can be confused with those of lichen planus, but are unrelated.[126]

Histopathology shows epithelioid granulomas constituted of small compact collections of histiocytes with variable numbers of lymphocytes in widened dermal papillae; slight epidermal hyperplasia is also frequently observed.[126]

1.2.2 Infectious

1.2.2.1 Mycobacterium tuberculosis

The most common histological finding in cutaneous tuberculosis is the tuberculoid granuloma in the dermis, observed in 57% to 96% of the samples.[127] Nevertheless, *Mycobacterium tuberculosis* causes several cutaneous lesions that also show the microscopic features of sarcoidal granulomas at some point in their development.[119]

Primary cutaneous tuberculosis initially appears as a suppurative ulcerated lesion (the tuberculous chancre), accompanied by painful regional lymphadenopathy. In these recent

lesions, there is the presence of necrotizing neutrophilic infiltrate with numerous acid-fast bacilli (AFB). At 3 to 6 weeks, epithelioid and giant cells begin to replace the initial inflammatory cells. The lesion acquires a granulomatous appearance with decrease number of bacilli. By 6 months, the lesion develops a sarcoidal appearance, with epithelioid and enlarged giant cells.[119,128] By the time the purified protein derivative (PPD) test becomes positive, bacilli are difficult to demonstrate in tissue sections and differentiation from other sarcoidal granulomas may be histologically impossible.[119]

Lupus vulgaris most commonly occurs in previously sensitized individuals, with delayed hypersensitivity reaction strongly positive to tuberculin.[129] Usually it occurs on the head and/or neck as red-brown plaques that conrain 1 mm papules with an "apple jelly" color appearance under diascopy.[119] Histology shows pseudoepitheliomatous hyperplasia and multiple, well-formed tuberculoid granulomas. The lymphocytic infiltrate is dense and caseous necrosis is rarely present, but it may occur in small foci centrally to the granuloma.[127] Sarcoidal granulomas may also be observed, but differentiation from sarcoidosis is suggested by the presence of a richer inflammatory mantle and caseation.[119] The observation of AFB is infrequent and mycobacterial culture is often negative.[127]

Tuberculids are controversial. Some are thought to result from a delayed hypersensitivity reaction to *M. tuberculosis* and others from dissemination of organisms or their products.[119] Lichen scrofulosorum is a rare tuberculid that presents as a lichenoid eruption of minute papules in children and adolescents with tuberculosis; it is a self-limiting disease that shows a perifollicular granulomatous infiltrate containing epithelioid granulomas without a prominent lymphocytic inflammation. The granulomas are smaller and less well defined than those of sarcoidosis.[119]

1.2.2.2 Atypical mycobacterial infections

Atypical (or nontuberculoid) mycobacteria infections of the skin are relatively common. In Europe, the most common variant of atypical mycobacteriosis is caused by *M. marinum* that results in the "fish tank" granuloma on the hands of aquarium hobbyists and "swimming pool" granulomas in the skin of swimmers.[119] These lesions evolve from a nonspecific mixed inflammatory cell infiltrate with epithelial hyperplasia to a sarcoidal

granulomatous pattern.[119,130] During the course of this evolution, organisms become less demonstrable in tissue sections. Culture of the lesion is necessary for specific diagnosis.[119]

1.2.2.3 Mycobacterium leprae

Mycobacterium leprae is the pathogen causing leprosy, a disease with the highest incidence in India and Brazil.[131]

Based on clinical and pathological characteristics, five types can be distinguished depending on individual immunity: tuberculoid (TT), borderline tuberculoid (BT), mid-borderline (BB), borderline lepromatous (BL), and lepromatous (LL).

The two forms that result from higher degrees of hypersensitivity to the bacteria demonstrate some or all of the features of sarcoidal granulomas. In tuberculoid leprosy, well-organized epithelioid granulomas containing Langhans giant cells and a moderate lymphocytic infiltrate are seen.[119] The granulomas of tuberculoid leprosy show small areas of central necrosis more often than those of sarcoidosis. In addition, the granulomas of tuberculoid leprosy, in contrast with those of sarcoidosis, follow nerves and therefore often appear elongated.[119] Crucial clues to diagnosis tuberculoid leprosy include inflammation within and around peripheral nerves (where acid-fast *Mycobacterium leprae* occasionally may be demonstrated with special stains), elongated tubercles (reflecting nerve involvement), and denser collections of lymphocytes and plasma cells around the tubercles.[119,131]

Borderline tuberculoid leprosy shows similar clinical and histologic findings, except that the skin lesions are more numerous and less well defined. Microscopically, fewer lymphocytes and giant cells are seen.[119]

1.2.2.4 Deep fungal infections

Granulomatous fungal infections may produce a predominantly sarcoidal pattern, with fungal pathogens seen within langhans giant cells.[119] In particular, lobomycosis shows histiocytic granulomas associated with massive fibrosis.[119] Other fungal infections that may produce a sarcoidal granulomatous reaction are aspergillosis (*Aspergillus fumigatus*

and *A. niger*), cryptococcosis, coccidioidomycosis, and sporotrichosis.[119] Histologically, these conditions appear more commonly as suppurative granulomas.

It is important to recognize or exclude fungal infections localized to one system or disseminated; in particular, granulomatous fungal meningitis needs to be distinguished from sarcoidosis by all available laboratory techniques.[124]

1.2.2.5 Cutaneous leishmaniasis

Cutaneous leishmaniasis may also show sarcoidal granulomas. There are about twenty species of *Leishmania* that cause cutaneous leishmaniasis. Species that most common infect the skin include *Leishmania major, L. tropica*, and *L. mexicana*. The protozoan parasites are transmitted by sand flies (*Phlebotominae*); rodents, dogs, and other animals serve as natural reservoirs.[119]

The acute phase is histologically typically characterized by a diffuse infiltrate of lymphocytes, macrophages, and plasma cells. Neutrophils are found in variable numbers, especially in the upper dermis.[131]

Granuloma formation is more common in chronic lesions. Here, small tuberculoid granulomas and giant cells predominate, usually without caseation necrosis, while sarcoidal and palisading granulomas represent occasional findings.[131] Suppurative granulomas may also occur especially with ulceration or pseudoepitheliomatous hyperplasia.[119] The *Leishmania* organisms in chronic lesions are difficult to demonstrate, which is why *Leishmania* PCR should be included in the workup of suspicious granulomatous reaction patterns.[131]

1.2.2.6 Treponema pallidum

Due to the various clinical and histopathological manifestations it can be challenging to diagnose *Treponema pallidum* infections, especially secondary syphilis.

Granulomatous inflammation is a hallmark of tertiary syphilis, which also presents with massive caseating necrosis surrounded by lymphocytes, giant cells, fibroblasts, and plasma cells.[131] In addition, there is frequently endothelial swelling with small vascular proliferations.[131]

A granulomatous dermatitis is a rare histopathological manifestation of secondary syphilis.[119] It is thought to develop in longer existing skin lesions and may indicate a transition to the tertiary phase.[119] It can mimic other granulomatous skin diseases, such as granuloma annulare, sarcoidosis, leprosy, cutaneous leishmaniasis, atypical mycobacteriosis or deep fungal infection. The most prominent feature that differentiates to syphilis is the presence of numerous plasma cells. These are uncommon in the other dermatoses.[119]

STUDY RATIONALE

STUDY RATIONALE

Sarcoidosis is a multisystem disorder of unknown origin that is characterized by the presence of non-caseating epithelioid granulomas.[5,7] Cutaneous involvement occurs in approximately 25% of sarcoidosis patients[70,71] and is generally observed at the onset of the disease.

Although cutaneous lesions are not life threatening and lead to physical impairment in only a minority of patients, the psychological and social impact of the disease can be devastating, which supports the need for treatment. However, the decision to treat is complicated by the unpredictable course of the disease, the possibility of spontaneous remission, variability in patient responses to treatment, and the severe side effects associated with some systemic treatments.

In the literature there are few studies that report the clinicopathological correlation and prognostic significance of skin lesions in sarcoidosis. It is therefore of great interest to deal with the course and the prognosis of cutaneous sarcoidosis in order to provide the dermatologist with a basis for its decision about whether treatment should, in fact, be instituted.

The first part of this doctoral thesis (**Phase 1**) addresses the experience of the department of Dermatology at the Hospital Universitari Vall d´Hebron in the follow-up of patients with cutaneous sarcoidosis. It describes not only the main clinical and histopathological findings but also describes the clinicopathological correlation and prognostic significance of skin lesions in sarcoidosis.

The second part of the doctoral thesis comprises the study of etiological factors (especially infectious) implicated in the pathogenesis of Sarcoidosis (**Phase 2**), as well as, in a more comprehensive way, of sarcoidal granulomatous dermatitis (**Phase 3**).

It has been established that sarcoidosis is characterized by a chronic immune response, mediated by type 1 helper (Th1)-like cells and produced by exposure of genetically susceptible individuals to an undefined antigen.[11] Taking into account the worldwide distribution of sarcoidosis, more than a single causative agent may be implicated in its

pathogenesis.[7] Numerous infectious and non-infectious etiological agents have been incriminated.[132] Currently, the focus is on infectious agents, especially species of *Mycobacterium* and *Propionibacterium*.

Recently, Oswald-Richter *et al.*[133] suggested that several microorganisms acting complementarily to each other, rather than just one, may be involved in the etiology of sarcoidosis. They concluded that the observation of positive signals consistent with both propionibacteria and mycobacteria proteins within sarcoidosis granulomas, combine to suggest that these infectious agents may play an important role in the etiology of sarcoidosis.[133]

Other infectious agents such as *Borrelia burgdorferi*, *Rickettsia helvetica, Chlamydia pneumoniae*, viruses, fungal infections and others have all been investigated, but the results have been inconclusive or conflicting.[134-141]

Finally, Moravvej *et al.*,[142] using a PCR technique, detected the presence of *Leishmania major* (*L. major*) DNA sequences in eight out of twenty-five sarcoidosis samples, thus bringing up the question of the role of *L. major* in the etiology of sarcoidosis.

Considering the studies above mentioned, it is certain that more than a single infectious agent may be involved in the etiology of sarcoidosis. To clarify the role of infectious agents in sarcoidosis, further molecular, immunological or epidemiological studies are needed.

Figure 8 summarizes the planning of this doctoral thesis, exemplifying its division into 3 phases.

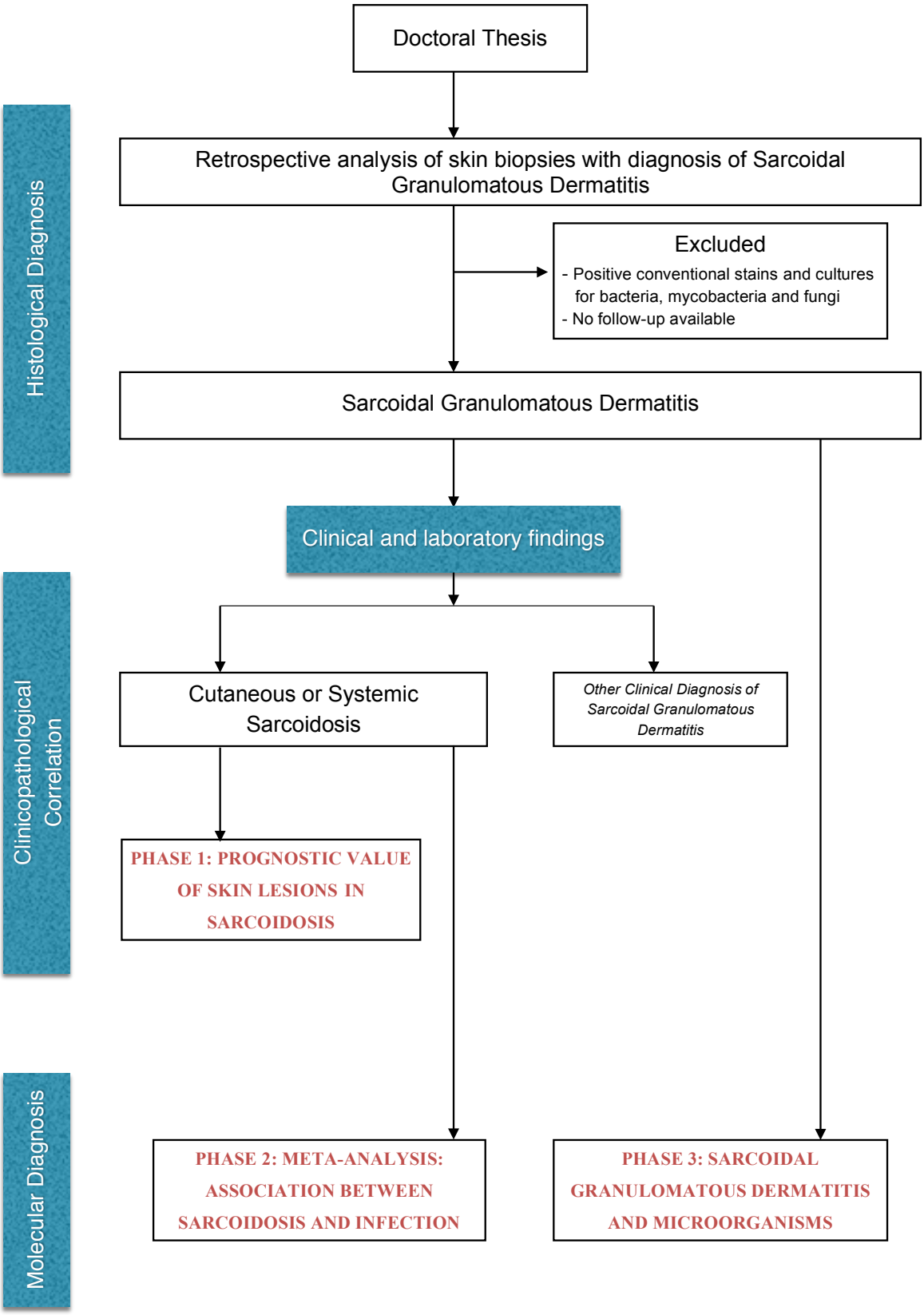


Fig. 8. *Planning of Doctoral Thesis*

OBJECTIVES

OBJECTIVES

The objectives of the study were:

1. **Provide novel data about the clinicopathological correlation in patients with cutaneous sarcoidosis:**
    a. Describe the main clinical and histopathological findings associated with cutaneous sarcoidosis, as well as, their clinicopathological correlation**.**
    b. Investigate the relationship of cutaneous lesions with systemic involvement.
    c. Determine the prognostic significance of skin lesion in sarcoidosis.

2. **Perform a systematic review and meta-analysis in order to:**
    a. Evaluate the role that infectious agents may have in sarcoidosis pathogenesis.

3. **Evaluate the role of microorganisms in the pathogenesis of sarcoidal granulomatous dermatitis using current molecular biology techniques.**
    a. Investigate the usefulness of molecular biology techniques in the study of sarcoidal granulomatous dermatitis and their differential diagnoses.
    b. Evaluate the potential role of mycobacteria, bacteria and *Leishmania* as the etiological factor for cutaneous lesions showing histologically a sarcoidal granulomatous dermatitis with no demonstrable pathogens in cultures and special stains.
    c. Correlate the molecular findings with the clinicopathological outcomes.
    d. Determine the real clinical significance of the molecular results.

PHASE 1: PROGNOSTIC VALUE OF SKIN LESIONS IN SARCOIDOSIS: CLINICAL AND HISTOPATHOLOGICAL CLUES

PHASE 1: PROGNOSTIC VALUE OF SKIN LESIONS IN SARCOIDOSIS: CLINICAL AND HISTOPATHOLOGICAL CLUES

1. INTRODUCTION

Although tremendous advances have been made, a significant gap exists between the vast knowledge accumulated concerning sarcoidosis in recent years and our understanding of this disease.

In the literature, most studies of cutaneous sarcoidosis (CS) have been drawn from populations with defined pulmonary involvement or other systemic organ disease.[21,143] We report our experience of 41 patients seen in a dermatopathology department with cutaneous sarcoidal granulomas.

2. MATERIAL AND METHODS

A retrospective analysis of skin biopsies received in the Department of Pathology at the Hospital Universitari Vall d´Hebron in Spain over a 23-year period (1991–2013) was performed.

The medical records corresponding to patients with skin biopsies diagnosed as sarcoidal granulomatous dermatitis were initially retrieved. On the basis of clinicopathological correlations and laboratory findings, 41 cases were diagnosed of cutaneous sarcoidosis. Only patients with specific sarcoidosis skin lesions were included. Biopsies of non-specific skin lesions (e.g., erythema nodosum) were excluded from this study. All of these cases exhibited non-caseating granulomas in formalin-fixed and paraffin-embedded skin biopsy specimens. Special stains (like periodic-acid Schiff, alcian blue, Ziehl Neelsen stain and others) and cultures for bacteria, mycobacteria, and fungi were negative, and other granulomatous diseases were excluded.[20]

Medical records, clinical photos at the time of biopsy, pathological slides, and laboratory data related to the documentation of extra-cutaneous involvement were reviewed.

The ACCESS (A Case Control Etiologic Study of Sarcoidosis) instrument[144] was used to define the extent of organ involvement. The pulmonary involvement of Sarcoidosis was confirmed by chest radiography, chest computed tomography, respiratory function tests and, in some cases, bronchoscopy, bronchoalveolar lavage and pulmonary or transbronchial lymph node biopsy. Pulmonary involvement in sarcoidosis was staged radiographically[145] as follows: stage 0 was normal; stage 1 exhibited bilateral hilar lymphadenopathy without infiltration; stage 2 demonstrated bilateral hilar lymphadenopathy with infiltration; stage 3 exhibited infiltration alone; and stage 4 showed fibrotic bands, bullae, hilar retraction, bronchiectasis, and diaphragmatic tenting.

Other laboratory tests, including routine biochemistry, urinalysis, Ga-67 scan, tuberculin skin test, and serum levels of angiotensin-converting enzyme (ACE) were performed on some patients based on clinical settings.

Central nervous system involvement was considered to exist if a lesion was confirmed by computed tomography (CT) or magnetic resonance imaging (MRI) and diagnosed by a

consultant neurologist. For evidence of ocular sarcoidosis, an ophthalmologist screened all patients.

The clinical variables assessed were sex and age, as well as the type and localization of lesions. The treatment outcomes and recurrences were also evaluated.

Histologically, the following characteristics were assessed:

1. Epidermis (normal, atrophic, ulcerated, focal lichenoid infiltrate);
2. Characteristics of the granulomatous infiltrate: Extent (+ mild, ++ moderate, +++ severe); Localization (superficial dermis, mid dermis, deep dermis and hypodermis); Distribution (perivascular, periannexial, irregular/interstitial); Coalescence of granulomas;
3. Characteristics of the granulomas: Type (naked sarcoidal or tuberculoid); Cellular types such as multinucleate giant cells (0 absence, + mild, ++ moderate, +++ numerous); Other cellular types; Presence of necrosis and fibrosis; Foreign material (0 absence, + presence); Schaumann bodies (0 absence, + presence); Asteroid bodies (0 absence, + presence).
4. Interstitial infiltrate (type and density).

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software (SPSS for Windows, version 19.0). Statistical comparisons were performed using the two-tailed Pearson's chi-square test and the Fisher's Exact test. A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

3.1 Clinical outcomes

In all, 41 cases of cutaneous sarcoidosis were analyzed. The clinical characteristics of these patients are summarized in Table 4.

Characteristics	n	%
Sex		
Female	34	83
Male	7	17
Age (year)		
Mean (± Std. Dev.)	52 ± 10	
Range	27-71	
Specific cutaneous lesion		
Maculopapular	23	56
Nodules	16	39
Plaque	9	22
Scar sarcoidosis	5	12
Lupus pernio	4	9.7
Ulcerated form	1	2.4
Cicatricial alopecia	1	2.4
Lesion distribution		
Lower limbs	26	63.4
Head and neck	19	46.3
Upper limbs	12	29.2
Trunk	7	17
Extracutaneous involvement	24/41 (58.5%)	
Lung	22	91.6
Lymph nodes	10	41.6
Liver	3	12.5

Central nervous system	2	8.3
Ocular involvement	1	4.2
First manifestation	n=24	
Cutaneous lesion	21	87.5
Extracutaneous manifestation	3	12.5
Radiological staging		
Stage 0	19	46.3
Stage I	8	19.5
Stage II	8	19.5
Stage III	5	12.2
Stage IV	1	2.4
Laboratory data		
Negative mantoux test	20/22	90.9
Elevated ACE levels	13/28	46.4

Table 4. *Clinical characteristics of 41 patients with cutaneous sarcoidosis.*

At presentation, the age of the patients ranged from 27–71 years, with a mean age of 52±10 years. The patients included 34 females (83%) and 7 males (17%). Ethnicity was documented for all patients; 38 were white and 3 Afro-American.

Clinically, the skin lesions consisted of macules or papules in 23 patients (56%), nodules in 16 patients (39%), plaques in 9 patients (22%), a scar sarcoidosis variant in 5 patients (12%), and lupus pernio in 4 patients (9.7%). The less common presentations of cutaneous sarcoidal lesions in our series were the ulcerated form (1 patient) and cicatricial alopecia (1 patient). Fourteen patients had more than one type of skin lesion (Fig. 9).

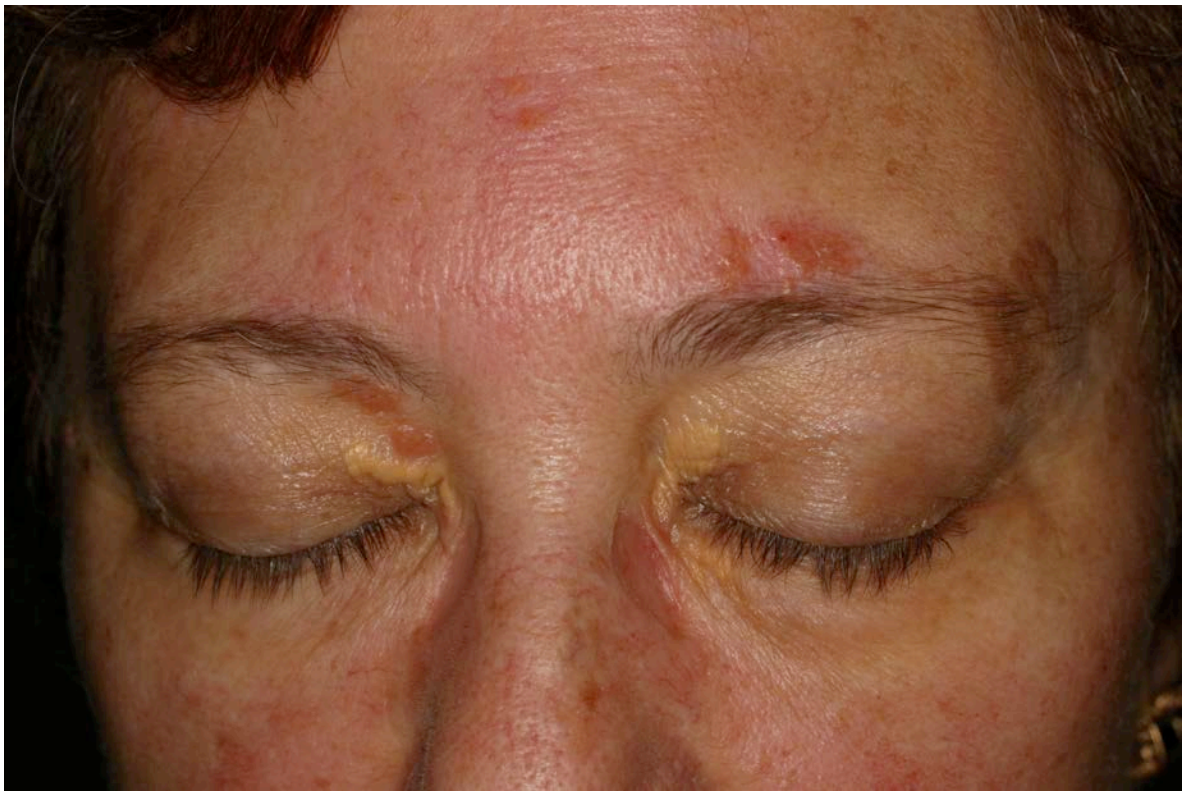


Fig. 9. *Cutaneous sarcoidosis: This patient presented a maculopapular eruption on the forehead and two paques in the upper right eyelid. The association of different clinical manifestations in the same patient makes the classification of the disease more difficult.*

With respect to localization, the most frequently affected areas were the lower limbs (26 patients, with 13 of these patients affected in the knees), the head and neck (19 patients, with 4 of these affected in the nose), the upper limbs (12 patients, with 3 affected in the elbows), and the trunk (7 patients). Lesions were present in more than one location in 15 patients.

Systemic organ involvement was observed in 24 of the 41 cases (58.5%). Among these patients, cutaneous lesions were the first manifestation in 87.5% (21 patients) and respiratory complaints were the first sign in 12.5% of cases (3 patients). The most frequent extracutaneous manifestation was dyspnea followed by arthralgia, fever and fatigue.

The most affected systemic organ was the lung (22 patients), followed by the lymph nodes (10 patients), the liver (3 patients), and the central nervous system (2 patients); one patient had ocular involvement. Ten patients had involvement of multiple organs.

Systemic disease occurred frequently in patients with lupus pernio (4/4), cicatricial alopecia (1/1), plaques (7/9), and the nodular variant (11/16). The clinical presentation of macules and papules and the scar sarcoidosis variant were less frequently associated with systemic involvement (Table 5).

	Systemic involvement	Radiological Staging					Disease activity	
		0	I	II	III	IV	< 2 years	> 2 years
Maculopapular (n=23)	13/23	11	6	2	3	1	10	13
Plaque (n=9)	7/9	4	2	1	2	-	2	7
Nodules (n=16)	11/16	7	2	4	2	1	2(*)	14(*)
Lupus pernio (n=4)	4/4	1	-	3	-	-	-	4
Scar sarcoidosis (n=5)	2/5	3	-	1	1	-	3	2
Cicatricial alopecia (n=1)	1/1	1	-	-	-	-	-	1
Ulcers (n=1)	-	1	-	-	-	-	1	-

Table 5. *Cutaneous sarcoidosis: relation between each type of cutaneous lesion and the radiological staging, disease activity and systemic involvement.*

** Statistically significant at $p<0.05$*

Chest radiography was performed in all patients with CS. During radiological staging at the time of diagnosis, the majority of patients (46.3%) had normal chest radiographs (stage 0), followed by stage I (19.5%), stage II (19.5%), stage III (12.2%), and stage IV (2.4%) (Table 4).

The most frequent CT findings were hilar and mediastinal adenopathy, followed by widespread nodules distributed among the bronchovascular bundles or subpleurally, interstitial infiltrates, and consolidation and architectural distortion with bronchiectasis and fibrotic changes of the lung parenchyma.

Of the 4 patients receiving Ga-67 scan, 3 showed abnormal findings compatible with sarcoidosis. All of these 3 patients had normal chest radiography at the time of initial clinical investigation (Fig. 10).

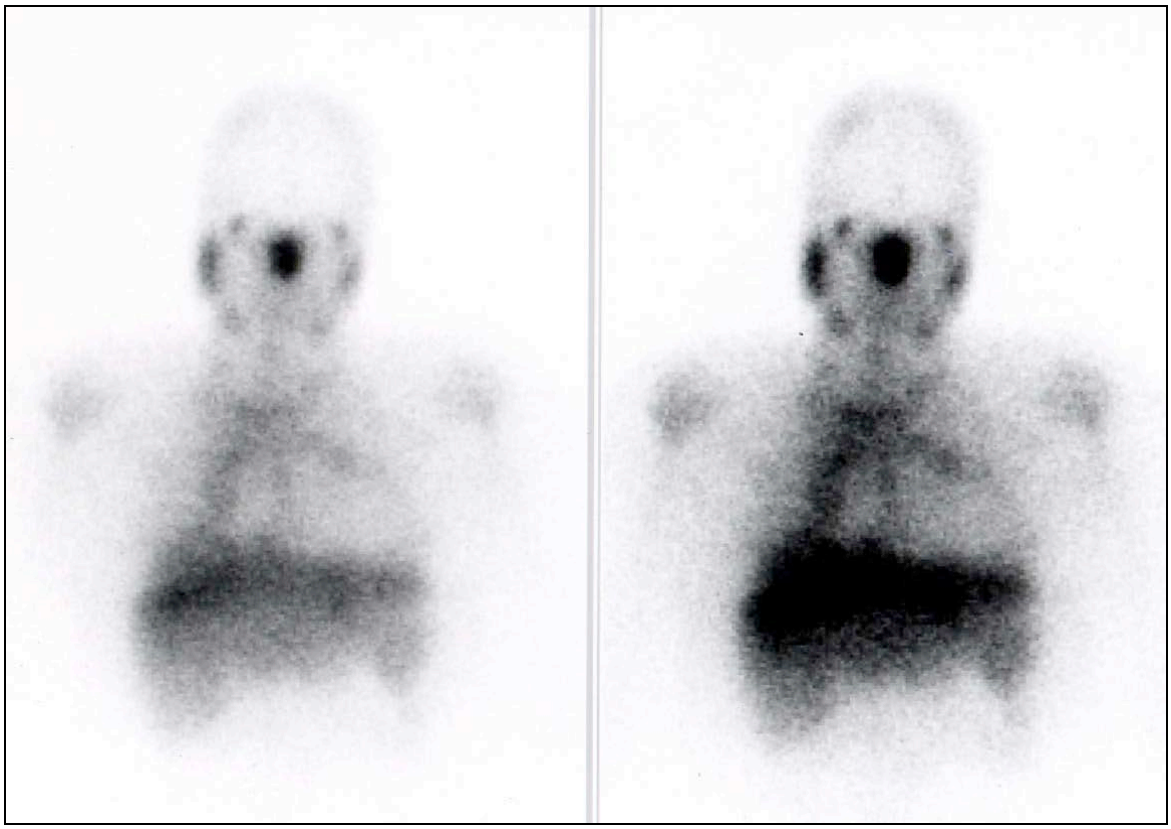


Fig. 10. *Ga-67 scan from a patient with systemic sarcoidosis, demonstrating the panda and lambda signs.*

Among the 28 patients with routine blood biochemistry, 13 (46.4%) had high serum angiotensin-converting enzyme (ACE) levels (>40 U/L). In all cases, the ACE levels were less than twice the normal value. Systemic disease was identified more commonly in patients with raised serum ACE levels (84.6% vs. 40%) ($p<0.05$). Mantoux test was

performed in 22 patients, of which 20 were negative. The overall findings in these two cases (positive tuberculin test) were consistent with sarcoidosis, possibly in association with old pulmonary tuberculosis.

The duration of follow-up ranged from 3 months–21 years (median 4 years). Of the 41 patients studied, the disease followed a chronic course with persistence of disease activity for more than 2 years in 27 patients (65.9%), of which 22 presented with systemic organ involvement of sarcoidosis (Table 6).

		Only cutaneous involvement (n=17)	Cutaneous and systemic involvement (n=24)
Disease activity	**<2 years (n=14)**	12 (70.6%)**	2 (8.3%)**
	>2 years (n=27)	5 (29.4%)**	22 (91.6%)**
Course of skin disease	**Complete resolution of skin lesions (n=21)**	13 (76.5%)*	8 (33.3%)*
	Persistence or progression of skin disease (n=20)	4 (23.5%)*	16 (66.6%)*
Treatment options	**No**	2	-
	CS top or intra	14	17
	CS oral	2	16
	MTX	-	3
	Chloroquine	1	4
	Others	-	4

Table 6. *Distribution of patients with isolated cutaneous involvement and systemic involvement according to the disease course and treatment options.*

CS, corticosteroids; intra, intralesional; top, topical.

** Statistically significant at p<0.05; ** statistically significant at p<0.001*

Concerning the course of skin disease, persistence or progression of cutaneous lesions was observed in 20 cases (20/41); however the lesions fully resolved in 21 cases (21/41). At the last evaluation, 15 patients had on-going systemic sarcoidosis. One patient died of liver cirrhosis caused by the hepatitis C virus, and another patient died of breast cancer.

In terms of treatment received and response to treatment, our data are limited, as records, particularly documenting treatment response, were sometimes incomplete. Intralesional or high-potency topical corticosteroids were the most frequent treatments used (31 patients), followed by systemic corticosteroids (18 patients), chloroquine phosphate (5 patients), methotrexate (3 patients), doxycycline (2 patients), metronidazole (1 patient), and thalidomide (1 patient).

3.2 Histological outcomes

A total of 42 formalin-fixed and paraffin-embedded skin biopsy specimens from the 41 patients with specific sarcoidosis skin lesions were reviewed. Forty patients had a single cutaneous biopsy, and one patient had two biopsies performed on separate occasions. The pathological findings are summarized in Table 7.

	N	%
Epidermis		
Atrophy	13	31
Acanthosis	11	26.2
Focal lichenoid infiltrate	1	2.4
Normal	17	40.4
Type of granuloma		
Naked granuloma with few lymphocytes	25	59.5
Naked granuloma without lymphocytes	17	40.5
Granulomatous infiltrate		
Mild	10	23.8
Moderate	17	40.5

Severe	15	35.7
Deepness		
Superficial dermis	6	14.3
Superficial and mid dermis	16	38.1
Mid dermis	5	11.9
Entire dermis	13	30.9
Hypodermis	2	4.8
Distribution		
Perivascular	3	7.1
Periannexial	4	9.5
Perivascular and Periannexial	7	16.7
Irregular/Interstitial	28	66,7
Coalescence		
Present	32	76.2
Absent	10	23.8
Multinucleated giant cells		
Absent	1	2.4
Mild	34	80.9
Moderate	7	16.7
Foreign material		
Present	10	23.8
Absent	32	76.2
Asteroid bodies		
Present	1	2.4
Absent	41	97.6
Schaumann bodies		
Present	2	4.8
Absent	40	95.2
Grenz zone		
Present	20	47.6
Absent	22	52.4

Table 7. *Pathological findings of 42 skin biopsy specimens of cutaneous sarcoidosis*

3.2.1 Epidermis

Twenty-five specimens (60%) showed epidermal changes, including slight epidermal atrophy in 13 cases (31%), acanthosis in 11 cases (26.2%) and focal lichenoid infiltrate in 1 case (2.4%).

3.2.2 Characteristics of the granulomatous infiltrate

The extent of the granulomatous infiltrate was mild in 10 cases (23.8%), moderate in 17 cases (40.5%), and severe in 15 cases (35.7%). Sarcoidal granulomas were located in the superficial dermis in 6 of 42 biopsies (14.3%), in the superficial and mid dermis in 16 cases (38.1%), in the mid dermis in 5 cases (11.9%), in the entire dermis in 13 cases (30.9%), and hypodermis in 2 cases (4.8%) (Fig. 11). The presence of a grenz zone (Fig. 12) was observed in 20 cases (47.6%).

Perivascular distribution of granulomas was seen in three cases (7.1%) (Fig. 13) and periannexial distribution in four samples (9.5%). However, in 7 cases, we observed perivascular and periannexial distribution, simultaneously (16.7%). In the remaining cases (66.7%), the distribution was irregular/interstitial.

Concerning the two cases of subcutaneous fat involvement, they showed a lobular distribution as well as a septal distribution.

Coalescence of granulomas was observed in 32 biopsies (76.2%).

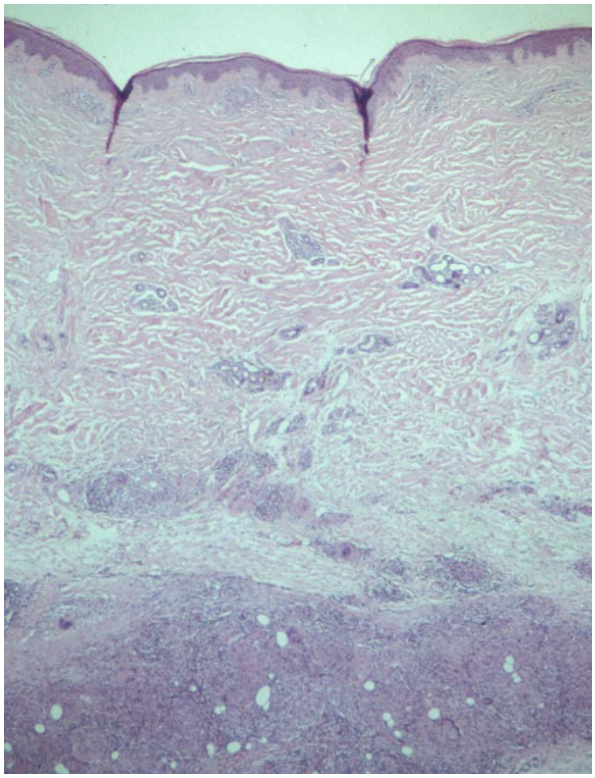


Fig. 11. *Hypodermis involvement of sarcoidal granulomas (HE; x50)*

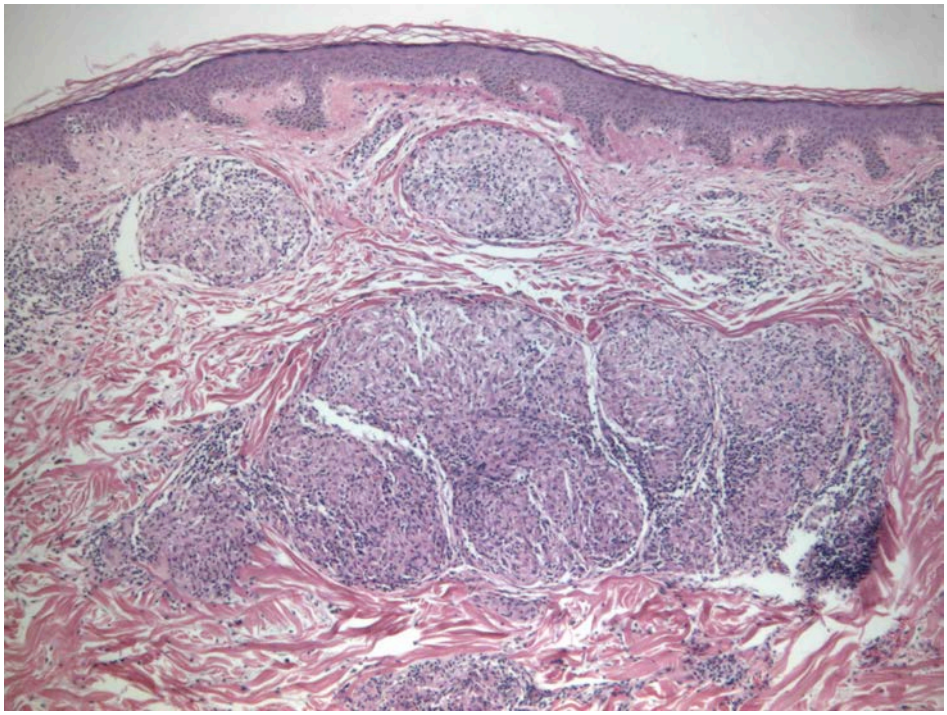


Fig. 12. *Histopathology in cutaneous sarcoidosis shows naked sarcoidal granulomas and the presence of a Grenz zone (hematoxylin and eosin stain; x50).*

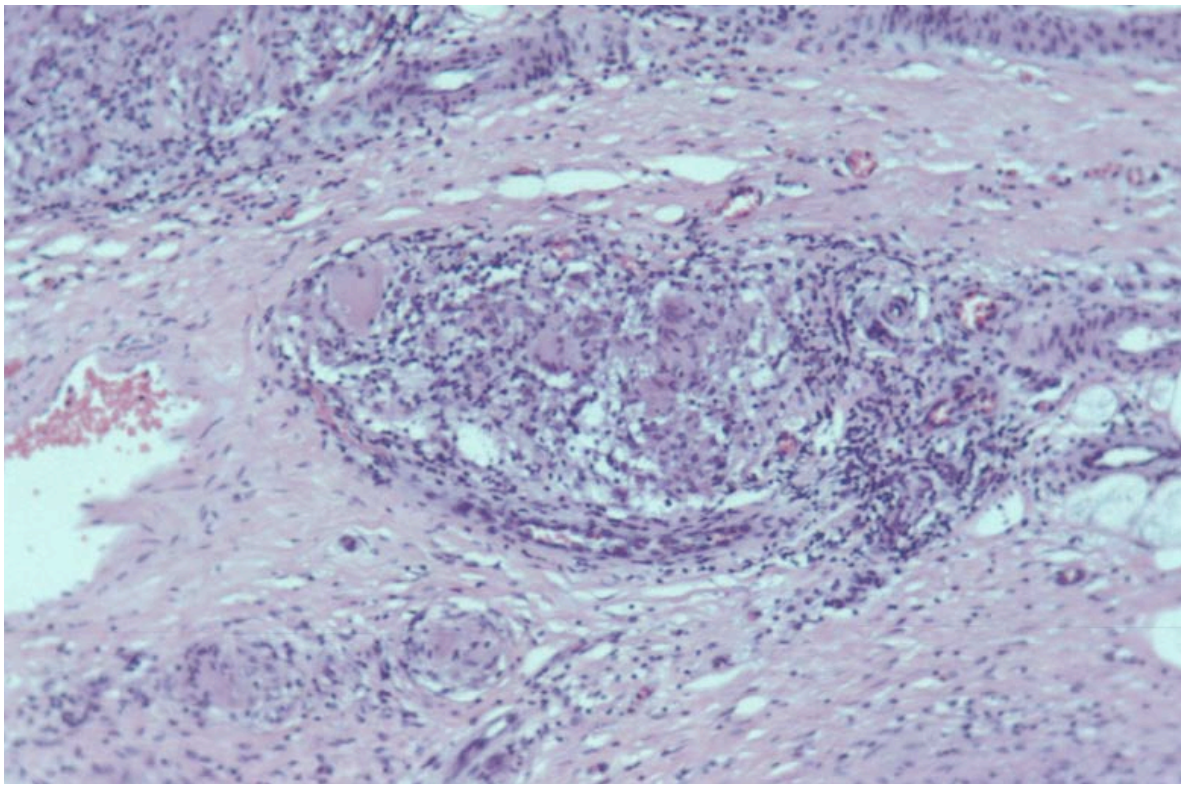


Fig. 13. *Sarcoidal granulomas with perivascular distribution (HE; x100)*

3.2.3 Characteristics of the granulomas

The inflammatory infiltrate consisted of nodular aggregates of epithelioid histiocytes with variable amounts of multinucleated giant cells and lymphocytes. In all cases, naked sarcoidal granulomas, with or without few lymphocytes, were present in the absence of mycobacterial and fungal organisms (Fig. 12).

Multinucleated giant cells were found in 41 biopsies (98%). The presence of these cells was mild in 34 cases (81%) and moderate in 7 (17%).

Focal fibrinoid necrosis among granulomas was present only in one case. We didn't observe any case with the presence of fibrosis.

Foreign material was detected in 10 biopsies (23.8%) without the use of polarized light microscopy. Schaumann bodies were observed in 2 cases, and Asteroid bodies were found in 1 case (Fig. 14).

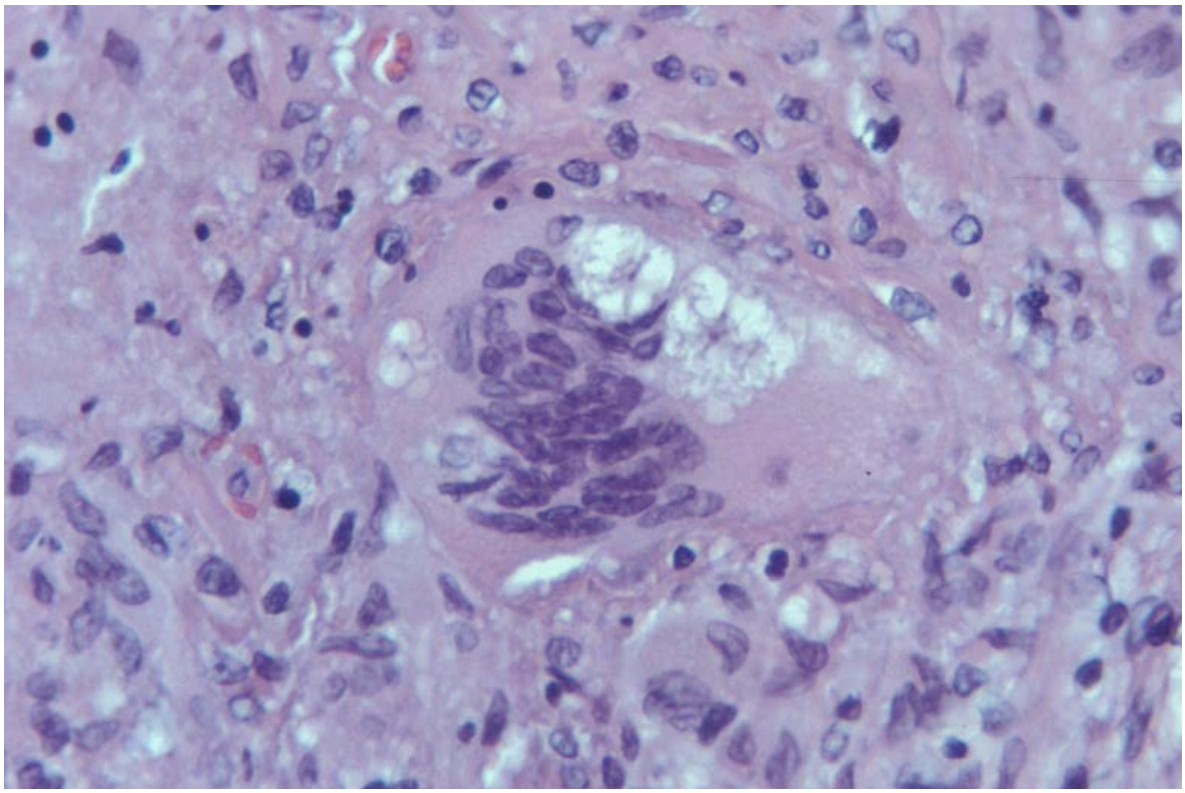


Fig. 14. *Asteroid bodies in multinucleated giant cells (HE; x200).*

3.3 Clinicopathological correlation

Concerning the clinicopathological correlation, we identified that more superficial (maculopapular) skin lesions also had more superficial granulomas, whereas the infiltrated lesions, especially the nodular form, presented with granulomas deep in the dermis. However, plaques, lupus pernio, and scar sarcoid lesions could contain granulomas in any localization. Interestingly, we also noted that the number of granulomas correlated well with a more or less extensive clinical presentation, as well as with the clinical course of skin disease (Table 8).

		Mild granulomatous infiltrate (n=10)	Moderate/severe granulomatous infiltrate (n=32)
Clinical presentation	**Isolated or localized skin lesions**	7	11
	Multiple or generalized skin lesions	3	21
Course of skin disease	**Complete resolution of skin lesions**	7	14
	Persistence or progression of skin disease	3	18
Systemic involvement		5/10 (50%)	20/32 (62.5%)

Table 8. *Cutaneous sarcoidosis: relation between the extension of the granulomatous infiltrate and the severity/extension of the clinical presentation, clinical course of skin disease and systemic involvement.*

4. DISCUSSION

4.1 Clinical outcomes

Sarcoidosis affects all races and ages and both sexes, with a slight preponderance among females.[18] This female predominance is particularly high (ranging from 71–76%) in patients with CS.[20-23] In our study, there was a marked female predominance (83%), which is consistent with other series. In Hanno's investigation,[116] 83% of the patients were female, and in Yanardag's[82] series of 170 patients with CS, 80% of the patients were female. Estrogen may play a role in the development of skin lesions,[20] which could explain the predominance of women in reports of CS. Another factor which may explain this difference between the two sexes, is that women may be more likely than males to notice and report cutaneous lesions.

CS lesions are classified as "specific" (i.e., the histological examination shows typical sarcoid granulomas) or "nonspecific" (i.e., a nondiagnostic inflammatory reaction pattern is observed on histological evaluation). Macular and papular lesions are the most common specific cutaneous manifestations of sarcoidosis.[74,76,77] In our cases, macules and papules were the most common type of cutaneous lesion (56%). It is worth noting that cutaneous involvement in sarcoidosis can manifest through multiple skin lesions. In addition, different clinical forms can occur in the same patient during the course of the disease, as observed in our study. The association of different clinical manifestations in the same patient makes the classification of the disease more difficult.

With respect to the distribution of the lesions, the lower limbs (63.4%) and the head and neck region (46.3%) were the most frequent localizations in our series. In western reports,[20,22] approximately 25% of CS occurs in the head region. In contrast, head involvement was much more frequent (46.3%) in our series; similarly, high rates have been reported in other series from Taiwan (50–58%)[146-148] and India (65%).[149]

Skin involvement occurred at the onset of sarcoidosis in the majority of patients (87.5%), as previously described.[21,74] This fact provides a valuable opportunity for early diagnosis of this disease due to the accessibility of skin biopsy.

Systemic involvement was detected in 58.5% of our patients. The lung (91.6%) and lymph nodes (41.6%) were the two most commonly affected extracutaneous organs. In contrast,

in 17 out of the 41 cases (41.5%), demonstrable sarcoidosis skin involvement was documented without extracutaneous symptoms. In these 17 patients, despite the exhaustive investigation and an extended follow-up (medium: 4 years), systemic symptoms were not detected. Therefore, these patients did not meet the diagnostic criteria for sarcoidosis and were considered to have an isolated cutaneous sarcoid. There are currently no established guidelines for monitoring patients for the development of extracutaneous sarcoidosis who have disease limited to the skin. Our results suggest that extracutaneous symptoms might occur much later (>4/5 years) in some patients with apparently isolated cutaneous disease. In view of this observation, the authors do not recommend performing open follow-up appointments in these patients (especially those with macules and papules) but rather conducting annual periodic screening in dermatology consultations to detect any possible systemic involvement in the early stages. However, our findings are consistent with Veien's report[117] and other series.[116,150] Hanno and colleagues[116] found that 33% of the patients had no systemic involvement; in a series of 25 patients in Singapore,[150] 60% had no systemic involvement.

Concerning the prognostic significance of specific skin lesions, in our study, we found that the macular and papular variant had a better prognosis. This variant was associated with systemic involvement in 56.5% of patients (13/23), with radiological stage 0/I in 17/23 patients and a duration of <2 years of sarcoidosis activity in 10/23 patients. In contrast, our 4 cases of lupus pernio were associated with systemic involvement (4/4), radiological stage II (3/4), and a duration of >2 years of sarcoidosis activity. This observation was also reported previously.[74,82] In addition, we found that as in lupus pernio, plaque-type and nodular lesions were more common in chronic forms of the disease and were more persistent, as shown in Table 5. These observations are also consistent with previous reports.[74,82,151]

Serum ACE levels are elevated in 30–80% of patients with sarcoidosis.[114,152-154] However, ACE elevations are not specific for sarcoidosis alone as many diseases have this feature.[114,152-154] Thus, the value of serum ACE levels in the diagnosis or management of sarcoidosis remains controversial. Elevated levels of serum ACE were observed in 46.4% of our patients (13/28). We also noted that systemic disease was identified more commonly in patients with raised serum ACE levels (84.6% vs. 40%) ($p<0.05$).

In our study, complete resolution of skin lesions was not uncommon (21/41). Curiously, the treatment response in our sample depended upon the association with systemic symptoms (Table 6). Among the 21 patients who showed complete resolution of cutaneous lesions, 13 had no associated systemic symptoms ($p<0.05$). Our study also indicated that patients with CS lesions that were associated with systemic symptoms had a more chronic form of the disease than patients with only cutaneous lesions (91.6% vs. 29.4%) ($p<0.001$) (Table 6).

4.2 Histological outcomes

Epidermal changes are usually non-specific and consequently of little diagnostic value in sarcoidosis. However, they are relatively frequent, as previously described.[155] We found epidermal alterations in 25 specimens (60%), mostly atrophy (13 cases) and acanthosis (11 cases).

Histologically, we found that in all cases in our study, naked sarcoidal granulomas were present in the absence of mycobacterial and fungal organisms. This finding confirms that typical naked non-caseating granulomas are a sensitive and characteristic histological finding in patients with CS. However, this type of granuloma is not specific for sarcoidosis and may be found in other diseases.[20]

Perivascular and/or periannexial distribution of granulomas was observed in 14 cases (33.3%). Perineural granulomas have been recently described in CS;[20,22,89,156] however, in our study, we did not observe granulomas with perineural tropism.

In our series, the location of the granulomas correlated well with the clinical form; the location was deeper for more infiltrated skin lesions, particularly the nodular form. These findings were also previously reported.[21,22,156] A novel interesting histopathological feature observed in our study was the correlation between the number of granulomas and the severity and extension of the clinical presentation. We found that patients with a moderate/severe granulomatous infiltrate in their biopsies had a more severe clinical presentation during the course of the disease, with a more generalized skin involvement (Table 8). Additionally, we also noted a correlation between the extension of the granulomatous infiltrate and the clinical course of the skin disease. Of the 10 patients with

mild granulomatous infiltrate in their biopsies, 7 of them showed complete resolution of skin lesions (Table 8). To the best of our knowledge, these correlations have never been reported in cutaneous sarcoidosis. Thus, in our opinion, these findings are very important because they could modify our clinical approach, particularly with regard to the follow-up, early diagnosis, and treatment of CS.

Another interesting histopathological finding observed in our study was the presence of a grenz zone (Fig. 12) in 20 cases (47.6%). Ishak *et al.*[156] recently described this feature in cutaneous sarcoidosis. Our case is the second study to evaluate the presence of a grenz zone in skin biopsies of CS. The prevalence of this finding in our study was considerably higher than that of the results published by Ishak *et al*. (47.6% vs. 5%). Although this histological finding is also present in other pathologies, we suspect this parameter may be much more frequent in cutaneous sarcoidosis than previously thought. Thus, we suggest that the histopathological spectrum of cutaneous sarcoidosis should be expanded to include the presence of a grenz zone.

Schaumann bodies were observed in 2 cases and Asteroid bodies only in 1 case, indicating that these histological findings are insensitive and non-specific.[20] In contrast, the presence of foreign material could be demonstrated in 23.8% of our biopsies, which is consistent with other reports (22–77%).[20,157] Foreign bodies may serve as an inciting factor to induce granuloma formation,[20,157] and the presence of either birefringent or non-birefringent foreign bodies should not be interpreted as evidence to exclude the diagnosis of sarcoidosis.[20-22,148] In fact, sarcoidosis and foreign material can exist together.[157-159]

Focal fibrinoid necrosis among granulomas was present only in one case. Obviously, when necrosis is found within granulomas, caution needs to be taken in excluding an infection, particularly tuberculosis.[20] However, we concur with other authors that the presence of this uncommon histological feature should not automatically exclude the diagnosis of sarcoidosis.[19]

PHASE 2: ASSOCIATION BETWEEN SARCOIDOSIS AND INFECTIOUS AGENTS: A SYSTEMATIC REVIEW AND META-ANALYSIS

PHASE 2: ASSOCIATION BETWEEN SARCOIDOSIS AND INFECTIOUS AGENTS: A SYSTEMATIC REVIEW AND META-ANALYSIS

1. INTRODUCTION

During the last few years, investigators have debated the role that infectious agents may have in sarcoidosis pathogenesis. Currently, some clinical trials are being conducted in order to evaluate the role of antimicrobial agents in the course and prognostic of sarcoidosis.

Meta-analysis is a statistical method that integrates the results of a number of different studies in order to provide a common conclusion. It remains an essential tool for summarizing previous studies until large studies become available.

In the literature there are only two relevant meta-analyses, one about Mycobacteria[160] and the other about *Propionibacterium acnes*[161], which address the causal relationship of these infectious agents in sarcoidosis. Since then, more than 20 new investigations have been published, thus adding new relevant data to the discussion. This meta-analysis is the first to evaluate all infectious agents that may be involved in sarcoidosis.

2. MATERIAL AND METHODS

2.1 Search strategy

This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA-P 2015) statement from the Cochrane collaboration guidelines.[162] Since this study was a literature review and meta-analysis of previously reported studies, ethical approval or additional consent from participants was not required. Four different databases (Medline, Scopus, Web of Science and Cochrane Database) were searched for all original articles without language restriction published from January 1980 to May 2015, using the search strategy described in Table 9.

Search Strategy for MEDLINE via OVID	
1	exp SARCOIDOSIS/
2	sarcoid$.mp.
3	1 or 2
4	exp INFECTIONS, BACTERIAL/
5	exp BACTERIA/
6	infectio$.mp.
7	mycobacteri$.mp.
8	propionibacterium.mp.
9	exp DISEASES, VIRUS/
10	exp VIRUSES/
11	virus$.mp.
12	herpesvirus.mp.
13	exp MYCOSES/
14	fungus.mp.
15	exp DISEASES, PARASITIC/
16	parasit$.mp.
17	4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16
18	3 and 17
+ RCT filter tested by Cochrane Collaboration	

Table 9. *Search strategy for MEDLINE via OVID.*

2.2 Inclusion criteria

The inclusion criteria were as follows: (i) the diagnosis of sarcoidosis was made according to the classical criteria: a compatible clinical and radiological picture, histopathological demonstration of non-caseating granulomas with negative stains for mycobacterium and fungi, and exclusion of other granulomatous diseases;[5] (ii) case-control studies that reported the presence of microorganisms in samples, both histological and cellular, of patients with sarcoidosis, using either culture methods (direct isolation of the organism) or molecular biology techniques (analysis of DNA, RNA or proteins); (iii) odds ratios (OR) and the corresponding confidence intervals (CI) or sufficient information to calculate them; (iv) patients without sarcoidosis were used as a reference group.

2.3 Exclusion criteria

Studies involving other techniques (e.g. ELISA, immunohistochemistry and immunofluorescence) were excluded from the analysis.

2.4 Data extraction

First, two independent authors (T. Esteves and V. Garcia-Patos) reviewed all titles and abstracts. A second selection was based on a full-text review of potentially relevant articles and any disagreement was resolved by discussion between the three authors of this meta-analysis. A standardized data collection form was used to extract the following items: author(s), title of article, study design, year of publication, country of origin, study size, details of molecular or other techniques used.

2.5 Statistical analysis and methodological quality assessment

The measure of interest was the OR and 95% CI calculated from each study, in order to assess the presence of microorganisms in sarcoidosis samples versus controls. Data analyses were performed using Stata Statistical Software 2015 (StataCorp LP, College Station, Texas, USA). We used a random-effects model to calculate the OR and 95% CI from each study.[163]

We assessed the heterogeneity among studies using Cochran's Q test,[164] complemented by the I^2-test.[165] An I^2 value of 76-100% represents high heterogeneity, 51-75% moderate heterogeneity and 0-50% low or insignificant heterogeneity.[165] If the result of the Chi-square heterogeneity test was not significant (p>0.10), we used the fixed-effects model described by Mantel and Haenszel[166] to calculate the pooled OR estimate. Additionally, sensitivity and subgroup analyses were performed in order to explore the heterogeneity among studies.

3. RESULTS

3.1 Studies included

A total of 2,465 articles were identified from the initial electronic search using the outlined search term parameters (Table 9). Among these, 2,401 studies were excluded because they did not meet the inclusion criteria. A total of 64 articles were identified as investigating the role of infectious agents in sarcoidosis using either microbial culture or molecular methods. Six of these were later excluded since they were descriptive studies without a control group. Therefore, 58 case-control studies were qualified for the analysis according to the inclusion and exclusion criteria. Figure 15 summarizes the study flow.

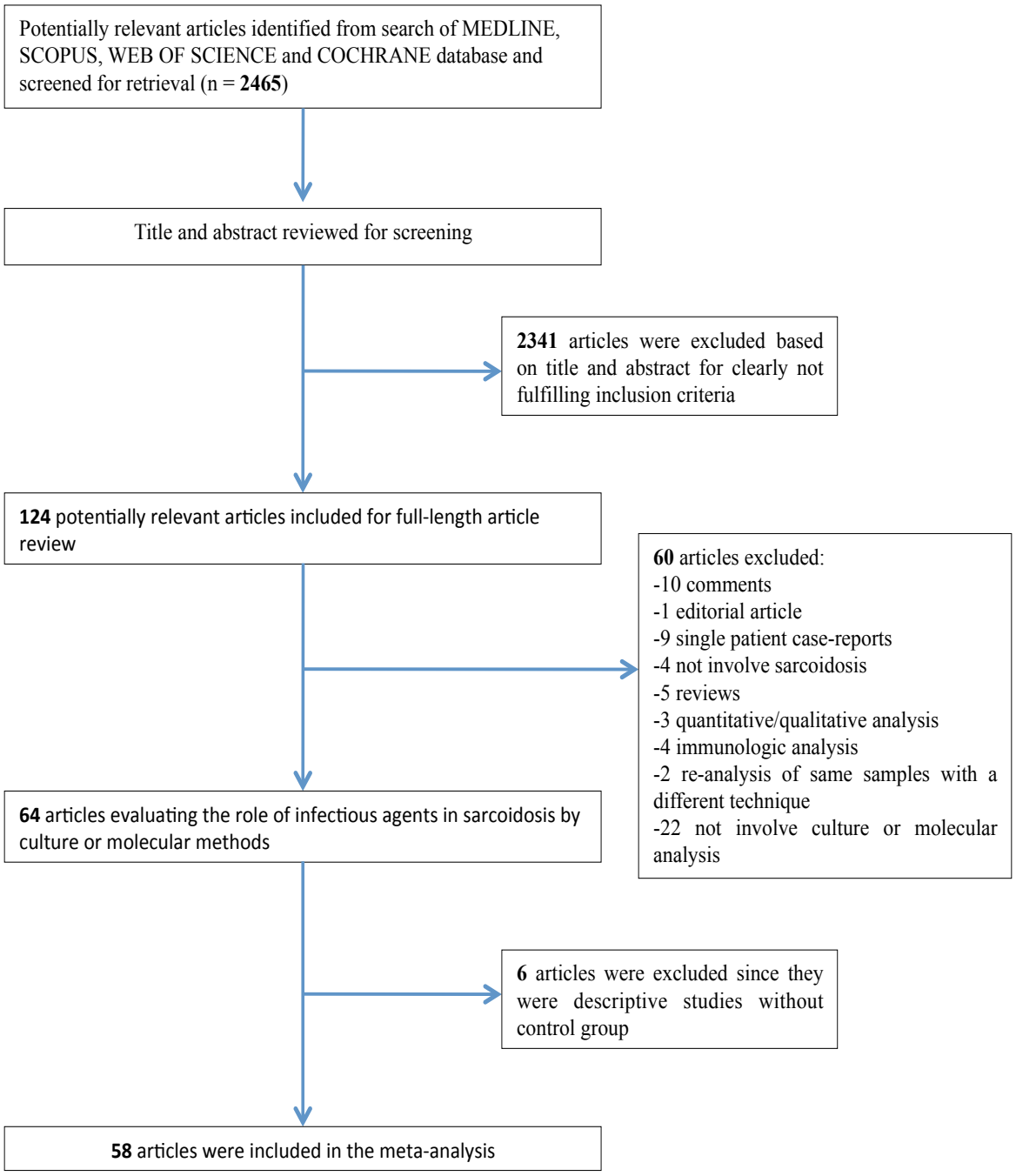


Fig. 15. *Flow diagram of the current meta-analysis*

In total, the 58 studies involved 2,467 samples from patients with proven sarcoidosis and 3,656 samples from control patients with other non-sarcoid disorders. All studies used molecular techniques to identify the different types of infectious agents except for two, which used microbial culture in their analyses.[167,168]

With regard to the infectious agents investigated, 36 studies evaluated the presence of mycobacteria[61,69,133,147,168-199] (Table 10), 11 evaluated *P. acnes*[69,133,167,170,172,178,182,200-203] (Table 11), 7 evaluated human herpesvirus-8 (HHV-8)[170,184,204-208] (Table 12), and 6 evaluated *Borrelia* species[135,209-213] (Table 13). Other infectious agents were investigated in some of the studies included, but there were insufficient cases to perform a meta-analysis. Three studies evaluated the presence of *Rickettsia* species, and one found a strong association between *Rickettsia helvetica* and sarcoidosis[214] (OR 21.72; CI:1.23–384.74). The second study did not reveal a significant association[134] (OR 0.43; CI:0–23.23), while in the third, all real-time PCR analyses for the detection of Rickettsia were negative.[215] None of the studies reported a significant association with *Chlamydia pneumonia,*[138,139,216] Epstein-Barr virus,[184] or retrovirus.[217]

Table 10. *Case-control studies evaluating the role of mycobacteria in sarcoidosis.*

First author/Year (Ref.)	Country	Molecular Technique	Sarcoidosis Patients		Non-sarcoidosis Controls		OR (95% CI)
			n/N	Type of microorganisms	n/N	Type of microorganisms	
Bocart, 1992 (171)	France	PCR of 65 kDa mycobacterial antigen and IS6110	2/22	MTBC	0/22	-	5.49 (0.25-121.18)
Hofland, 2014 (168)	Netherlands	NAAT for Mycobacteria and Culture	0/32	-	2/86	1 MTBC, 1NTM	0.52 (0.02-11.13)
Robinson, 2013 (172)	USA	PCR for 16S rDNA, hsp65 and rpoB	2/30	NTM	1/30	NTM	2.07 (0.18-24.15)
Oswald-Richter, 2012 (133)	USA	MALDI-IMS for ESAT-6	5/15	Mycobacterium spp	0/4	-	4.71 (0.21-104.49)
Svendsen, 2011 (173)	Denmark	BD ProbeTec IS6110 amplification	1/52	MTBC	0/50	-	2.94 (0.12-73.93)
Mootha, 2010 (174)	India	PCR of 65 kDa mycobacterial antigen and IS6110	13/27	10 MTBC, 3 NTM	2/40	NTM	17.64 (3.53-88.25)
Zhou, 2008 (175)	China	Real-time PCR of IS986 and human β-globin gene	20/104	MTBC	7/55	MTBC	1.63 (0.64-4.14)
Dubaniewicz, 2006 (176)	Poland	BD ProbeTec IS6110 amplification	3/50	MTBC	0/10	-	1.55 (0.07-32.27)
Fite, 2006 (177)	Spain	PCR of IS6110 and Southern blot hybridization	9/23	MTBC	1/23	MTBC	14.14 (1.61-124.11)
Yasuhara, 2005 (178)	Japan	PCR of IS6110	0/6	-	0/6	-	-
Song, 2005 (61)	USA	PCR of MTB 16S rRNA	6/16	MTBC	0/16	-	20.43 (1.04-401.67)
Marcoval, 2005 (179)	Spain	NAAT for rRNA of MTBC	0/35	-	0/39	-	-
Yu-Yun Lee, 2002 (147)	Taiwan	Nested PCR for mycobacterial hsp65 DNA	7/21	NTM	0/16	-	17.07 (0.89-325.59)
Drake, 2002 (169)	USA	PCR of 16S rRNA, rpoB and IS6110	15/25	11 MTBC, 3 NTM, 1 both	0/25	-	75.29 (4.12-1377.06)
Gazouli, 2002 (170)	Greece	PCR of IS6110/IS1245/IS900/IS901, 16S rRNA, MPB64 and mtp40	33/46	MTBC	0/20	-	101.74 (5.74-1804.62)
Eish, 2002 (69)	Japan	PCR of IS6110/IS900	5/108	MTBC	2/86	MTBC	2.04 (0.39-10.78)
Klemen, 2000 (180)	Austria	PCR of IS6110 and mycobacterial chaperonin	3/4	NTM	0/39	-	184.33 (6.26-5425.48)
Li, 1999 (181)	USA	PCR of 65 kDa mycobacterial antigen and RFLP analysis	16/20	2 MTBC, 14 NTM	0/20	-	150.33 (7.54-2997.83)
Ishige, 1999 (182)	Japan	PCR of IS6110	3/15	MTBC	1/15	MTBC	3.50 (0.32-38.23)

Wilsher, 1998 (183)	NZ	PCR of IS6110, nested PCR to amplify 85 bp sequence within the 123 bp product	0/31	-	0/10	-	-
Di Alberti, 1997 (184)	Italy	Heminested PCR for 16S rRNA	17/38	4 NTM, 13 Mycobacterium	39/113	39 Mycobacterium spp	1.54 (0.73-3.24)
Vokurka, 1997 (185)	France	PCR of IS6110 and DR region	0/15	-	0/27	-	-
Ozcelik, 1997 (186)	Turkey	PCR of IS6110	5/11	MTBC	2/15	MTBC	5.42 (0.81-36.36)
Popper, 1997 (187)	Austria	PCR of 65 kDa mycobacterial antigen and IS6110	11/35	NTM	0/39	-	37.08 (2.09-657.90)
El-Zaatari, 1996 (188)	USA	PCR of IS900/IS902, MAC-specific PCR assay and Western blot	7/7	NTM	13/38	NTM	28.33 (1.50-534.74)
Fidler, 1993 (189)	UK	PCR of 65 kDa mycobacterial antigen and IS6110	7/16	MTBC	1/16	MTBC	11.67 (1.23-110.95)
Thakker, 1992 (190)	UK	PCR of groEL	1/14	MTBC	1/11	MTBC	0.77 (0.04-13.87)
Gerdes, 1992 (191)	Germany	PCR of 16S rDNA	0/14	-	0/10	-	-
Mitchell, 1992 (192)	UK	Mycobacterial rRNA detection by liquid phase hybridization	5/5	MTBC	0/5	-	121 (2.02-7259.18)
Saboor, 1992 (193)	UK	PCR of IS986/IS6110 and groEL	14/20	10 MTBC, 4 NTM	5/22	3 MTBC, 2 NTM	7.93 (1.99-31.59)
Lisby, 1993 (194)	Denmark	Nested PCR for IS900	0/18	-	0/18	-	-
Grosser, 1999 (195)	Germany	PCR of IS986/IS6110	35/65	MTBC	1/34	MTBC	38.50 (4.96-298.57)
Vago, 1998 (196)	Italy	PCR of IS6110	2/30	MTBC	0/17	-	3.07 (0.14-67.75)
Richter, 1996 (197)	Germany	PCR of mycobacterial 16S rDNA	1/24	MTBC	3/57	MTBC	0.78 (0.08-7.93)
Ghossein, 1994 (198)	USA	PCR of 65 kDa mycobacterial antigen	0/10	-	0/10	-	-
Cannone, 1997 (199)	Italy	PCR of IS6110	2/30	MTBC	0/10	-	1.84 (0.08-41.62)

n: Mycobacteria-positive samples; **N**: total samples; **PCR**: polymerase chain reaction; **65 kDa**: 65-Kilodalton mycobacteria antigen; **IS6110**: insertion sequence to identify *Mycobacterium tuberculosis* complex (MTBC); **NTM**: non-tuberculous mycobacteria; **NAAT**: nucleic acid amplification test; **16S rDNA**: ribosomal DNA common to all mycobacteria; **rpoB**: RNA polymerase β-subunit gene; **MALDI-IMS**: matrix-assisted laser desorption ionization as a mass spectrometry imaging; **ESAT-6**: 6 kDa early secretory antigenic target produced by Mycobacterium tuberculosis; **IS986**: insertion sequence to identify MTBC; **rRNA**: ribosomal RNA; **IS1245/IS900/IS901/IS902**: insertion sequence to identify *Mycobacterium avium* complex; **MPB64**: mycobacterial protein; **mtp40**: Specific primers of MTB species; **RFLP**: restriction fragment length polymorphism; **DR**: direct repeat; **groEL**: gene encoding 65kDa antigen.

Table 11. *Case-control studies evaluating the role of P. acnes in sarcoidosis*

First author/Year (Ref.)	Country	Molecular Technique	Sarcoidosis	Controls	OR (95% CI)
			n/N	n/N	
Robinson, 2013 (172)	USA	PCR for bacterial 16S rDNA	7/30	1/30	8.83 (1.01-76.96)
Oswald-Richter, 2012 (133)	USA	MALDI-IMS for propionibacterial proteins	7/15	1/4	2.63 (0.22-31.35)
Yasuhara, 2005 (178)	Japan	PCR for 16S rRNA	2/6	0/6	7.22 (0.28-189.19)
Gazouli, 2002 (170)	Greece	PCR for 16S rRNA	0/46	0/20	-
Eish, 2002 (69)	Japan	PCR for 16S rRNA	93/108	25/86	15.13 (7.39-30.99)
Ishige, 1999 (182)	Japan	Quantitative PCR for 16S rRNA	12/15	3/15	16 (2.67-95.75)
Negi, 2012 (200)	Japan	Immunohistochemical methods (PAG and TIG antibodies) and western blot	149/196	0/79	500.43 (30.44-8226.20)
Yamada, 2002 (201)	Japan	Quantitative real-time PCR for 16S rRNA	8/9	2/9	28 (2.07-379.25)
Eishi, 1994 (202)	Japan	PCR for P. acnes DNA	36/39	12/29	17 (4.23-68.28)
Abe, 1984 (167)	Japan	Isolation of P acnes in culture	31/40	38/180	12.87 (5.65-29.34)
Hiramatsu, 2003 (203)	Japan	Nested PCR for 16S rRNA	21/30	7/30	7.67 (2.42-24.24)

16S rDNA: ribosomal DNA; **MALDI-IMS**: matrix-assisted laser desorption ionization as a mass spectrometry imaging; **rRNA**: ribosomal RNA.

Table 12. *Selected studies on the association between HHV-8 and sarcoidosis*

First author/Year (Ref.)	Country	Molecular Technique	Sarcoidosis	Controls	OR (95% CI)
			n/N	n/N	
Knoell, 2005 (204)	USA	PCR for HHV-8 DNA	0/8	0/8	-
Gazouli, 2002 (170)	Greece	PCR for HHV-8 DNA	0/46	0/20	-
Fredricks, 2002 (205)	USA	PCR for HHV-8 ORF 26 DNA	0/18	0/4	-
Maeda, 2000 (206)	Japan	Hemi-nested PCR for HHV-8 DNA	4/119	4/120	1.01 (0.25-4.13)
Sugaya, 1999 (207)	Japan	Nested PCR for HHV-8 ORF 26 DNA	0/12	1/1	0.01 (0.00-0.95)
Bélec, 1998 (208)	France	Nested PCR for HHV-8 ORF 25/26 DNA	0/14	2/17	0.21 (0.01-4.84)
Di Alberti, 1997 (184)	Italy	Nested PCR for HHV-8 ORF 26 DNA and Heminested PCR for HHV-8 ORF 25 DNA	38/39	6/113	677.67 (79.01-5812.52)

HHV-8: Human Herpesvirus 8; **ORF 25/26 DNA**: insertion sequence to identify HHV-8

Table 13. *Selected studies on the association between Borrelia species and sarcoidosis*

First author/Year (Ref.)	Country	Molecular Technique	Sarcoidosis Patients		Non-sarcoidosis Controls		OR (95% CI)
			n/N	Type of microorganisms	n/N	Type of microorganisms	
Derler, 2009 (135)	Austria	Focus-floating microscopy and Borrelia-specific PCR DNA	13/35	Borrelia sp.	1/61	Borrelia sp.	35.45 (4.38-287.16)
Ishihara, 1998 (209)	Japan	Dot-blot analysis (Dotblot Borrelia Kit)	15/46	Borrelia sp.	2/100	Borrelia sp.	23.71 (5.14-109.46)
Martens, 1997 (210)	Germany	Western blot for Borrelia burgdorferi	1/60	Borrelia burgdorferi	27/1000	Borrelia burgdorferi	0.61 (0.08-4.57)
Lian, 1995 (211)	China	PCR for Borrelia burgdorferi DNA	6/49	Borrelia burgdorferi	2/28	Borrelia burgdorferi	1.81 (0.34-9.66)
Xu, 1996 (212)	China	In situ PCR for Borrelia burgdorferi DNA	0/23	-	0/23	-	-
Ishihara, 1996 (213)	Japan	Elisa and Dot-blot analysis for Borrelia sp.	1/38	Borrelia sp.	1/80	Borrelia sp.	2.14 (0.13-35.08)

3.2 Meta-analysis

3.2.1 Mycobacteria (Table 10)

Both *Mycobacterium tuberculosis* complex (MTBC) and nontuberculous mycobacteria (NTM) were investigated in most of the 36 relevant studies, although some used primers to detect only *M. tuberculosis,*[61,173,175-179,182,183,185,186,195,196,199] and others detected only nontuberculous mycobacteria.[188,194]

Figure 16 provides a forest plot for sarcoidosis and mycobacteria based on a total of 1,034 sarcoidosis patients and 1,054 controls. Of the 1,034 sarcoidosis cases, 173 were positive for MTBC, and 58 were positive for NTM. It was not possible to identify the type of mycobacteria involved in 18 samples, while both types of mycobacteria DNA were present in one sample. In total, 250 sarcoidosis samples were positive for some form of mycobacteria DNA sequence for a positive signal rate of 24.2%. We found a significant association between sarcoidosis and mycobacteria with an OR of 6.8 (95% CI:3.73–12.39). A strong association was also found between sarcoidosis and NTM alone with an OR of

10.39 (95% CI:5.25–20.56), as well as for *M. tuberculosis* complex (OR 4.29; CI:2.60-7.08). There was moderate heterogeneity among studies (I^2 test 52.1%; p=0.001), although all but three studies estimated a risk above unity with significance in most cases.

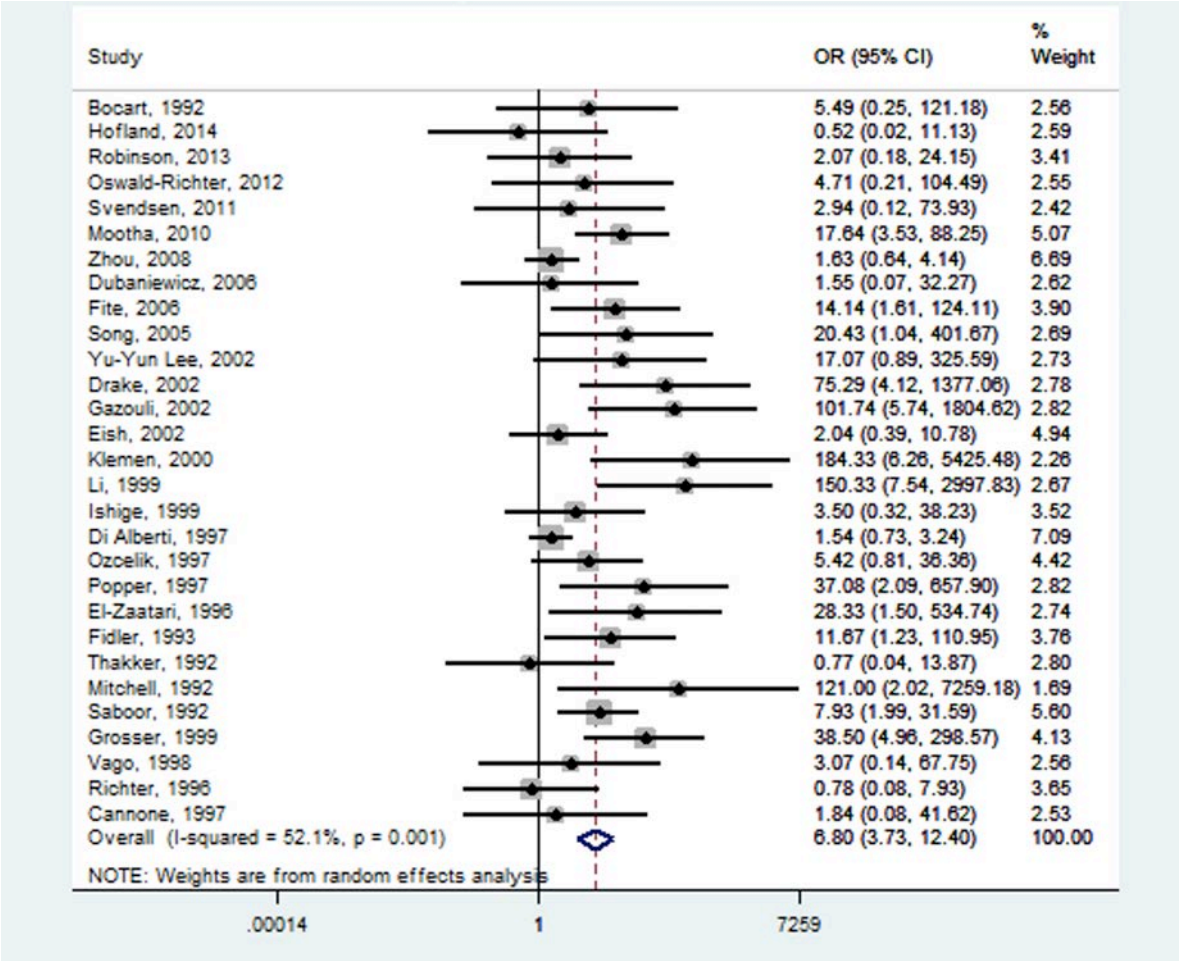


Fig. 16. *Forest plot of studies that show the presence of mycobacteria in sarcoidosis patients versus controls.*

3.2.2 P. acnes (Table 11)

The risk of sarcoidosis associated with *P. acnes* was provided by the study design (Fig. 17). The OR derived from 11 studies with 534 cases and 488 controls was 18.80 (95% CI:12.62–28.01), and there was low heterogeneity (I^2 test 25.9%; p=0.206). There was a positive signal rate of 68.54% for *P. acnes* (366 positive samples from 534 patients). When

accounting for the source of biological samples studied, we found that nine of the 11 studies[69,133,167,170,172,182,200-202] evaluated the presence of *P. acnes* in lymph node samples, of which seven evaluated this location exclusively.[69,167,172,182,201,202] This could justify the low heterogeneity among studies, contrary to what was observed in the forest plot of mycobacteria, where the studied biological samples were more heterogeneous.

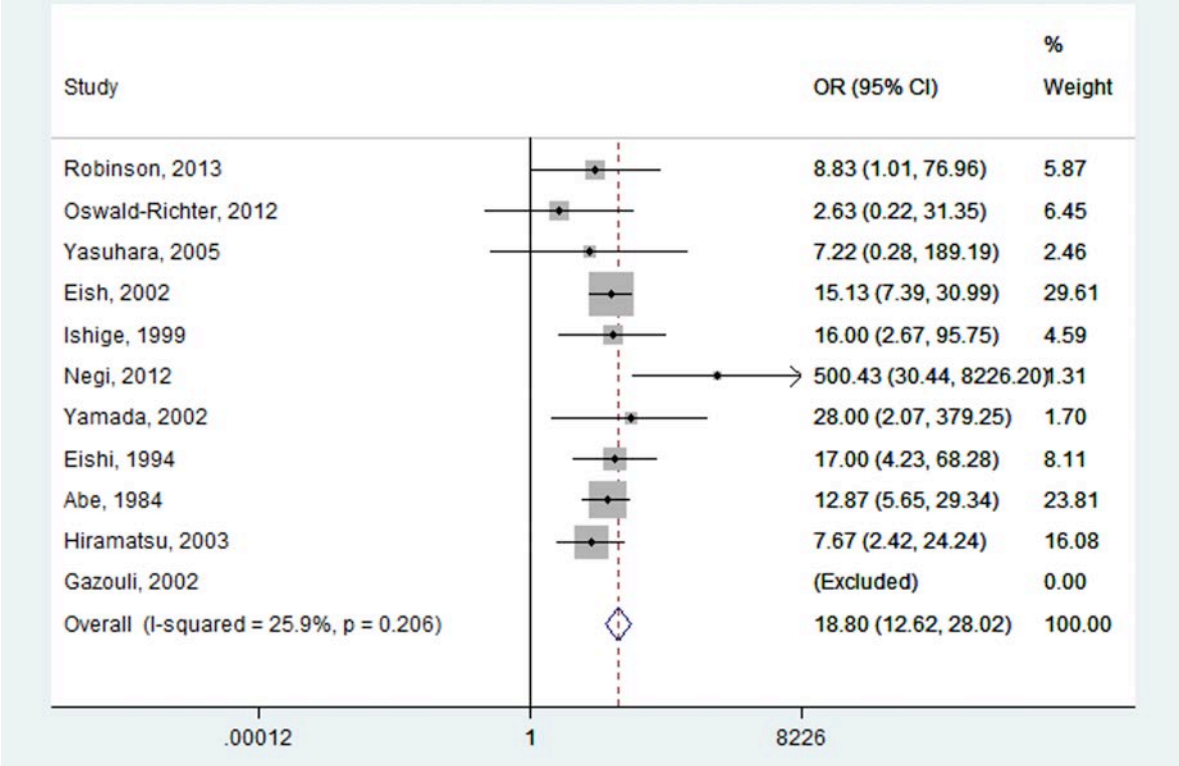


Fig. 17. *Forest plot of studies that show the presence of P. acnes in sarcoidosis patients versus controls.*

3.2.3 Borrelia and HHV-8 (Table 12 and 13)

Of the six articles assessing the presence of *Borrelia* in sarcoidosis tissues, three used polymerase chain reaction (PCR) techniques for DNA amplification of *B. burgdorferi*,[210-212] whereas the other three did not specify which species of *Borrelia* were involved.[135,209,213] The pooled OR derived from these six studies with 251 cases and 1,292 controls was 4.82, but this result did not reach statistical significance (95% CI:0.98–23.81). Statistical heterogeneity was moderate with an I^2 of 70% and p=0.01 (Fig. 18).

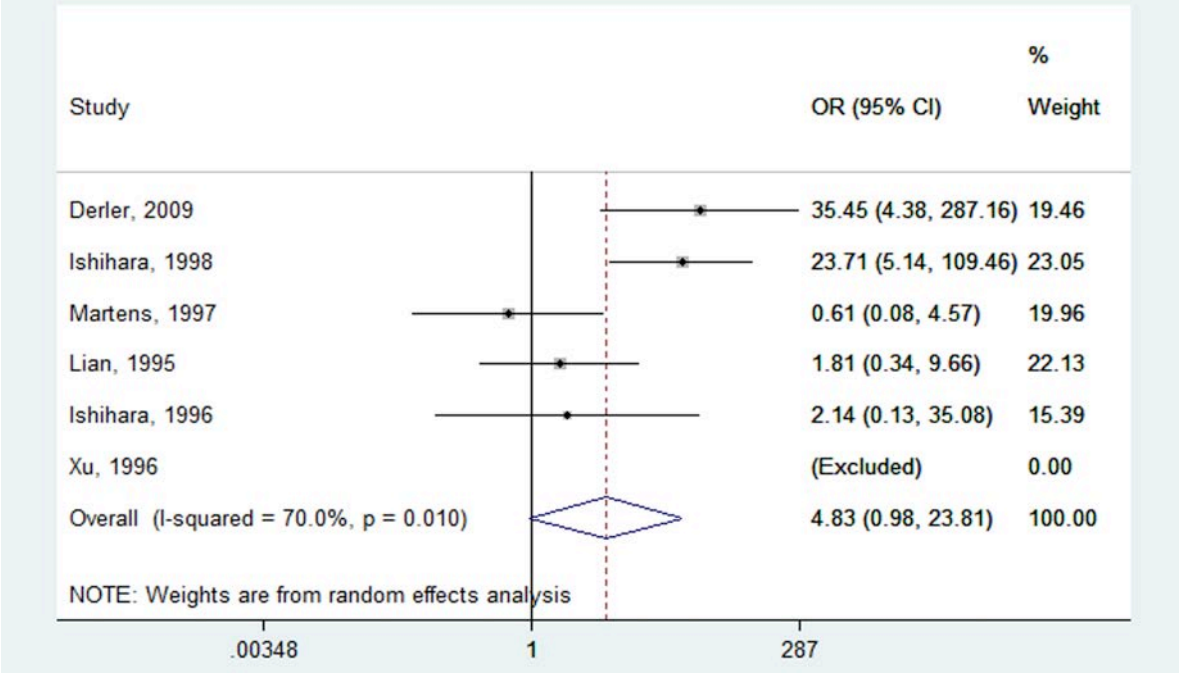


Fig. 18. *Forest plot: summary OR for the presence of Borrelia species in sarcoidosis patients*

Di Alberti et al[184] were the only ones to report a significant association between sarcoidosis and HHV-8 in comparison with controls. However, the remaining six studies refuted those results.[170,204-208] Overall, there was no significant association between sarcoidosis and HHV-8 (OR 1.47; CI:0.02–110.06), and there was high heterogeneity among studies (I^2 test 92%; p=0.000) (Fig. 19).

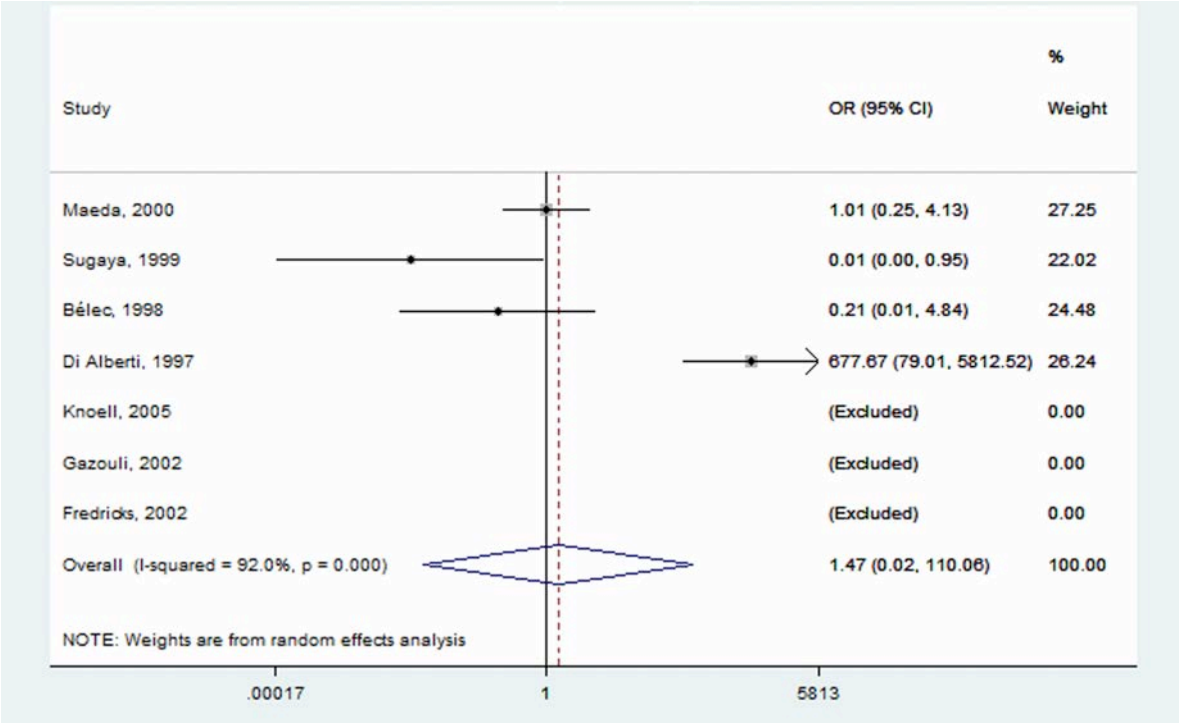


Fig. 19. *Forest plot: summary OR for the presence of HHV-8 in sarcoidosis patients Evaluation of publication bias.*

We performed funnel plots to evaluate publication bias (Fig. 20). The funnel plots of HHV-8 and mycobacteria showed evidence of publication bias (Fig. 20B and Fig. 20C), while the graphs regarding the presence of *Borrelia* and *P. acnes* are fairly symmetrical (Fig. 20A and Fig. 20D). Thus, no suggestion of publication bias is indicated in these cases.

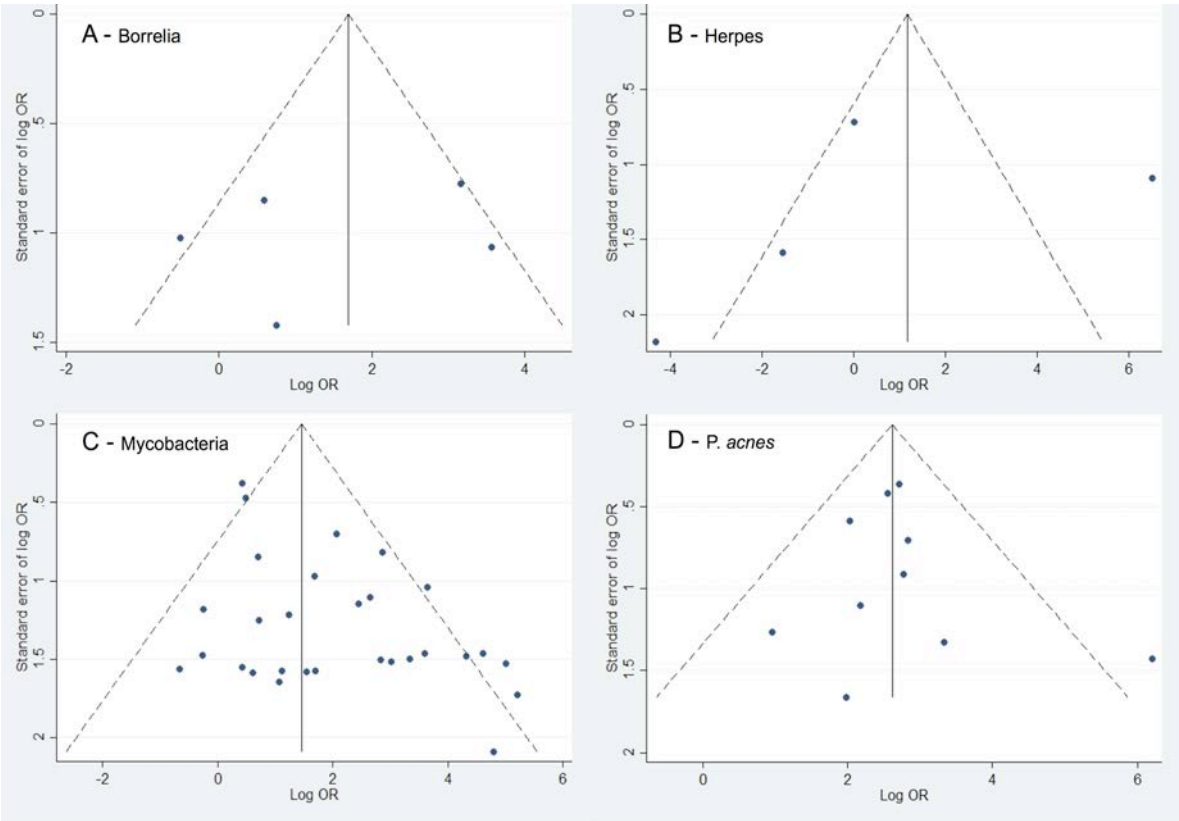


Fig. 20. *Funnel plot of all studies*

3.3 Sensitivity and subgroup analysis

To verify the robustness of the results, as well as the potential sources of heterogeneity, subgroup and sensitivity analyses were performed (especially for mycobacteria).

3.3.1 Subgroup analysis

Concerning the studies of mycobacteria, we conducted subgroup meta-analysis by various study characteristics (Table 14). The pooled OR was calculated in subgroups of studies according to geographical area, publication year, type of study, and molecular technique used. There was a significant association between sarcoidosis and mycobacteria in all subgroups, except in three studies included in the subgroup of molecular techniques (BD ProbeTec and culture). The pooled OR was significantly higher with some covariates, however almost all of the ORs derived from these subgroup data were significantly above unity.

Table 14. *Subgroup and sensitivity analysis of the association between sarcoidosis and mycobacteria.*

	No. of studies	OR (95%CI)	*P*-value heterogeneity	I^2 (%)
SUBGROUP ANALYSIS				
1 - Geographical region				
Europe	22	6.92 (3.05, 15.71)	0.004	53.8
USA	7	18.21 (4.64, 71.53)	0.238	26.2
Asia	7	4.09 (1.38, 12.12)	0.093	49.8
2 - Publication year				
<2000	20	6.63 (2.84, 15.51)	0.006	54.2
>=2000	16	8.40 (3.31, 21.31)	0.012	53
3 - Type of study				
Prospective	10	11.91 (4.94, 28.69)	0.743	0.0
Retrospective	26	6.41 (3.14, 13.09)	0.001	57.1
4 - Molecular technique				
PCR	29	7.04 (3.57, 13.89)	0.000	58.8
Hybridization	2	37.81 (3.40, 420.43)	0.484	0.0
Protein analysis	2	12.12 (1.44, 102.20)	0.408	0.0
BD ProbeTec	2	2.09 (0.23, 19.10)	0.776	0.0
Culture	1	0.52 (0.02, 11.13)	-	-
SENSITIVITY ANALYSIS				
1 - Biological samples				
Only lymph nodes	11	3.82 (1.53, 9.49)	0.384	4.0
Only lung	5	2.93 (1.09, 7.86)	0.098	56.9
Only skin	2	11.58 (0.06, 2016.91)	0.021	81.3
2 - Incidence of tuberculosis				
Only countries with low burden of TB	33	4.33 (2.06, 9.10)	0.042	45.7

CI: confidence interval, **OR**: odds ratio, **BD ProbeTec**: molecular detection based on strand displacement amplification (SDA) technology, **TB**: tuberculosis

Through the subgroup analyses, it was noted that the variables that most influenced the results of heterogeneity were: a) the study type, being null the heterogeneity in the 10 prospective studies, contrasting with the moderate heterogeneity in retrospective studies; b) the geographical location, verifying a low heterogeneity in studies conducted in the USA and Asia.

3.3.2 Sensitivity analysis

We also performed a sensitivity analysis to complement the subgroup analysis in order to better explain the heterogeneity between studies (Table 14).

Regarding sarcoidosis and mycobacteria, there was a strong and significant association (OR 4.33; CI:2.06–9.10) in subgroup analysis of geographic locations when we restricted to studies performed in countries with low burden of tuberculosis.[69,172,177,182,184,186,188-190,193,195,197] There was low heterogeneity among these studies (I^2 test 45.7%; p=0.042) (Table 14).

Another subgroup analysis compared the results according to the different biological samples used. Most of the studies included in our meta-analysis used biological samples from different locations, including the skin, lymph nodes, and lungs. However, when we restricted the analysis to include only studies that performed PCR on biological samples of the same type, the associations were also significant in both lung samples[177,184,189] (OR 2.93; CI:1.09–7.86; I^2=56.9%) and lymph node samples[69,172,182,184,190] (OR 3.82; CI:1.53–9.49; I^2=4%), but not skin biological specimens[181,184] (OR 11.58; CI:0.06–2016.91; I^2=81.3%). Once again, the heterogeneity among studies was low to moderate except in two studies performed on skin biopsies (Table 14).

In sensitivity analysis on studies of sarcoidosis and *P. acnes*, we found a significant association compared with the controls when we selected only studies performed outside Asia,[133,170,172] with a pooled OR of 5.5 (95% CI:1.13–27.42) and no heterogeneity among studies.

4. DISCUSSION

4.1 Meta-analysis

The present meta-analysis is the first to evaluate all infectious agents proposed to be associated with sarcoidosis and involving more than 6,000 patients in several countries. The results point to an etiological link between *P. acnes* and sarcoidosis with a positive signal rate of 68.54%. Also, almost one quarter of sarcoidosis patients show the presence of mycobacteria within the lesions. The associations are fairly specific, since *P. acnes* (OR 18.80) and mycobacteria (OR 6.8) were significantly increased in sarcoidosis patients, while *Borrelia* (OR 4.82; CI:0.98–23.81) and HHV-8 (OR 1.47; CI:0.02–110.06) were not associated with sarcoidosis, contrary to previous investigations.

Three decades ago, Abe et al[167] reported that *P. acnes* was the only bacterium isolated in lymph node biopsy samples taken from sarcoidosis patients. Studies published in recent years have confirmed that *P. acnes* could be a possible infectious agent implicated in the pathogenesis of sarcoidosis.[172,200,218-220] However, some studies suggest that *P. acnes* is not specific for sarcoidosis because it is a normal inhabitant of peripheral lung tissue and mediastinal lymph nodes, apart from the skin.[68] Despite this, the results of our meta-analysis show a significant quantitative difference in the presence of the *P. acnes* genome in sarcoidosis patients compared to control subjects. This suggests that this microorganism may be present abnormally or may proliferate ectopically in such sarcoid lesions.

However, it is important to note that most of the studies in our meta-analysis evaluating the role of *P. acnes* in sarcoidosis were by Japanese groups testing Japanese patients, while only very limited data exist for African American or Caucasian patients.[133,170,172] The results were conflicting in these three studies, but interestingly, the pooled OR was above unity and statistically significant (5.58; CI:1.13-27.42). Despite these surprising results, the ORs observed in studies with Japanese patients were far superior, and the results were more consistent and robust. Differences between these two groups may be due to the geographical, ethnic, or racial composition of the study population. Sarcoidosis in Japanese patients is characterized by a high rate of ocular, cutaneous, and cardiac involvement, while in Europe and the USA, this disease mainly affects the lungs.

In 2002, the first large, relevant study was published as a collaboration between several countries.[69] The results of this international study suggest an association between *P. acnes* and sarcoidosis in not only Japanese patients (positive signal rate of 89.2%), but also in Europeans (positive signal rate of 81.4%). However, more international corporative studies with quantitative PCR are needed to clarify the role of *P. acnes* in sarcoidosis and for better understanding of the phenotypic variability of this disease.

Recent years have witnessed substantial discussion among investigators about the role that mycobacteria may have in the pathogenesis of sarcoidosis, and the issue remains unsettled, if not controversial. With the emergence of new microbiological techniques, especially in the molecular biology area, several studies have been conducted in order to investigate this possible association more deeply.

In the present meta-analysis, we identified 36 studies assessing the presence of mycobacteria in a total of 1,034 sarcoidosis patients and 1,054 controls. The results suggest a strong association of sarcoidosis with NTM (OR 10.39; CI:5.25–20.56) and with MTBC (4.29; CI:2.60–7.08). However, to evaluate the possible relationship between mycobacteria and sarcoidosis, the current incidence of tuberculosis should be taken into account in general populations of the different countries where the studies of sarcoidosis were performed. In the sensitivity analyses, a significant association was also found (OR 4.33; CI:2.06–9.10) when we restricted the analysis to include only studies performed in countries with low prevalence of tuberculosis. This further confirms the robustness of the results and the relevance of this association worldwide.

Despite the heterogeneity of analyzed studies and the potential publication bias suggested by the mycobacteria funnel plots, most of the ORs derived from individual data were significantly above unity. Furthermore, sensitivity and subgroup analyses including only studies performed on lung samples or lymph nodes showed low heterogeneity. Therefore, it is important to account for the heterogeneity in sarcoidosis specimens (lung versus skin or lymph nodes). We found significant increased ORs in studies performed on lung or lymph node samples but not in skin specimens. Possible explanations for this include the following: 1) In the initial phase of the disease, systemic sarcoidosis primarily affects and spreads through the lymphatic system, following the lymphatic vessels to the hilar and mediastinal lymph nodes. 2) Lung and lymph node samples are obtained sterilely by endoscopy biopsies and thus avoid possible microorganism contamination, in contrast to

skin biopsies. 3) The two studies performed on sarcoidosis skin samples were both retrospective.[181,184] In such studies, there is a greater possibility of both contamination of the paraffin-embedded specimens and more DNA fragmentation. In contrast, several studies performed on lung and lymph node samples were prospective, and only fresh tissues were used. Additionally, when we conducted the subgroup analysis according to the type of study, it was found a low heterogeneity in the 10 prospective studies (I^2=0%), contrasting with the moderate heterogeneity in retrospective studies (I^2=57.1%).

The hypothesis that *B. burgdorferi* could be a possible causal infectious agent for sarcoidosis was first mentioned in 1989 in epidemiological studies.[221] Since then, several studies have been conducted using serological or molecular techniques in order to clarify the role of *Borrelia* in the pathogenesis of sarcoidosis. We identified six articles assessing the presence of *Borrelia* in sarcoidosis tissues using molecular techniques (251 cases and 1,292 controls), and we did not find a significant association (OR 4.82; CI:0.98–23.81). On the other hand, the two studies that reported a significant association between *Borrelia* and sarcoidosis[135,209] were both conducted in regions where Lyme disease is endemic, in contrast to the four other articles performed in non-endemic areas,[210-213] where the results did not reach statistical significance.

It is important to note that the frequency of exposure to *Borrelia spirochete* is different between patients living in regions where the disease is endemic and those in regions where it is not. Thus, in countries with elevated *B. burgdorferi* prevalence, a protective immunity against this microorganism has to be assumed in the general population. T-helper lymphocyte activity to this microorganism might be a trigger for the development of sarcoidosis in endemic regions, which could explain the positive results in studies published in Austria and Japan.[135,209] Apart from these two studies, the fact that significant positive PCR results could not be found argues against the hypothesis of a connection between *B. burgdorferi* infection and sarcoidosis. However, more studies are needed to clarify the possible association, especially in endemic areas.

4.2 Implications

There are several clinical implications of this study. Currently, immune suppression remains the primary treatment modality for sarcoidosis. Given our meta-analysis, it is worth exploring whether certain antibacterial or antimycobacterial drugs might alter the course of sarcoidosis. In the past, some clinical trials have been published with conflicting results using classical antituberculous drugs, such as isoniazid, amino-salicylic acid, and streptomycin.[222-225] Recently, Drake et al[226] conducted a double-blind, placebo-controlled study to investigate the efficacy of oral antimycobacterial therapy (levofloxacin, ethambutol, azithromycin, and rifampin) in patients with cutaneous sarcoidosis. The results were promising, with significant reductions in cutaneous lesion size. The same authors also conducted an open-label investigation using the same therapy regimen in pulmonary sarcoidosis patients, and the results were again very interesting with significant improvements in forced vital capacity from baseline to completion of therapy.[227]

Other antimicrobial agents such as minocycline and doxycycline have been shown to be quite effective in treating cutaneous sarcoidosis in some series.[228,229] However, the exact mechanism of action of these drugs it is not fully understood.[230]

Currently, other clinical trials are being done (NCT02024555 and NCT01245036) to clarify the role that antimicrobial agents might have in the treatment of sarcoidosis.

4.3 Limitations

Several limitations in our study should be recognized. First, one of the main potential limitations relates to the variability and heterogeneity of the results analyzed. It is important to consider that the majority of these studies were assessed retrospectively and that data were obtained from different databases and hospitals. This could lead to different types of bias in the included studies and to variability in the results. Second, the risk of contamination or DNA fragmentation in PCR techniques can lead to false positive or false negative results. In addition, PCR does not discriminate between living and dead microorganisms. Third, the patients had varied clinical manifestations of sarcoidosis; moreover, the non-sarcoidosis controls were comprised of different types of subjects across the studies, which may cause misclassification bias and heterogeneity.

PHASE 3: SARDOIDAL GRANULOMATOUS DERMATITIS AND MICROORGANISMS: A MOLECULAR APPROACH

PHASE 3: SARCOIDAL GRANULOMATOUS DERMATITIS AND MICROORGANISMS: A MOLECULAR APPROACH

1. INTRODUCTION

Typically found in sarcoidosis, sarcoidal granulomatous dermatitis may be also found in a wide variety of systemic and cutaneous diseases, including infections.[2] Some infectious agents, especially mycobacteria, fungi and *Leishmania* may also produce a sarcoidal reaction pattern.[2] Consequently, some clinicians may misinterpret this histological pattern as sarcoidosis, particularly in cases in which special stains and cultures for microorganisms are negative. Therefore, it is always important to remember that sarcoidosis is a diagnosis of exclusion.[5] Nowadays, given the emergence of new microbiological techniques, especially in the field of molecular biology, a significant number of cases that were previously interpreted as sarcoidosis may in fact be shown to be associated with other diagnoses, especially infectious disorders.[142,181,231-235]

Several epidemiological, immunological and molecular studies have been carried out in sarcoidosis, however no molecular studies exist on sarcoidal granulomatous dermatitis.

This study is the first described in the literature in evaluate the behavior of sarcoidal granulomatous dermatitis, its clinical, histological and molecular characteristics and its infectious causes and differential diagnoses.

2. MATERIAL AND METHODS

2.1 Patients and Samples

In this phase 3 of doctoral thesis, the study sample was distinct from phase 1. We included all patients with sarcoidal granulomas in their skin biopsies, over a longer period (1991-2014), which corresponded to patients not only with sarcoidosis but also with other clinical diagnosis.

The medical records corresponding to patients with skin biopsies diagnosed as sarcoidal granulomatous dermatitis were initially retrieved. Only those cases with demonstrable negative results in cultures for bacteria, mycobacteria and fungi were included in the study.

Sarcoidal granulomatous dermatitis was defined as circumscribed collections of epithelioid histiocytes with scarce mononuclear cell infiltrate ("naked" granulomas) and absence of necrosis and necrobiotic collagen.

A total of 48 formalin-fixed and paraffin-embedded skin biopsy specimens from 48 patients were included in the study. In each case, two pathologists independently reviewed the histopathological specimens. To exclude the presence of any infectious agent, special stains were also performed (i.e., Gram, Giemsa, Periodic Acid Schiff, Ziehl-Neelsen and Grocott's Methenamine Silver). All of the results were negative. When molecular biology techniques for microorganisms showed positive results, new microbiological stains were also performed, including immunohistochemical (IHC) detection of specific *Leishmania* spp. antigens.

2.2 Immunohistochemical analysis for detection of *Leishmania* amastigotes

Biopsy tissues were fixed in 5% buffered formalin, embedded in paraffin blocks, and cut into 4µm tissue sections. Samples were stained with hematoxylin and eosin (H&E) for routine diagnosis purposes.

Immunohistochemical detection of *Leishmania* amastigotes was performed on 4µm sections from formalin-fixed, paraffin-embedded tissues. The polyclonal antibody was obtained by immunization of rabbits from a *Leishmania* antigen. The antigen used for

immunized rabbit was a whole suspension of sonicated promastigotes of a *L. infantum* strain, zimodeme MON-1 at the concentration of 1 mg/ml (supplied by Department of Parasitology, Facultat de Farmàcia, Universitat de Barcelona, Spain).

Briefly, sections were deparaffinized and rehydrated through graded alcohol and water. Antigen retrieval with CC1 solution for 20 min was performed. Then, the sections were incubated in a humidified chamber with the antibody anti-*leishmania* diluted 1/1000 for 30min. Staining were performed with BenchMark XT (Ventana/Roche) using ultraVIEW Universal DAB Detection Kit (Ventana/Roche). Immunohistochemical procedures for the antibody were performed at the same time to avoid possible day-to-day variations in staining performance. Immunohistochemical analysis was performed in all samples by two dermatopathologist who were blinded to the patient groups.

2.3 DNA Extraction

DNA extraction from formalin-fixed, paraffin-embedded samples was performed according to the method described by Li *et al.*[181] with some modifications.

The DNA was extracted using the QIAsymphony DSP DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

To ensure PCR quality and to rule out contamination, the microtome blade was changed between each paraffin block. Additionally, both positive and negative genomic DNA controls were included in every batch of PCR. All of the tissue samples were also amplified with β-Actin primers (110-base-pair) as internal control to verify the adequacy and integrity of the template DNA.

The 16S rRNA primers used were of broad range for all bacteria. *Mycobacterium* spp. DNA was detected by hsp65 and/or 16S-23S rRNA gene primers. *Leishmania*-specific PCR primers were used to amplify a fragment of *Leishmania* kinetoplast DNA (kDNA) (Table 15).

Microorganism	Target	Primer sequence (5´- 3´)		Product size (bp)
		Forward	Reverse	
Panbacteria	16S rRNA	AGAGTTTGATCMTGGCTCAG	TACGGTTACCTTGTTACGACTT	Variable
***Mycobacterium* spp.**	hsp65	CGCCAAGGAGATCGAGCTGGA	GCCCTTGTCGAACCGCATACC	424
	16S-23S rRNA	GAAGTCGTAACAAGGTAGCCG	GATGCTCGCAACCACTATCCA	250-350
***Leishmania* spp.**	Kinetoplast DNA fragment of *Leishmania* spp.	CTTTTCTGGTCCTCCGGGTAGG	CCACCCGGCCCTATTTTACACCAA	120

Table 15. *Nucleotide sequences and product size of oligonucleotides for amplification of bacterial, mycobacterial and leishmania DNA.*

2.4 PCR Analysis and Primers (Table 15)

The investigators that performed the PCR analysis were blinded as to the identity of the specimens.

2.4.1 16S rRNA gene

Bacterial identification by 16S rRNA gene amplification and sequencing was performed by using universal primers 27 F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492 R (5′-TACGGTTACCTTGTTACGACTT-3′). Amplification was done by initial denaturation at 94 °C for 3 minutes, followed by 40 cycles of denaturation at 94 °C for 30 seconds, annealing temperature of 55 °C for 30 seconds and extension at 72 °C for 1 minute. The final extension was performed at 72 °C for 10 minutes.

2.4.2 hsp65 gene

For amplification of the hsp65 sequences, a nested PCR was carried out according to the method described by Ros Bascunana *et al.*[236] with some modifications. The outer primers used in the first PCR were M1 (5'-CCC CAC GAT CAC CAA CGA TG-3´) and M4 (5'-CGA GAT GTA GCC CTT GTC GAA CC-3'), which amplified a region of 450 bp. The inner primers M2 (5'-CGC CAA GGA GAT CGA GCT GGA-3') and M3 (5'-GCC CTT GTC GAA CCG CAT ACC-3') amplified a region of 424 bp.

The first amplification was performed in a 100µl reaction mixture containing 10 µl of the sample, 0,5 µM of each primer (primers M1 and M4), 50 mM KCl, 10 mM Tris-HCl (pH 8.4), 1,5 mM MgCl2, and 1,5 U of *Taq* DNA polymerase (*Expand High Fidelity PCR System, Roche Applied Science)*. The PCR technique was started with an initial denaturation step at 95°C for 5 min, followed by 40 cycles, each cycle consisting of a denaturation step at 94°C for 40 seconds, an annealing step at 60°C for 50 seconds, and an extension step at 72°C for 1 min. An additional extension step at 72°C for 10 min was performed in a 9600 thermal cycler (Perkin-Elmer). Subsequently, 1-µl amounts of the PCR mixture were transferred to new tubes containing freshly added reagents and the two internal primers M2 and M3. The conditions of the second round of amplification were identical to those of the first round. The amplified product (10 µl) was subjected to a Microchip Electrophoresis System for DNA/RNA Analysis MCE-202 *MultiNA* (Shimadzu Europa GmbH). The PCR product (50 µl) was purified by QIAquick PCR purification kit (QIAGEN GmbH).

2.4.3 16S-23S rRNA gene

For amplification of the 16S-23S rRNA gene, a hemi-nested PCR was performed using some modifications.[237,238] The hypervariable region of the ITS-1 region (approximately 250–350 bp) was amplified using a newly specified pan-*Mycobacterium* primer set. The forward primer ITS-A1 (5′-GAA GTC GTA ACA AGG TAG CCG-3′) amplified from the 3′ end of the 16S rRNA, and the reverse primer ITS-A6 (5′-G ATG CTC GCA ACC ACT ATC CA-3′) amplified from within the ITS-1 target.

The first amplification was performed using 10 µl of the sample in a total reaction volume of 100µl to include PCR buffer (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$),

0,3 µM (each) primer (primers ITS-A1 and ITS-A2), 1,5 mM $MgCl_2$, and 1,5 U of *Taq* DNA polymerase (*Expand High Fidelity PCR System, Roche Applied Science)*. The amplification was performed on a 9600 thermal cycler (Perkin-Elmer) starting with an initial denaturation step at 95°C for 5 min, followed by 40 cycles, each cycle consisting of a denaturation step at 95°C for 1 min, an annealing step at 59°C for 1 min, and an extension step at 72°C for 1 min. An additional extension step at 72°C for 7 min was performed after the last cycle. Subsequently, 1-µl amounts of the PCR mixture were transferred to new tubes containing freshly added reagents and the two primers Sp1 (5′-ACC TCC TTT CTA AGG AGC ACC-3′) and ITS-A6. The amplification of the second round was performed on a 9600 thermal cycler (Perkin-Elmer) starting with an initial denaturation step at 95°C for 5 min, followed by 40 cycles, each cycle consisting of a denaturation step at 95°C for 1 min, an annealing step at 55°C for 1 min, and an extension step at 72°C for 1 min. An additional extension step at 72°C for 7 min was performed after the last cycle. The amplified product (10 µl) was subjected to a Microchip Electrophoresis System for DNA/RNA Analysis MCE-202 *MultiNA* (Shimadzu Europa GmbH). The PCR product (50 µl) was purified by QIAquick PCR purification kit (QIAGEN GmbH).

2.4.4 FluoroType® MTB

The FluoroType® MTB (Hain Life Science, Nehren, Germany), a newly commercialized fluorescence-based molecular genetic test system, was also used for the detection of *Mycobacterium tuberculosis* complex.

FluoroType® MTB is based on the innovative FluoroType® technology. Mycobacterial DNA is extracted from the sample material and specifically amplified via PCR. During the isolation process an Internal Control is added to the sample, which ensures a correct DNA extraction. Then fluorescence-labeled probes are bound to single-stranded amplicons. In the subsequent melting curve analysis the decrease in fluorescence is measured. The evaluation is done by the test specific Fluoro-Software®. Amplification and detection run fully automated in the FluoroCycler® instrument. This guarantees maximum user-friendliness and efficient diagnostics with reliable results at one glance.

Additionally, an automated molecular test for *M. tuberculosis* and resistance to rifampin was used (Xpert MTB/RIF) to confirm the diagnosis.

2.4.5 Leishmania spp. DNA

The presence of *Leishmania* spp. DNA was analyzed by amplifying the kinetoplast minicircle DNA sequence via a real-time PCR technique, as previously described in the literature.[239] Each amplification was performed in a 25-µL-reaction mixture containing 2× Qiagen One Step RT-PCR kit (Qiagen. Germany), 10 pmol of forward primer (CTTTTCTGGTCCTCCGGGTAGG), 10 pmol of reverse primer (CCACCCGGCCCTATTTTACACCAA), 2.5 pmol labeled TaqMan probe (FAM-TTTTCGCAGAACGCCCCTACCCGC-TAMRA), and 5 µL of sample DNA. The Smartcycler (Cepheid) at 95°C and 55°C cycling over 45 cycles was used. The pre-developed reagent for the RNase P human gene (TaqMan Human RNase P detection reagent; Applied Biosystems) was included in the PCR reaction as an internal control of amplification. Previously known positive and negative samples were included in each run as external control. A sample was considered valid when the internal control was amplified, and was considered positive when the threshold (Ct) for the specific target were < 40.

2.5 Determination of DNA Sequence of Amplified Products

The DNA obtained was sequenced with the ABI *BigDye* Terminator v3.1 Cycle *Sequencing* Kit (Applied Biosystems, Inc., Foster City, Calif.). Sequencing products were purified using a Montage SEQ_{96} sequencing reaction cleanup kit (Millipore). The samples were passed through POP7 capillary columns on an Applied Biosystems 3130*xL* DNA Analyzer (Applied Biosystems, Inc.).

Alignments of all DNA sequences were performed using the Basic Local Alignment Search Tool (BLAST) program.[240]

2.6 Statistical Analysis

Comparisons of frequencies were made using χ^2 tests with Fisher`s exact test corrections.

3. RESULTS

3.1 Patient Demographics

Forty-eight biopsy specimens from 48 patients (37 females) were included in the study. At presentation, the age of the patients ranged from 25–90 years, with a mean age of 54±12 years (± Std. Dev.).

3.2 Clinical Outcomes

The skin lesions were single (18 patients) or multiple (30 patients) and consisted mainly of macules or papules in 24 patients, nodules in 20 patients and plaques in 15 patients. In more than half the patients, the course of skin lesions was chronic (> 2 years).

With respect to localization, the most frequently affected areas were the lower limbs (28 patients), the head and neck (21 patients), the upper limbs (17 patients) and the trunk (seven patients).

The clinical diagnosis included systemic or cutaneous sarcoidosis in the majority of the patients (n=44). The four other cases were submitted with a clinical diagnosis of erythema nodosum, atypical mycobacteriosis and two cases of panniculitis.

Systemic organ involvement was observed in 24 out of the 48 cases (50%). None of the patients presented concomitant HIV infection.

3.3 Histological Outcomes

Non-caseating, epithelioid cell granulomas were observed upon histopathological examination in all of the cases. The pathological findings are summarized in Table 16.

Table 16. *Histopathological findings from all cases and from those in which mycobacteria and leishmania DNA were demonstrated.*

	All included cases	PCR positive cases	
		Mycobacteria	*Leishmania*
Epidermis			
Atrophy	13/48	2/8	1/3
Acanthosis	11/48	1/8	0/3
Hyperplasia	4/48	0/8	0/3
Focal lichenoid infiltrate	2/48	0/8	0/3
Normal	18/48	5/8	2/3
Type of granuloma			
Naked granuloma with few lymphocytes	31/48	4/8	3/3
Naked granuloma without lymphocytes	17/48	4/8	0/3
Granulomatous infiltrate			
Mild	10/48	1/8	1/3
Moderate	21/48	5/8	1/3
Severe	17/48	2/8	1/3
Depth			
Superficial dermis	6/48	0/8	1/3
Superficial and mid dermis	16/48	4/8	2/3
Mid dermis	6/48	0/8	0/3
Entire dermis	17/48	4/8	0/3
Hypodermis	3/48	0/8	0/3
Distribution			
Perivascular	3/48	0/8	0/3
Periannexial	4/48	0/8	0/3
Perivascular and Periannexial	7/48	2/8	0/3
Diffuse	34/48	6/8	3/3
Coalescence			
Present	38/48	5/8	2/3
Absent	10/48	3/8	1/3
Multinucleated giant cells			
Absent	1/48	0/8	0/3
Mild	40/48	8/8	2/3
Moderate	7/48	0/8	1/3
Other inflammatory cells			
Lymphocytes/Histiocytes	42/48	8/8	3/3
Neutrophils	4/48	2/8	0/3
Plasma cells	9/48	0/8	3/3 *
Foreign material			
Present	10/48	1/8	0/3
Asteroid bodies			
Present	1/48	0/8	0/3
Schaumann bodies			
Present	2/48	0/8	0/3
Focal necrosis			
Present	4/48	1/8	0/3

* statistically significant at $p<0.01$

3.4 Polymerase Chain Reaction Results and Clinicopathological Correlation

Twelve out of the 48 histological specimens (25%) showed positive results for microorganism DNA detection by PCR. Of these twelve specimens, mycobacteria DNA was identified in 8 samples, *Leishmania*-specific DNA was identified in 3 cases and one sample revealed the presence of *Pseudomonas aeruginosa* DNA.

The microorganisms present in each group are listed in Table 17.

Table 17. *Clinical features and follow-up of the twelve cases that showed positive results for microorganism DNA detection by PCR.*

No	Age/Sex	Type of skin lesion	Site (single/multiple)	Underlying condition	Systemic involvement	PCR		Course of skin disease
						Microorganisms DNA	Positive amplified technique	
1	53/M	Maculopapular; Nodules	Trunk, back (m)	no	no	*Leishmania* spp.	Kinetoplast DNA	Persistence
2	58/F	Nodulo-plaques; Lupus pernio	Back, arm, ear (m)	DM	BHL	MTBC	16S-23S rRNA	Persistence
3	53/F	Maculopapular; Nodules	Arm, leg (s)	Chronic water exposure	no	*Leishmania* spp.	Kinetoplast DNA	Persistence
4	56/F	Maculopapular; Plaques	Shoulder, knee (m)	no	no	*Mycobacterium xenopi*	16S-23S rRNA	Complete resolution
5	65/F	Nodulo-plaques; Lupus pernio	Nose (s)	HTN	BHL	*Mycobacterium xenopi*	16S-23S rRNA	Complete resolution
6	53/M	Plaques	Ear, face (m)	HCV, Cirrhosis	BHL	MTBC	16S-23S rRNA and FluoroType® MTB Kit	Persistence
7	58/F	Maculopapular	Face (s)	no	no	*P. aeruginosa*	16S rRNA	Persistence
8	30/F	Maculopapular	Arm, leg, face (m)	Asthma, Hypothyroidism	BHL	MTBC	16S-23S rRNA	Persistence
9¶	44/M	Nodules	Leg (m)	HCV	BHL and lung involvement	MTBC	FluoroType® MTB Kit	Persistence
10	27/F	Nodules	Leg, forehead (m)	Hypothyroidism	no	MTBC	FluoroType® MTB Kit	Persistence
11	40/F	Nodules	Foot, hand (m)	DM	no	MTBC	16S-23S rRNA	Persistence
12	50/M	Nodulo-plaques	Hand, arm, face (m)	Chronic water exposure	no	*Leishmania* spp.	Kinetoplast DNA	Persistence

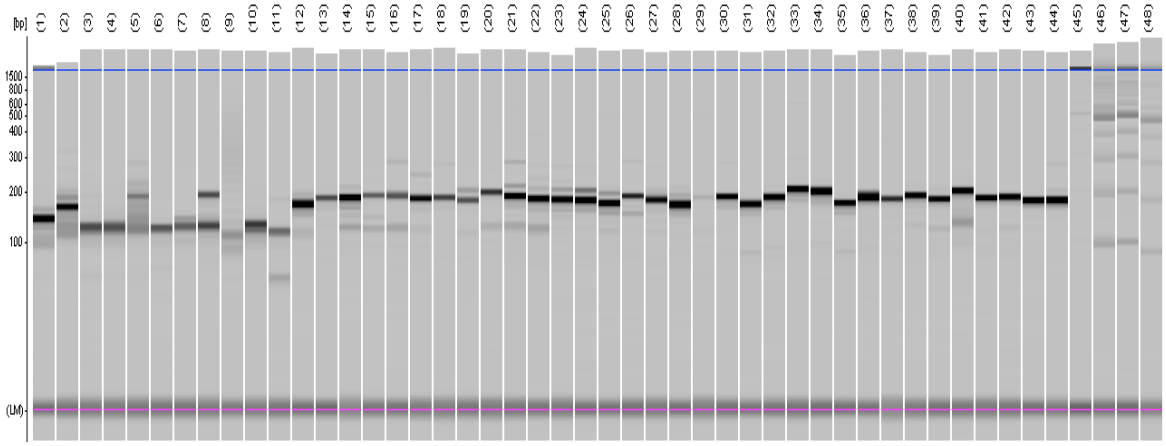
PCR, polymerase chain reaction; **M**, male; **F**, female; **DM**, diabetes mellitus; **BHL**, bilateral hilar lymphadenopathy; **MTBC**, *Mycobacterium tuberculosis* complex; **HTN**, hypertension; **HCV**, hepatitis c virus

¶ High serum angiotensin-converting enzyme (ACE) levels (>40 U/L)

3.4.1 Mycobacteria-positive cases according to PCR

All eight cases of mycobacteria were detected only via the amplification of the 16S-23S rRNA gene (Fig. 21) and/or FluoroType® Kit. Six of these (patients 2, 6, 8, 9, 10 and 11) exhibited DNA sequences related to *Mycobacterium tuberculosis* complex (MTBC), and the remaining two cases (patients 4 and 5) showed >90% homology to atypical mycobacteria (*Mycobacterium xenopi*).

(a) Beta-globin



(b) ITS

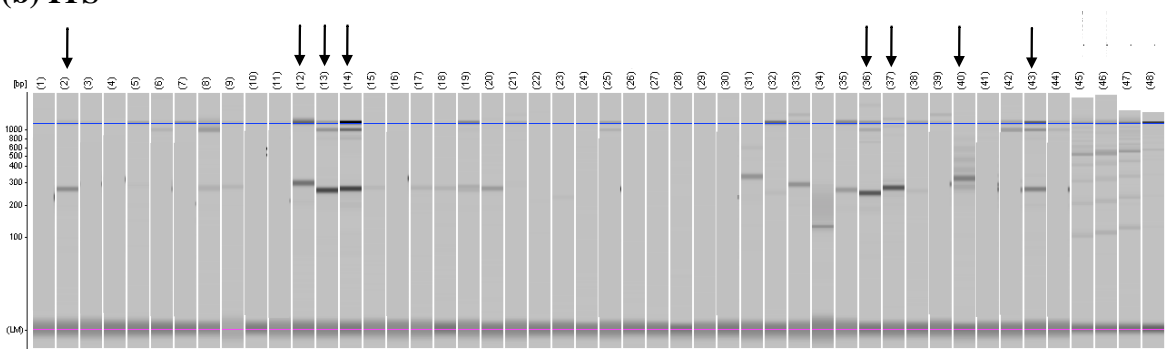


Fig. 21. *Gel electrophoresis of mycobacteria from PCR-amplified 16S-23S rRNA spacer sequences.* ***(a)*** *The quality of DNA samples was verified by amplifying a 110 bp fragment of beta-globin gene.* ***(b)*** *Using primers ITS-A1 and ITS-A6, we identified four cases of M. tuberculosis complex (lanes 2, 14, 36, 43) and two cases of M. xenopi (lanes 12 and 13); amplification bands were also observed in lanes 37 and 40, however the sequencing and identification of M. tuberculosis complex was only possible using the FluoroType® Kit;*

lanes 45 to 48 corresponded to molecular size markers (100-1000-bp); all positive cases for Mycobacterium tuberculosis (lanes 2, 14, 36, 37, 40, 43) were confirmed by the test of resistance to rifampin.

Clinically, most of these patients had multiple chronic skin lesions (at least a 2-year history) ($p<0.05$) consisting of nodules, plaques and lupus pernio located predominantly on the face (Fig. 22). Lymph node involvement (bilateral hilar lymphadenopathy) was frequently observed in these patients (5 of the 8 cases). Taking into account these clinical features as well as the presence of sarcoidal granulomas in their biopsies, the patients were diagnosed with sarcoidosis.

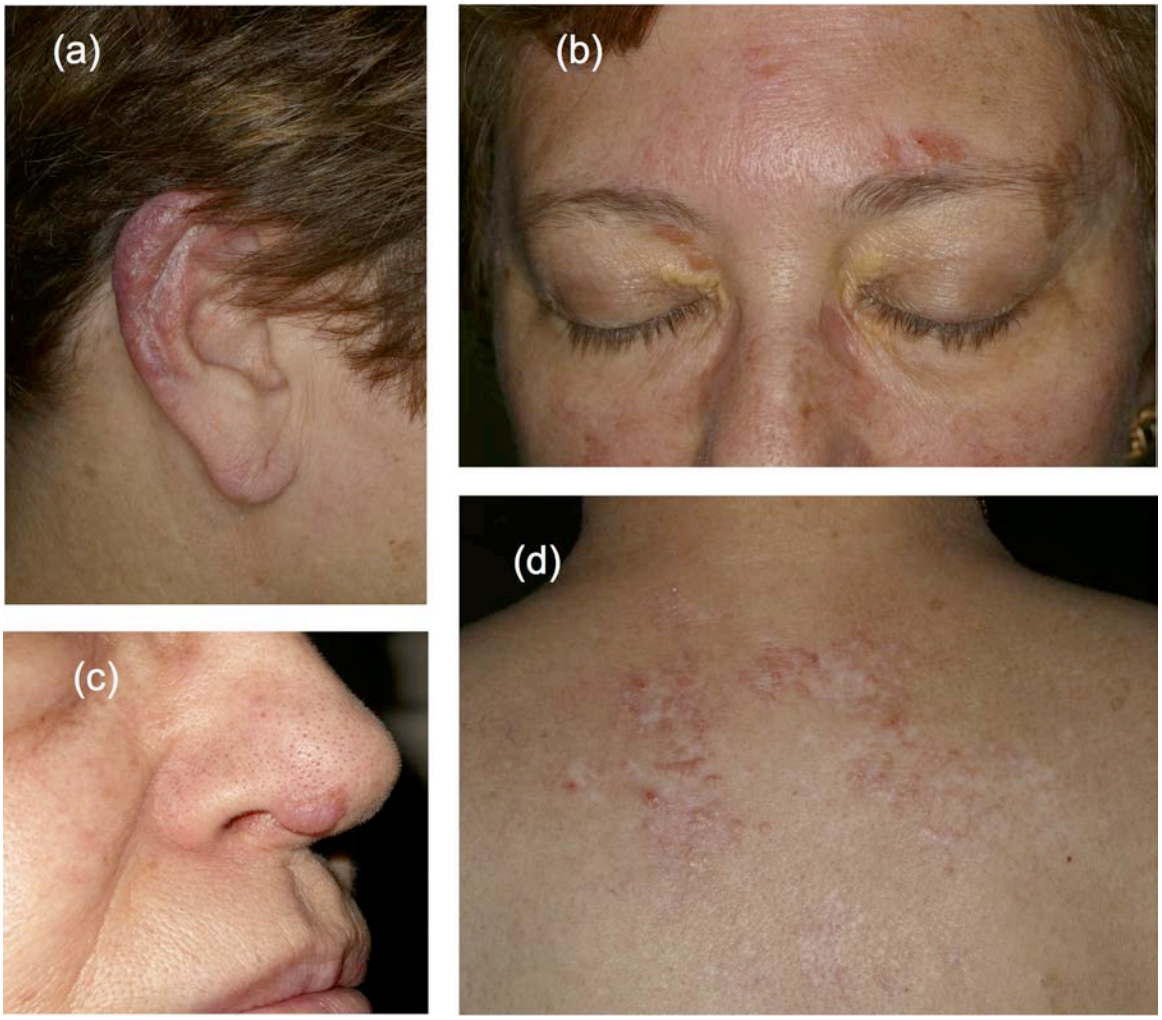


Fig. 22. *Cutaneous lesions of patients who presented positive PCR results for mycobacteria. The morphological pattern of skin lesions was varied and consisted of:*

lupus pernio (a), papules/plaques (b and d) and nodules (c). The clinical features of these patients strongly resemble sarcoidosis.

There was no past history of tuberculosis in any of these eight patients, and the mantoux test was negative in all of the patients. All of them had received immunosuppressive therapy for sarcoidosis (topical and oral corticosteroids) with partial clinical response in 6 of the 8 patients; however, one case is worthy of mention. A 53 year-old-male (case 6), with a past history of Hepatitis c virus (HCV) infection, presented with multiple plaque-type skin lesions of 5 months` duration on his face and ears. The chest X-ray revealed bilateral hilar lymphadenopathy, without ring enhancement or caseation. His serum ACE level was normal and tuberculin test was negative. The skin biopsy revealed noncaseating granulomas consistent with the diagnosis of sarcoidosis. He was managed with oral corticosteroids with partial clinical response. Considering his underlying condition (HCV) as well as the successive recurrences of skin lesions, a treatment with RIPE (rifampicin, isoniazid, pyrazinamide and ethambutol) was added over the course of six months; he subsequently achieved a good clinical response.

Histologically, epidermal changes such as acanthosis and atrophy were observed in 3 samples (38%). All of the specimens exhibited epithelioid granulomas without necrosis and a dermal inflammatory infiltrate of lymphocytes and histiocytes throughout the reticular dermis. Multinucleated giant cells were present in the granulomas of all cases; however, plasma cells were absent. The presence of neutrophils was found only in the two biopsies in which *M. xenopi* was identified (patients 4 and 5). Only one biopsy (patient 2) demonstrated focal necrosis (Table 16 and Fig. 23). Special stains for mycobacteria were again negative in all cases.

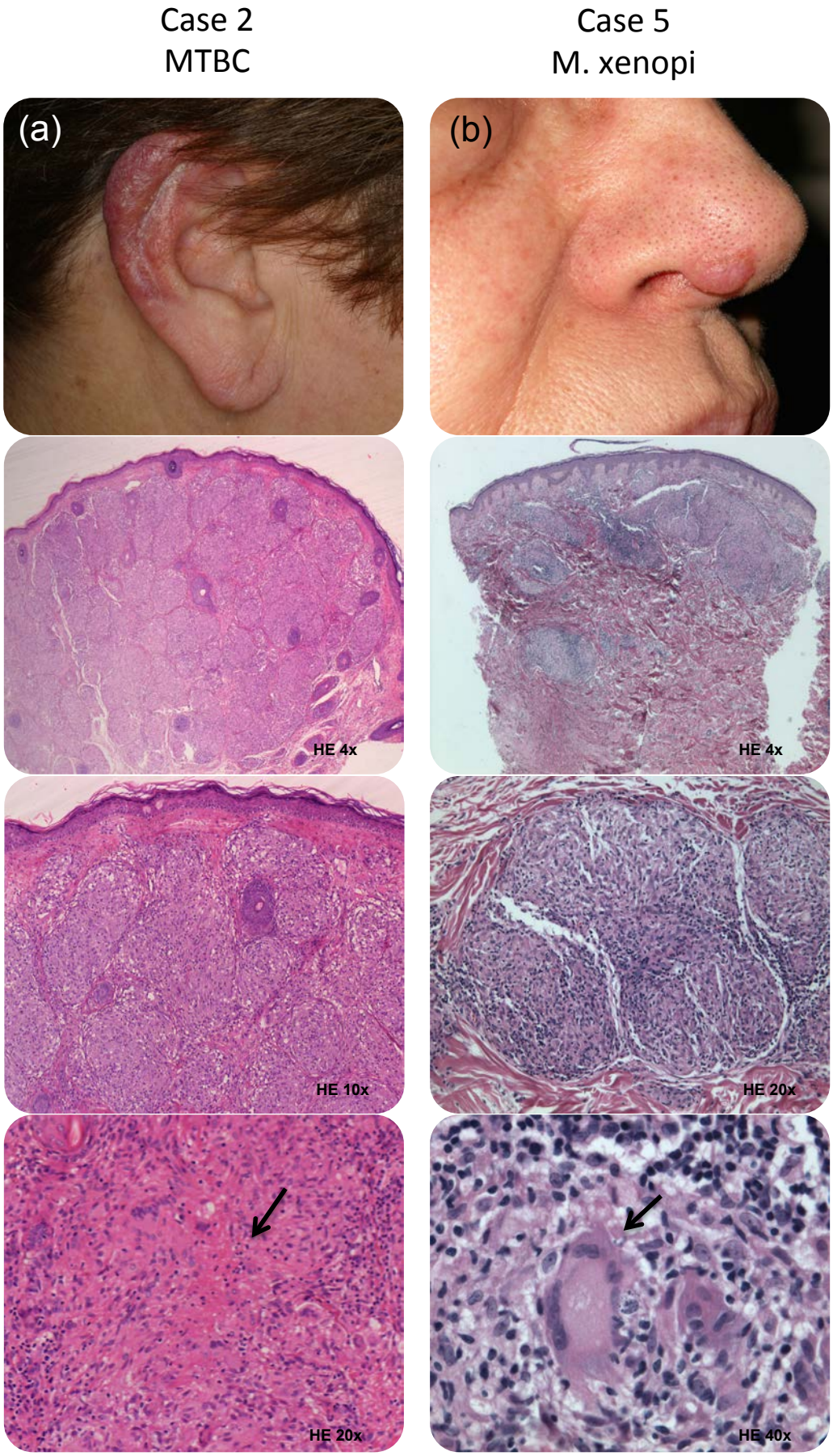

Case 2
MTBC
Case 5
M. xenopi
(a)
(b)
HE 4x
HE 4x
HE 10x
HE 20x
HE 20x
HE 40x

Fig. 23. *Histopathological features from 2 patients in which mycobacteria DNA was demonstrated.* ***(a)*** *case 2, M. tuberculosis complex.* ***(b)*** *case 5, M. xenopi. Both cases exhibited sarcoidal granulomas ("naked") without necrosis and associated with a dermal inflammatory infiltrate of lymphocytes and histiocytes. The epidermis was normal* ***(b)*** *or atrophic* ***(a)****. The granulomatous infiltrate was moderate* ***(b)*** *or severe, throughout the entire dermis* ***(a)****. The presence of multinucleated giant cells* ***(b)*** *was the rule, as observed in higher magnification (x40) (arrow). Focal necrosis* ***(a)*** *was occasionally observed (x20) (arrow).*

3.4.2 Leishmania-positive cases according to PCR

Specific *Leishmania* PCR amplification was positive in three biopsies (patients 1, 3 and 12). Clinically, these three patients had chronic cutaneous lesions with at least 2-year history ($p<0.05$) but without any other organ involvement. Cutaneous lesions were single or few nodules, without ulceration, situated predominantly on the extremities (Fig. 24).

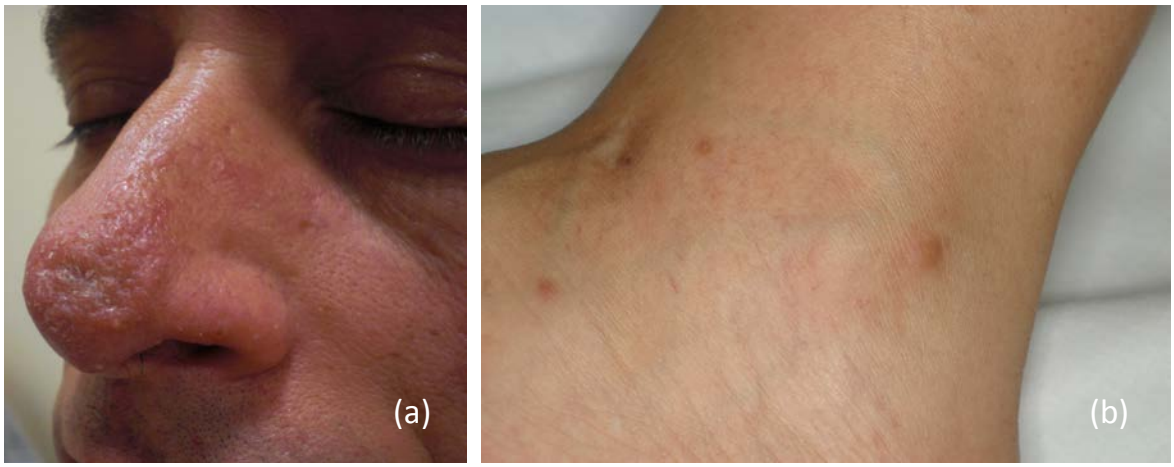


Fig. 24. *Cutaneous lesions of patients who presented positive PCR results for Leishmania spp.* ***(a)*** *case 12, chronic cutaneous lesion on the nose.* ***(b)*** *case 3, multiple papules on the leg.*

All cases were diagnosed as cutaneous sarcoidosis. However, in two of these cases (patients 3 and 12), a clinical differential diagnosis of atypical mycobacteriosis was suggested since those patients had cutaneous lesions on their extremities and histories of chronic water exposure. A clinical diagnosis of cutaneous leishmaniasis (CL) was not made in any of the patients.

Histologically, all three cases shared the same features: the epidermis was almost unaltered; the presence of naked granulomas with few lymphocytes around them; the inflammatory infiltrate of lymphocytes and histiocytes was diffuse and observed in the superficial or mid dermis; the presence of multinucleated giant cells and plasma cells ($p<0.01$) was the rule; and the absence of foreign materials and focal necrosis (Table 16 and Fig. 25).

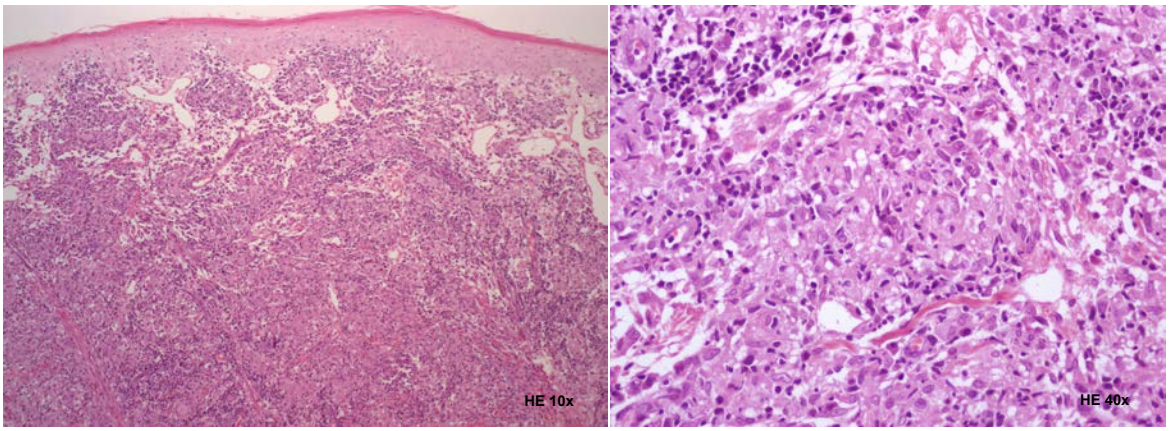


Fig. 25. *Histopathological features from one patient (case 3) in which Leishmania DNA was demonstrated. Epithelioid granulomas with a diffuse infiltrate of lymphocytes and histiocytes in the upper and mid dermis.*

IHC evaluation for the detection of intracytoplasmic amastigotes, also performed in all of the samples, was positive only in patient 3 (Fig. 26).

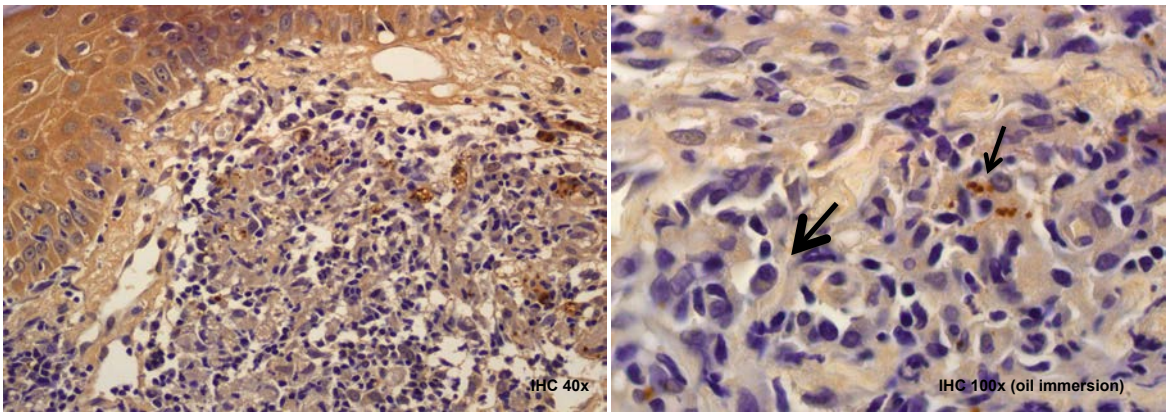


Fig. 26. *Immunohistochemical evaluation from patient 3 for the detection of intracytoplasmic amastigotes (brownish structures). As we can see in higher magnification (x100), only few amastigotes were identifiable (thin arrow), which may explain the negative results with Giemsa stain; plasma cells (thick arrow) are another important histological features that can help clinicians to obtain a correct diagnosis of CL.*

4. DISCUSSION

To the best of our knowledge, this study is the first to evaluate the behavior of sarcoidal granulomatous dermatitis and its infectious causes using current molecular biology techniques. This investigation is also the first to perform molecular analysis for mycobacteria, bacteria and *leishmania* in these patients and to correlate those results with the clinical and histological outcomes in order to identify important subtle clues that might help clinicians obtain a correct diagnosis.

Among the 48 patients of our study, we showed a 25% overall positivity of microorganisms DNA in sarcoidal granulomatous dermatitis. *M. tuberculosis* complex (6 cases), *Leishmania* spp. (3 cases) and *Mycobacterium xenopi* (2 cases) represented almost all DNA identified. Additionally, in one patient, we also found a positive PCR result for *P. aeruginosa* DNA, which, in our opinion, has no clinical significance. This result was likely due to contamination during processing. All cases were diagnosed clinical and histologically as cutaneous sarcoidosis.

4.1 Leishmania-specific DNA

Cutaneous leishmaniasis (CL) may present unusual clinical or histopathological features, thereby representing a diagnostic dilemma, particularly in endemic areas such as in Spain.

Consistent with previous studies,[142,234,235,241,242] our results demonstrate that CL may be misinterpreted as other granulomatous disorders, especially in chronic forms where *Leishmania* amastigotes are difficult to detect or are altogether absent and the clinical picture is often non-specific. Real-time PCR for *Leishmania* spp. enabled the diagnosis of three cases of CL that the clinicopathological correlation as well as H&E and Giemsa stains had missed. In two of these cases, immunohistochemical analysis for detection of *Leishmania* amastigotes was also negative, which represents a low sensitivity compared with previous reports.[235,243-245] Despite these results, it is worth remembering the concept of leishmaniasis without the presence of leishmanias and the possible spontaneous involution as an expression of a good immune response.

All of the cases were misinterpreted as cutaneous sarcoidosis, but a clinical and histological re-evaluation of these cases allowed us to find some subtle elements that served as a guide for establishing the correct diagnosis: (1) the predominance of few cutaneous lesions (<3 lesions), with chronic evolution ($p<0.05$), without systemic involvement and located on the extremities; (2) the presence of unusual histopathological features such as unaltered epidermis and sarcoidal granulomas does not exclude the diagnosis of CL, as evidenced in our study and consistent with previous reports;[142,234,235,241,242] (3) the presence of a diffuse infiltrate of lymphocytes and histiocytes in the superficial dermis, multinucleated giant cells and especially plasma cells ($p<0.01$) are important subtle histological features that can lead to the correct diagnosis.

4.2 Mycobacterium xenopi DNA

Atypical mycobacterial infections may also prove to be a diagnostic challenge. Their clinical presentation is also varied and histologically the lesions may evolve from a nonspecific mixed inflammatory cell infiltrate with epithelial hyperplasia to a sarcoidal granulomatous pattern with less demonstrable microorganisms.[2,246,247]

Mycobacterium xenopi is a slow-growing mycobacteria and a well cause of nontuberculous mycobacterial pulmonary disease, particularly in Western Europe and Canada.[248,249] Extrapulmonary and disseminated infections have also been recorded,[250,251] but reports of cutaneous infections remain rare especially in immunocompetent hosts.[252] Therefore, when *M. xenopi* is identified by molecular techniques from a human sample such as skin, its clinical significance needs to be determined. It is of greatest importance to differentiate true infection from pseudo infection or contamination because treatment of *M. xenopi* infections is often complicated and time-consuming.[253,254] In a retrospective study to assess the clinical relevance of *M. xenopi*, Van Ingen *et al*.[254] used the American Thoracic Society (ATS) diagnostic criteria to differentiate true infection from pseudo infection. Interestingly, they concluded that the clinical isolation of *M. xenopi* was relevant for 51% of the patients.

A clinical and histological re-evaluation of our cases allowed us to find some features that served to confirm that the molecular isolation of *M. xenopi* was not clinically relevant and

consequently did not correspond to a true infection: (1) the clinical appearance of the lesions was variable and included nodules, papules, plaques and lupus pernio, strongly resemble sarcoidosis; (2) the two patients were immunocompetent, had no histories of chronic water exposure and the mantoux test was negative in all of them; (3) no demonstrable signs of pulmonary involvement (especially cavitation) or pre-existing pulmonary disease; (4) treatment with oral corticosteroids resulted in the complete resolution of skin lesions; (5) as evidenced in our study and consistent with previous reports,[20,22,255] the presence of neutrophils in small amounts is an histopathological finding occasionally observed in sarcoidosis and does not necessarily indicate infection.

4.3 Mycobacterium tuberculosis complex DNA

During the last few years, investigators have debated the role that *Mycobacterium tuberculosis* may have in sarcoidosis pathogenesis, and actually the issue continues to be controversial. In order to explore this possible association more deeply, several studies investigating the presence of mycobacterial DNA employing molecular biological techniques have been carried out in sarcoidosis.

A recent meta-analysis by us[43] (described during phase 2 of the doctoral thesis) compiled the published evidence for the presence of microbial DNA in sarcoidosis patients by molecular biological techniques. We concluded that the balance of evidence from pooled analysis favors association between some microorganisms (i.e. mycobacteria and *Propionibacterium acnes*) and sarcoidosis. However, many of those studies have been criticized for their small sample size and high incidence of false-positive PCR.[173,175] Despite multiple studies that demonstrate mycobacterial DNA in sarcoid lesions, at this time, there is a striking lack of evidence that sarcoidosis is associated with an active, replicating mycobacterial infection.[256] Furthermore, the inability to identify mycobacteria by culture from sarcoidosis tissues continues to be one of the strongest arguments against a potential role for mycobacteria.[62,64] In view of the above considerations, currently no consensus exists on the pathogenic mechanisms by which microbial infection can cause sarcoidosis.

An alternative hypothesis to explain these observations would be that sarcoidosis and tuberculosis could coexist in the same patient. Sarcoidosis is known to follow or even coexist with tuberculosis[257,258] and tuberculosis following sarcoidosis is a common occurrence due to the immunosuppressive effects of oral corticosteroids.[259] However, the diagnosis of co-existent disease is challenging and needs to be supported by strong bacteriological evidence of MTBC on smear examination or culture associated with compatible clinico-radiological findings. In one study,[260] a positive mantoux test in active sarcoidosis was also found to be a good indicator of concurrent tuberculosis. Concerning our study, there was no past history of tuberculosis and the mantoux test was negative in all of the patients where MTBC DNA was identified. Additionally, almost all of them had the presence of symmetrical hilar lymphadenopathy, without necrosis or cavitation, which is a radiological finding frequently observed in sarcoidosis and uncommon in tuberculosis.

Lastly, in view of the current scarce evidence available, it might be appropriate to consider that sarcoidosis and tuberculosis may represent a spectrum of the same disease. Analogous to leprosy, sarcoidosis might represent the tuberculoid form of the pathological responses to mycobacteria while pulmonary tuberculosis would represent the opposite end of the same spectrum. This viewpoint has already been suggested previously[261] and was recently taken up by Agrawal *et al*.[262] who proposed the development of a subdivision classification system for tuberculosis and sarcoidosis. According to the authors, S (Sarcoidosis) and TB (Tuberculosis) would comprise the opposite pure forms of the same spectrum, whereas ST (Sarcoid-Tuberculous) and TS (Tuberculous-Sarcoid) would represent mixed features of both diseases, with TS being predominantly tuberculous with sarcoid features and ST being predominantly sarcoid with tuberculous features.[262] For this classification the authors had take into account not only the clinicopathological features but also molecular and immunological aspects of each disease.

Thus, we believe that such a classification system may explain in part the results obtained in our study. The clinical and histopathological manifestations of our six patients strongly resemble sarcoidosis. However, in the presence of chronic cutaneous lesions, bilateral hilar lymphadenopathy, positive PCR results for MTBC DNA and an absence of response to classical treatments for sarcoidosis, one should consider the “grey zone” entities like TS and ST. This statement holds true, even after observing a histological pattern of sarcoidal

granulomatous dermatitis with special stains and cultures negatives for microorganisms. These patients (TS or ST) should be treated with a combination therapy, with both immunosuppressants and antitubercular therapy so as to allow treatment of the underlying alternative disease adequately, such as it was possible to verify in one of our patients (case 6).

4.4 Implications

The results of our study allow us to make a few final relevant points: (1) one strength of our study was its inclusion of a large series of patients; furthermore, our investigation was the first to evaluate the behavior of sarcoidal granulomatous dermatitis; (2) PCR techniques performed on paraffin-embedded tissues proved to be a reliable tool for obtaining an accurate diagnosis, especially for MTBC and *Leishmania spp*. However, in order to assess the real clinical significance of the molecular results, it is always necessary to correlate those results with the clinicopathological features. In some instances the microorganisms are pathogenic, but in others they may just be a commensal or theoretically they might even be attracted to the area of granulomatous inflammation; (3) currently, for mycobacterial identification, there are several PCR assays that encode different genes (e.g., hsp65, 16S rRNA, rpoB).[62,64,257] However, not all PCR techniques have the same sensitivity and specificity. Our study demonstrated that the internal transcribed spacer (ITS) sequence between the 16S rRNA and 23S rRNA genes and the FluoroType® Kit have a higher degree of accuracy for detection and identification of *Mycobacterium tuberculosis* complex, consistent with previous reports;[237,238] (4) in regarding *Leishmania*, the IHC methods are not sufficient to obtain a correct diagnosis, especially in chronic lesions, given the low concordance between IHC results and real-time PCR. (5) working under the hypothesis that sarcoidosis and tuberculosis may potentially be two ends of the same disease and given that *Leishmania* is widely distributed (i.e., endemic in more than 70 countries),[258,259] we consider that, especially in endemic areas, PCR for *Mycobacterium tuberculosis* and *Leishmania*-specific DNA should be performed in any granulomatous disease, in particular sarcoidal granulomatous skin disease compatible with

sarcoidal/naked granuloma, regardless of its clinical presentation and even when dermatologists and dermatopathologists do not suspect an infectious cause.

4.5 Limitations

One of the primary potential limitations of our study relates to the retrospective nature of the analyzed biopsies. The DNA was extracted from formalin-fixed, paraffin-embedded samples and not from fresh tissues. Therefore, the risk of DNA fragmentation is greater in these cases, which can lead to false-negative results. Moreover, the risk of contamination in PCR techniques during the paraffin processing can also lead to false-positive results. However, the DNA extraction technique used by our team has been meticulously designed in order to reduce the risk of contamination.

CONCLUSIONS

CONCLUSIONS

Regarding the first part of the study, the conclusions are:

1. The clinical spectrum of skin lesions exhibited a good correlation with the location of the granulomas in the biopsies. Additionally, the number of granulomas correlated well with a more or less extensive clinical presentation.
2. Cutaneous involvement in sarcoidosis is not only useful for diagnosis but may also discriminate progressive disease. Specific lesions, such as lupus pernio and nodules, may have prognostic significance. Furthermore, the prognosis of cutaneous lesions depends on the existence of systemic symptoms.
3. The presence of a grenz zone in biopsies of cutaneous sarcoidosis is a novel histological feature that may be useful for the diagnosis and management of this disease.

According to the meta-analysis performed in the second part of this doctoral thesis, we can conclude:

4. The meta-analysis results suggest a significant association between sarcoidosis and some infectious agents, taking into account the marked difference in the percentage of microbial DNA-positive samples in sarcoidosis patients versus controls, especially mycobacteria and *P. acnes*.
5. What seems clear is that more than one infectious agent might be implicated in the pathogenesis of sarcoidosis; probably the patient's geographical location might dictate which microorganisms are more involved.

Regarding the third part of the study, the conclusions are:

6. The current molecular diagnostic techniques in microbiology suggest that some sarcoidal granulomatous dermatitis may be related to microorganisms.
7. Especially in endemic regions, we consider that PCR for *Mycobacterium tuberculosis* and *Leishmania* should be performed in any sarcoidal granulomatous skin disease.

FUTURE LINES OF INVESTIGATION

FUTURE LINES OF INVESTIGATION

Recent molecular, genetic, and immunologic studies from independent laboratories support an association between sarcoidosis and microorganisms. The balance of evidence favors mycobacteria or its products as a trigger for inciting immune responses leading to sarcoidosis in a proportion of patients.

Future efforts should involve delineating whether the nucleic acids or proteins detected in sarcoidosis patients reflect actively replicating organisms or persistent antigens. Genotypic and phenotypic factors that are necessary for these antigens to trigger the immune response need to be further studied. Focus on other mycobacterial virulence factors would also provide greater insight into the pathogenic mechanisms.

On the other hand, working under the hypothesis that sarcoidosis and tuberculosis may potentially be two ends of the same disease, future clinical trials need to be done, especially focusing on current available diagnostic investigations for tuberculosis and sarcoidosis on sarcoid and tuberculoid patients respectively, so as to determine if these modalities can truly distinguish between the two entities and whether there are any associations between the two. Moreover, response to anti-tuberculous therapy amongst patients in “grey zone” or with sarcoidosis can also be examined.

In the meantime, differentiating between the two conditions will continue to be challenging.

PUBLICATIONS

PUBLICATIONS

1. ARTICLES

1.1 Article 1

Title: Prognostic value of skin lesions in sarcoidosis: clinical and histopathological clues

Authors: Esteves TC, Aparicio G, Ferrer B, Garcia-Patos V.

Journal: European Journal of Dermatology

JCR Impact Factor: 2.069

Eur J Dermatol. 2015 Nov-Dec;25(6):556-62.

Doi: 10.1684/ejd.2015.2666.

1.2 Article 2

Title: Is there any association between Sarcoidosis and infectious agents?: a systematic review and meta-analysis

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Journal: BMC Pulmonary Medicine

JCR Impact Factor: 2.329

BMC Pulm Med. 2016 Nov 28;16(1):165.

Esteves et al. BMC Pulmonary Medicine (2016) 16:165
DOI 10.1186/s12890-016-0332-z

BMC Pulmonary Medicine

RESEARCH ARTICLE Open Access

Is there any association between Sarcoidosis and infectious agents?: a systematic review and meta-analysis

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Abstract

Background: During the last few years, investigators have debated the role that infectious agents may have in sarcoidosis pathogenesis. With the emergence of new molecular biology techniques, several studies have been conducted; therefore, we performed a meta-analysis in order to better explain this possible association.

Methods: This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement from the Cochrane collaboration guidelines. Four different databases (Medline, Scopus, Web of Science, and Cochrane Collaboration) were searched for all original articles published from 1980 to 2015. The present meta-analysis included case–control studies that reported the presence of microorganisms in samples of patients with sarcoidosis using culture methods or molecular biology techniques. We used a random effects or a fixed-effect model to calculate the odds ratio (OR) and 95% confidence intervals (CI). Sensitivity and subgroup analyses were performed in order to explore the heterogeneity among studies.

Results: Fifty-eight studies qualified for the purpose of this analysis. The present meta-analysis, the first, to our knowledge, in evaluation of all infectious agents proposed to be associated with sarcoidosis and involving more than 6000 patients in several countries, suggests an etiological link between *Propionibacterium acnes* and sarcoidosis, with an OR of 18.80 (95% CI 12.62, 28.01). We also found a significant association between sarcoidosis and mycobacteria, with an OR of 6.8 (95% CI 3.73, 12.39). *Borrelia* (OR 4.82; 95% CI 0.98, 23.81), HHV-8 (OR 1.47; 95% CI 0.02, 110.06) as well as *Rickettsia helvetica*, *Chlamydia pneumoniae*, Epstein-barr virus and Retrovirus, although suggested by previous investigations, were not associated with sarcoidosis.

Conclusion: This meta-analysis suggests that some infectious agents can be associated with sarcoidosis. What seems clear is that more than one infectious agent might be implicated in the pathogenesis of sarcoidosis; probably the patient's geographical location might dictate which microorganisms are more involved. Future investigations and more clinical trials are need to bring these evidences to a more global level.

Keywords: Sarcoidosis, Propionibacterium acnes, Mycobacteria, Infection, Meta-analysis

Background

Sarcoidosis is a systemic disorder of unknown origin that is characterized by the presence of non-caseating granulomas. With worldwide distribution, more than one causative agent may be implicated in its pathogenesis [1], with numerous infectious and non-infectious etiological agents having been identified [2]. Currently, the focus is on infectious agents, especially species of *Mycobacterium* and *Propionibacterium*. Other infectious agents have been investigated with inconclusive or conflicting results, such as *Borrelia burgdorferi*, *Rickettsia helvetica*, *Chlamydia pneumoniae*, viruses, fungal infections, and *Leishmania* species [3–11].

There are only two relevant meta-analyses in the literature [12, 13], which address the causal relationship of some infectious agents in sarcoidosis. Since then, more than 20 new investigations have been published, thus adding new relevant data to the discussion. This meta-

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analysis is the first to evaluate all infectious agents that may be involved in sarcoidosis.

Methods

Search strategy

This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement from the Cochrane collaboration guidelines. A checklist is available (Additional file 1). Since this study was a literature review and meta-analysis of previously reported studies, ethical approval or additional consent from participants was not required. Four different databases (Medline, Scopus, Web of Science and Cochrane Database) were searched for all original articles without language restriction published from January 1980 to May 2015, using the search strategy described in online supplementary data (Additional file 2).

Inclusion criteria

The inclusion criteria were as follows: (i) the diagnosis of sarcoidosis was made according to the classical criteria: a compatible clinical and radiological picture, histopathological demonstration of non-caseating granulomas with negative stains for mycobacterium and fungi, and exclusion of other granulomatous diseases; [14] (ii) case–control studies that reported the presence of microorganisms in samples, both histological and cellular, of patients with sarcoidosis, using either culture methods (direct isolation of the organism) or molecular biology techniques (analysis of DNA, RNA or proteins); (iii) odds ratios (OR) and the corresponding confidence intervals (CI) or sufficient information to calculate them; (iv) patients without sarcoidosis were used as a reference group.

Exclusion criteria

Studies involving other techniques (e.g. ELISA, immunohistochemistry and immunofluorescence) were excluded from the analysis.

Data extraction

First, two independent authors (T. Esteves and V. Garcia-Patos) reviewed all titles and abstracts. A second selection was based on a full-text review of potentially relevant articles and any disagreement was resolved by discussion between the three authors of this meta-analysis. A standardized data collection form was used to extract the following items: author(s), title of article, study design, year of publication, country of origin, study size, details of molecular or other techniques used.

Statistical analysis and methodological quality assessment

The measure of interest was the OR and 95% CI calculated from each study, in order to assess the presence of microorganisms in sarcoidosis samples versus controls. Data analyses were performed using Stata Statistical Software 2015 (StataCorp LP, College Station, Texas, USA). We used a random-effects model to calculate the OR and 95% CI from each study [15].

We assessed the heterogeneity among studies using Cochran's Q test [16], complemented by the I^2-test. [17] An I^2 value of 76–100% represents high heterogeneity, 51–75% moderate heterogeneity and 0–50% low or insignificant heterogeneity [17]. If the result of the Chi-square heterogeneity test was not significant ($p > 0.10$), we used the fixed-effects model described by Mantel and Haenszel [18] to calculate the pooled OR estimate. Additionally, sensitivity and subgroup analyses were performed in order to explore the heterogeneity among studies.

Results

Studies included

A total of 2465 articles were identified from the initial electronic search using the outlined search term parameters (Additional file 2). Among these, 2401 studies were excluded because they did not meet the inclusion criteria. A total of 64 articles were identified as investigating the role of infectious agents in sarcoidosis using either microbial culture or molecular methods. Six of these were later excluded since they were descriptive studies without a control group. Therefore, 58 case–control studies were qualified for the analysis according to the inclusion and exclusion criteria. Additional file 3 summarizes the study flow.

In total, the 58 studies involved 2467 samples from patients with proven sarcoidosis and 3656 samples from control patients with other non-sarcoid disorders. All studies used molecular techniques to identify the different types of infectious agents except for two, which used microbial culture in their analyses [19, 20].

With regard to the infectious agents investigated, 36 studies evaluated the presence of mycobacteria [20–55] (Table 1), 11 evaluated *P. acnes* [19, 22, 24, 25, 31, 35, 38, 56–59] (Table 2), seven evaluated human herpesvirus-8 (HHV-8) [22, 40, 60–64] (Table 3), and six evaluated *Borrelia* species [4, 65–69] (Table 4). Other infectious agents were investigated in some of the studies included, but there were insufficient cases to perform a meta-analysis. Three studies evaluated the presence of *Rickettsia* species, and one found a strong association between *Rickettsia helvetica* and sarcoidosis [70] (OR 21.72; CI:1.23–384.74). The

Table 1 Case–control studies evaluating the role of mycobacteria in sarcoidosis

First author/Year (Ref.)	Country	Molecular technique	Sarcoidosis patients		Non-sarcoidosis controls		OR (95% CI)
			n/N	Type of microorganisms	n/N	Type of microorganisms	
Bocart, 1992 [23]	France	PCR of 65 kDa mycobacterial antigen and IS6110	2/22	MTBC	0/22	-	5.49 (0.25–121.18)
Hofland, 2014 [20]	Netherlands	NAAT for Mycobacteria and Culture	0/32	-	2/86	1 MTBC, 1NTM	0.52 (0.02–11.13)
Robinson, 2013 [24]	USA	PCR for 16S rDNA, hsp65 and rpoB	2/30	NTM	1/30	NTM	2.07 (0.18–24.15)
Oswald-Richter, 2012 [25]	USA	MALDI-IMS for ESAT-6	5/15	Mycobacterium spp	0/4	-	4.71 (0.21–104.49)
Svendsen, 2011 [26]	Denmark	BD ProbeTec IS6110 amplification	1/52	MTBC	0/50	-	2.94 (0.12–73.93)
Mootha, 2010 [27]	India	PCR of 65 kDa mycobacterial antigen and IS6110	13/27	10 MTBC, 3 NTM	2/40	NTM	17.64 (3.53–88.25)
Zhou, 2008 [28]	China	Real-time PCR of IS986 and human β-blobin gene	20/104	MTBC	7/55	MTBC	1.63 (0.64–4.14)
Dubaniewicz, 2006 [29]	Poland	BD ProbeTec IS6110 amplification	3/50	MTBC	0/10	-	1.55 (0.07–32.27)
Fite, 2006 [30]	Spain	PCR of IS6110 and Southern blot hybridisation	9/23	MTBC	1/23	MTBC	14.14 (1.61–124.11)
Yasuhara, 2005 [31]	Japan	PCR of IS6110	0/6	-	0/6	-	-
Song, 2005 [32]	USA	PCR of MTB 16S rRNA	6/16	MTBC	0/16	-	20.43 (1.04–401.67)
Marcoval, 2005 [33]	Spain	NAAT for rRNA of MTBC	0/35	-	0/39	_	-
Yu-Yun Lee, 2002 [34]	Taiwan	Nested PCR for mycobacterial hsp65 DNA	7/21	NTM	0/16	-	17.07 (0.89–325.59)
Drake, 2002 [21]	USA	PCR of 16S rRNA, rpoB and IS6110	15/25	11 MTBC, 3 NTM, 1 both	0/25	_	75.29 (4.12–1377.06)
Gazouli, 2002 [22]	Greece	PCR of IS6110/IS1245/IS900/IS901, 16S rRNA, MPB64 and mtp40	33/46	MTBC	0/20	-	101.74 (5.74–1804.62)
Eish, 2002 [35]	Japan	PCR of IS6110/IS900	5/108	MTBC	2/86	MTBC	2.04 (0.39–10.78)
Klemen, 2000 [36]	Austria	PCR of IS6110 and mycobacterial chaperonin	3/4	NTM	0/39	_	184.33 (6.26–5425.48)
Li, 1999 [37]	USA	PCR of 65 kDa mycobacterial antigen and RFLP analysis	16/20	2 MTBC, 14 NTM	0/20	_	150.33 (7.54–2997.83)
Ishige, 1999 [38]	Japan	PCR of IS6110	3/15	MTBC	1/15	MTBC	3.50 (0.32–38.23)
Wilsher, 1998 [39]	NZ	PCR of IS6110, nested PCR to amplify 85 bp sequence within the 123 bp product	0/31	_	0/10	-	-
Di Alberti, 1997 [40]	Italy	Heminested PCR for 16S rRNA	17/38	4 NTM, 13 Mycobacterium spp	39/113	39 Mycobacterium spp	1.54 (0.73–3.24)

Table 1 Case–control studies evaluating the role of mycobacteria in sarcoidosis *(Continued)*

Vokurka, 1997 [41]	France	PCR of IS6110 and DR region	0/15	_	0/27	_	-
Ozcelik, 1997 [42]	Turkey	PCR of IS6110	5/11	MTBC	2/15	MTBC	5.42 (0.81-36.36)
Popper, 1997 [43]	Austria	PCR of 65 kDa mycobacterial antigen and IS6110	11/35	NTM	0/39	-	37.08 (2.09–657.90)
El-Zaatari, 1996 [44]	USA	PCR of IS900/IS902, MAC-specific PCR assay and Western blot	7/7	NTM	13/38	NTM	28.33 (1.50–534.74)
Fidler, 1993 [45]	UK	PCR of 65 kDa mycobacterial antigen and IS6110	7/16	MTBC	1/16	MTBC	11.67 (1.23–110.95)
Thakker, 1992 [46]	UK	PCR of groEL	1/14	MTBC	1/11	MTBC	0.77 (0.04–13.87)
Gerdes, 1992 [47]	Germany	PCR of 16S rDNA	0/14	-	0/10	-	-
Mitchell, 1992 [48]	UK	Mycobacterial rRNA detection by liquid phase hybridisation	5/5	MTBC	0/5	-	121 (2.02–7259.18)
Saboor, 1992 [49]	UK	PCR of IS986/IS6110 and groEL	14/20	10 MTBC, 4 NTM	5/22	3 MTBC, 2 NTM	7.93 (1.99–31.59)
Lisby, 1993 [50]	Denmark	Nested PCR for IS900	0/18	-	0/18	-	-
Grosser, 1999 [51]	Germany	PCR of IS986/IS6110	35/65	MTBC	1/34	MTBC	38.50 (4.96–298.57)
Vago, 1998 [52]	Italy	PCR of IS6110	2/30	MTBC	0/17	-	3.07 (0.14–67.75)
Richter, 1996 [53]	Germany	PCR of mycobacterial 16S rDNA	1/24	MTBC	3/57	MTBC	0.78 (0.08–7.93)
Ghossein, 1994 [54]	USA	PCR of 65 kDa mycobacterial antigen	0/10	-	0/10	-	-
Cannone, 1997 [55]	Italy	PCR of IS6110	2/30	MTBC	0/10	-	1.84 (0.08–41.62)

n Mycobacteria-positive samples, *N* total samples, *PCR* polymerase chain reaction, *65 kDa* 65-Kilodalton mycobacteria antigen, *IS6110* insertion sequence to identify *Mycobacterium tuberculosis* complex (MTBC), *NTM* non-tuberculous mycobacteria, *NAAT* nucleic acid amplification test, *16S rDNA* ribosomal DNA common to all mycobacteria, *rpoB* RNA polymerase β-subunit gene, *MALDI-IMS* matrix-assisted laser desorption ionization as a mass spectrometry imaging, *ESAT-6* 6 kDa early secretory antigenic target produced by Mycobacterium tuberculosis, *IS986* insertion sequence to identify MTBC, *rRNA* ribosomal RNA, *IS1245/IS900/IS901/IS902* insertion sequence to identify *Mycobacterium avium* complex, *MPB64* mycobacterial protein, *mtp40* Specific primers of MTB species, *RFLP* restriction fragment length polymorphism *DR* direct repeat, *groEL* gene encoding 65 kDa antigen

Table 2 Case–control studies evaluating the role of *P. acnes* in sarcoidosis

First author/Year (Ref.)	Country	Molecular technique	Sarcoidosis n/N	Controls n/N	OR (95% CI)
Robinson, 2013 [24]	USA	PCR for bacterial 16S rDNA	7/30	1/30	8.83 (1.01–76.96)
Oswald-Richter, 2012 [25]	USA	MALDI-IMS for propionibacterial proteins	7/15	1/4	2.63 (0.22–31.35)
Yasuhara, 2005 [31]	Japan	PCR for 16S rRNA	2/6	0/6	7.22 (0.28–189.19)
Gazouli, 2002 [22]	Greece	PCR for 16S rRNA	0/46	0/20	-
Eish, 2002 [35]	Japan	PCR for 16S rRNA	93/108	25/86	15.13 (7.39–30.99)
Ishige, 1999 [38]	Japan	Quantitative PCR for 16S rRNA	12/15	3/15	16 (2.67–95.75)
Negi, 2012 [56]	Japan	Immunohistochemical methods (PAG and TIG antibodies) and western blot	149/196	0/79	500.43 (30.44–8226.20)
Yamada, 2002 [57]	Japan	Quantitative real-time PCR for 16S rRNA	8/9	2/9	28 (2.07–379.25)
Eishi, 1994 [58]	Japan	PCR for P. acnes DNA	36/39	12/29	17 (4.23–68.28)
Abe, 1984 [19]	Japan	Isolation of P acnes in culture	31/40	38/180	12.87 (5.65–29.34)
Hiramatsu, 2003 [59]	Japan	Nested PCR for 16S rRNA	21/30	7/30	7.67 (2.42–24.24)

16S rDNA ribosomal DNA, *MALDI-IMS* matrix-assisted laser desorption ionization as a mass spectrometry imaging, *rRNA* ribosomal RNA

second study did not reveal a significant association [3] (OR 0.43; CI:0–23.23), while in the third, all real-time PCR analyses for the detection of Rickettsia were negative [71]. None of the studies reported a significant association with *Chlamydia pneumonia* [7, 8, 72], Epstein-Barr virus [40], or retrovirus [73].

Meta-analysis

Mycobacteria (Table 1)

Both *Mycobacterium tuberculosis* complex (MTBC) and nontuberculous mycobacteria (NTM) were investigated in most of the 36 relevant studies, although some used primers to detect only *M. tuberculosis* [26, 28–33, 38, 39, 41, 42, 51, 52, 55], and others detected only nontuberculous mycobacteria [44, 50].

Figure 1 provides a forest plot for sarcoidosis and mycobacteria based on a total of 1034 sarcoidosis patients and 1054 controls. Of the 1034 sarcoidosis cases, 173 were positive for MTBC, and 58 were positive for NTM. It was not possible to identify the type of mycobacteria involved in 18 samples, while both types of mycobacteria DNA were present in one sample. In total, 250 sarcoidosis samples were positive for some form of mycobacteria DNA sequence for a positive signal rate of 24.2%. We found a significant association between sarcoidosis and mycobacteria with an OR of 6.8 (95% CI:3.73–12.39). A strong association was also found between sarcoidosis and NTM alone with an OR of 10.39 (95% CI:5.25–20.56), as well as for *M. tuberculosis* complex (OR 4.29; CI:2.60–7.08). There was moderate heterogeneity among studies (I^2 test 52.1%; $p = 0.001$), although all but three studies estimated a risk above unity with significance in most cases.

P. acnes (Table 2)

The risk of sarcoidosis associated with *P. acnes* was provided by the study design (Fig. 2). The OR derived from 11 studies with 534 cases and 488 controls was 18.80 (95% CI:12.62–28.01), and there was low heterogeneity (I^2 test 25.9%; $p = 0.206$). There was a positive signal rate of 68.54% for *P. acnes* (366 positive samples from 534

Table 3 Selected studies on the association between HHV-8 and sarcoidosis

First author/Year (Ref.)	Country	Molecular technique	Patients n/N	Controls n/N	OR (95% CI)
Knoell, 2005 [60]	USA	PCR for HHV-8 DNA	0/8	0/8	-
Gazouli, 2002 [22]	Greece	PCR for HHV-8 DNA	0/46	0/20	-
Fredricks, 2002 [61]	USA	PCR for HHV-8 ORF 26 DNA	0/18	0/4	-
Maeda, 2000 [62]	Japan	Hemi-nested PCR for HHV-8 DNA	4/119	4/120	1.01 (0.25–4.13)
Sugaya, 1999 [63]	Japan	Nested PCR for HHV-8 ORF 26 DNA	0/12	1/1	0.01 (0.00–0.95)
Bélec, 1998 [64]	France	Nested PCR for HHV-8 ORF 25/26 DNA	0/14	2/17	0.21 (0.01–4.84)
Di Alberti, 1997 [40]	Italy	Nested PCR for HHV-8 ORF 26 DNA and Heminested PCR for HHV-8 ORF 25 DNA	38/39	6/113	677.67 (79.01–5812.52)

HHV-8 Human Herpesvirus 8, *ORF 25/26 DNA* insertion sequence to identify HHV-8

Table 4 Selected studies on the association between *Borrelia* species and sarcoidosis

First author/Year (Ref.)	Country	Molecular Technique	Sarcoidosis		Controls		OR (95% CI)
			n/N	Type of microorganism	n/N	Type of microorganism	
Derler, 2009 [4]	Austria	Focus-floating microscopy and Borrelia-specific PCR DNA	13/35	Borrelia sp.	1/61	Borrelia sp.	35.45 (4.38–287.16)
Ishihara, 1998 [65]	Japan	Dot-blot analysis (Dotblot Borrelia Kit)	15/46	Borrelia sp.	2/100	Borrelia sp.	23.71 (5.14–109.46)
Martens, 1997 [66]	Germany	Western blot for Borrelia burgdorferi	1/60	Borrelia burgdorferi	27/1000	Borrelia burdorferi	0.61 (0.08–4.57)
Lian, 1995 [67]	China	PCR for Borrelia burgdorferi DNA	6/49	Borrelia burgdorferi	2/28	Borrelia burgdorferi	1.81 (0.34–9.66)
Xu, 1996 [68]	China	In situ PCR for Borrelia burgdorferi DNA	0/23	-	0/23	-	-
Ishihara, 1996 [69]	Japan	Elisa and Dot-blot analysis for Borrelia sp.	1/38	Borrelia sp.	1/80	Borrelia sp.	2.14 (0.13–35.08)

patients). When accounting for the source of biological samples studied, we found that nine of the 11 studies [19, 22, 24, 25, 35, 38, 56–58] evaluated the presence of *P. acnes* in lymph node samples, of which seven evaluated this location exclusively [19, 24, 35, 38, 57, 58]. This could justify the low heterogeneity among studies, contrary to what was observed in the forest plot of mycobacteria, where the studied biological samples were more heterogeneous.

Borrelia and HHV-8 (Tables 3 and 4)

Of the six articles assessing the presence of *Borrelia* in sarcoidosis tissues, three used polymerase chain reaction (PCR) techniques for DNA amplification of *B. burgdorferi*

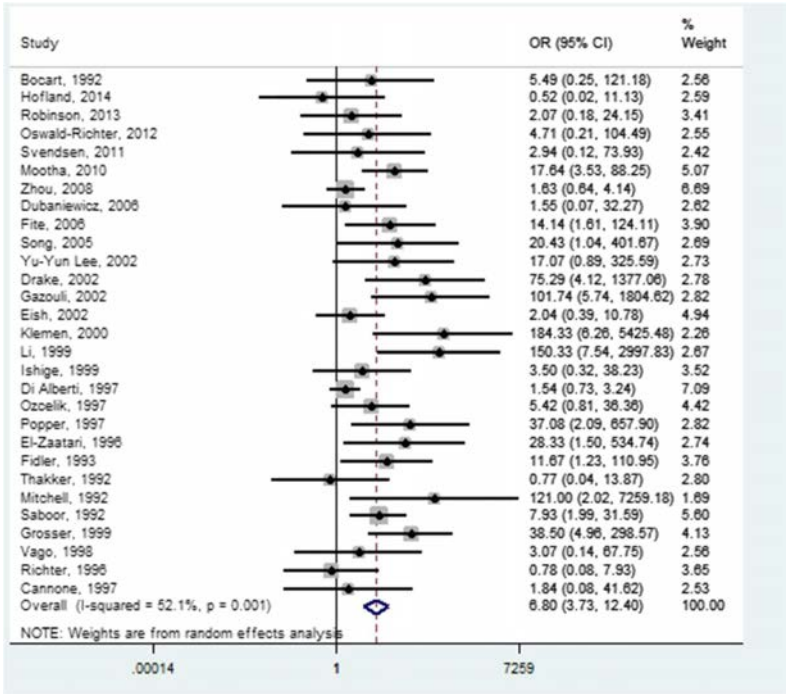

Fig. 1 Forest plot of studies that show the presence of mycobacteria in sarcoidosis patients versus controls

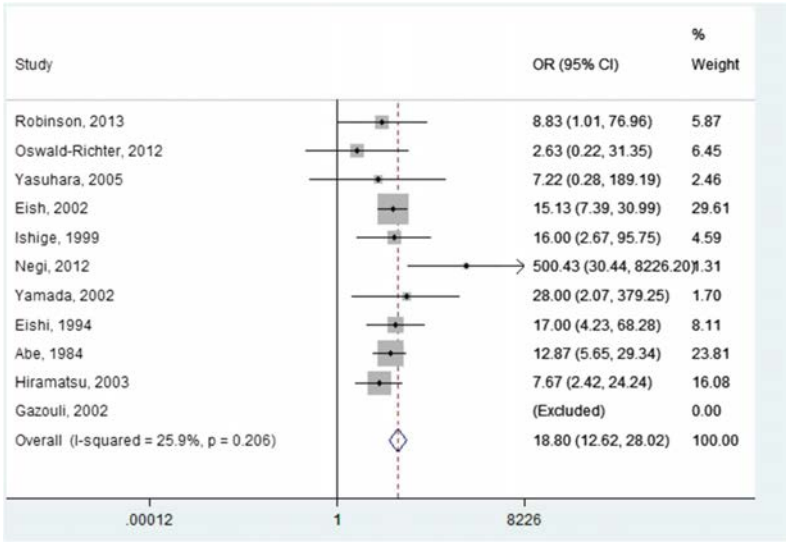

Fig. 2 Forest plot of studies that show the presence of *P. acnes* in sarcoidosis patients versus controls

[66–68], whereas the other three did not specify which species of *Borrelia* were involved [4, 65, 69]. The pooled OR derived from these six studies with 251 cases and 1292 controls was 4.82, but this result did not reach statistical significance (95% CI:0.98–23.81). Statistical heterogeneity was moderate with an I^2 of 70% and $p = 0.01$ Fig. 3 a).

Di Alberti et al [40] were the only ones to report a significant association between sarcoidosis and HHV-8 in comparison with controls. However, the remaining six studies refuted those results [22, 60–64]. Overall, there was no significant association between sarcoidosis and HHV-8 (OR 1.47; CI:0.02–110.06), and there was high heterogeneity among studies (I^2 test 92%; $p = 0.000$) (Fig. 3 b).

Evaluation of publication bias

We performed funnel plots to evaluate publication bias (Fig. 4). The funnel plots of HHV-8 and mycobacteria showed evidence of publication bias (Fig. 4b and c), while the graphs regarding the presence of *Borrelia* and *P. acnes* are fairly symmetrical (Fig. 4a and d). Thus, no suggestion of publication bias is indicated in these cases.

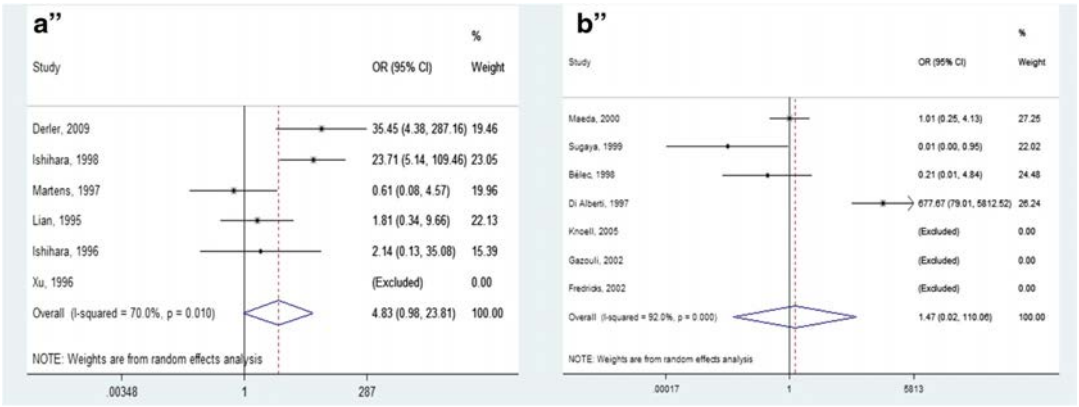

Fig. 3 Forest plot: **a** summary OR for the presence of *Borrelia* species in sarcoidosis patients; **b** summary OR for the presence of HHV-8 in sarcoidosis patients

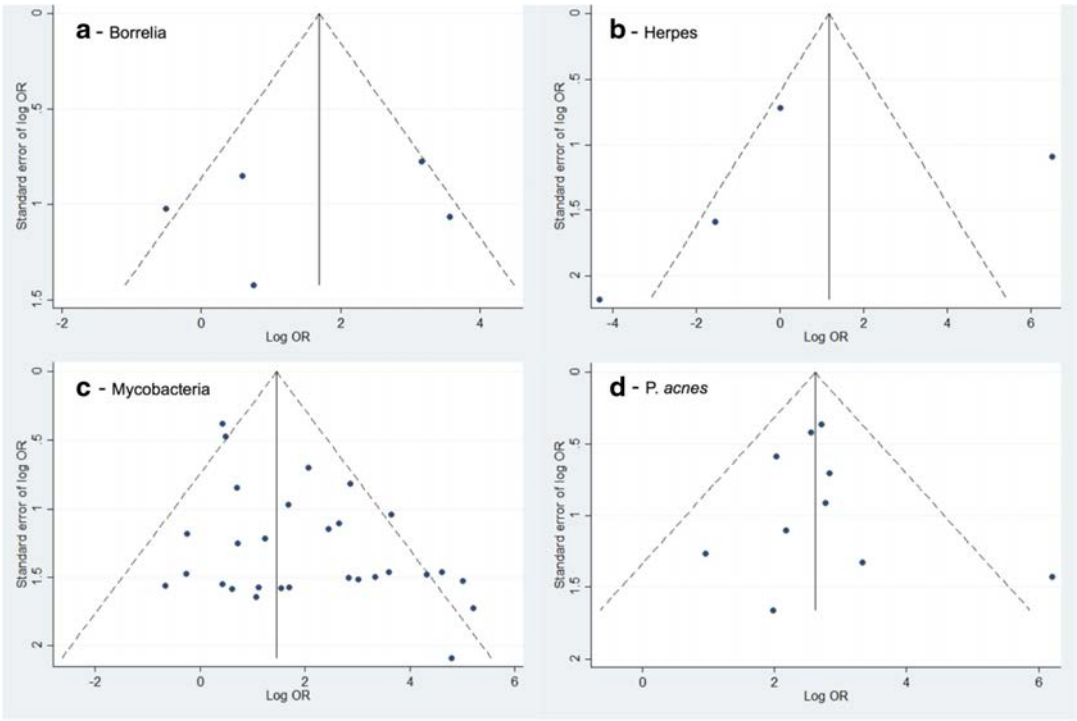

Fig. 4 Funnel plot of all studies: (a) Borrelia; (b) Herpes; (c) Mycobacteria; (d) P. acnes

Sensitivity and subgroup analysis

To verify the robustness of the results, as well as the potential sources of heterogeneity, subgroup and sensitivity analyses were performed (especially for mycobacteria).

Subgroup analysis

Concerning the studies of mycobacteria, we conducted subgroup meta-analysis by various study characteristics (Table 5). The pooled OR was calculated in subgroups of studies according to geographical area, publication year, type of study, and molecular technique used. There was a significant association between sarcoidosis and mycobacteria in all subgroups, except in three studies included in the subgroup of molecular techniques (BD ProbeTec and culture). The pooled OR was significantly higher with some covariates, however almost all of the ORs derived from these subgroup data were significantly above unity.

Through the subgroup analyses, it was noted that the variables that most influenced the results of heterogeneity were: a) the study type, being null the heterogeneity in the ten prospective studies, contrasting with the moderate heterogeneity in retrospective studies; b) the geographical location, verifying a low heterogeneity in studies conducted in the USA and Asia.

Sensitivity analysis

We also performed a sensitivity analysis to complement the subgroup analysis in order to better explain the heterogeneity between studies (Table 5).

Regarding sarcoidosis and mycobacteria, there was a strong and significant association (OR 4.33; CI:2.06–9.10) in subgroup analysis of geographic locations when we restricted to studies performed in countries with low burden of tuberculosis [24, 30, 35, 38, 40, 42, 44–46, 49, 51, 53]. There was low heterogeneity among these studies (I^2 test 45.7%; $p = 0.042$) (Table 5).

Another subgroup analysis compared the results according to the different biological samples used. Most of the studies included in our meta-analysis used biological samples from different locations, including the skin, lymph nodes, and lungs. However, when we restricted the analysis to include only studies that performed PCR on biological samples of the same type, the associations were also significant in both lung samples [30, 40, 45] (OR 2.93; CI:1.09–7.86; $I^2 = 56.9\%$) and lymph node samples [24, 35, 38, 40, 46] (OR 3.82; CI:1.53–9.49; $I^2 =$

Table 5 Subgroup and sensitivity analysis of the association between sarcoidosis and mycobacteria

	No. of studies	OR (95%CI)	*P*-value heterogeneity	I^2 (%)
Subgroup analysis				
1 - Geographical region				
Europe	22	6.92 (3.05, 15.71)	0.004	53.8
USA	7	18.21 (4.64, 71.53)	0.238	26.2
Asia	7	4.09 (1.38, 12.12)	0.093	49.8
2 - Publication year				
< 2000	20	6.63 (2.84, 15.51)	0.006	54.2
> =2000	16	8.40 (3.31, 21.31)	0.012	53
3 – Type of study				
Prospective	10	11.91 (4.94, 28.69)	0.743	0.0
Retrospective	26	6.41 (3.14, 13.09)	0.001	57.1
4 - Molecular technique				
PCR	29	7.04 (3.57, 13.89)	0.000	58.8
Hybridization	2	37.81 (3.40, 420.43)	0.484	0.0
Protein analysis	2	12.12 (1.44, 102.20)	0.408	0.0
BD ProbeTec	2	2.09 (0.23, 19.10)	0.776	0.0
Culture	1	0.52 (0.02, 11.13)	-	-
Sensitivity analysis				
1 - Biological samples				
Only lymph nodes	11	3.82 (1.53, 9.49)	0.384	4.0
Only lung	5	2.93 (1.09, 7.86)	0.098	56.9
Only skin	2	11.58 (0.06, 2016.91)	0.021	81.3
2 – Incidence of tuberculosis				
Only countries with low burden of TB	33	4.33 (2.06, 9.10)	0.042	45.7

CI confidence interval, *OR* odds ratio, *BD ProbeTec* molecular detection based on strand displacement amplification (SDA) technology, *TB* tuberculosis

4%), but not skin biological specimens [37, 40] (OR 11.58; CI:0.06–2016.91; $I^2 = 81.3\%$). Once again, the heterogeneity among studies was low to moderate except in two studies performed on skin biopsies (Table 5).

In sensitivity analysis on studies of sarcoidosis and *P. acnes*, we found a significant association compared with the controls when we selected only studies performed outside Asia [22, 24, 25], with a pooled OR of 5.5 (95% CI:1.13–27.42) and no heterogeneity among studies.

Discussion

The present meta-analysis is the first to evaluate all infectious agents proposed to be associated with sarcoidosis and involving more than 6000 patients in several countries. The results point to an etiological link between *P. acnes* and sarcoidosis with a positive signal rate of 68.54%. Also, almost one quarter of sarcoidosis patients show the presence of mycobacteria within the lesions. The associations are fairly specific, since *P. acnes* (OR 18.80) and mycobacteria (OR 6.8) were significantly increased in sarcoidosis patients, while *Borrelia* (OR 4.82; CI:0.98–23.81) and HHV-8 (OR 1.47; CI:0.02–110.06) were not associated with sarcoidosis, contrary to previous investigations.

Three decades ago, Abe et al [19] reported that *P. acnes* was the only bacterium isolated in lymph node biopsy samples taken from sarcoidosis patients. Studies published in recent years have confirmed that *P. acnes* could be a possible infectious agent implicated in the pathogenesis of sarcoidosis [24, 56, 74–76]. However, some studies suggest that *P. acnes* is not specific for sarcoidosis because it is a normal inhabitant of peripheral lung tissue and mediastinal lymph nodes, apart from the skin [77]. Despite this, the results of our meta-analysis show a significant quantitative difference in the presence of the *P. acnes* genome in sarcoidosis patients compared to control subjects. This suggests that this microorganism may be present abnormally or may proliferate ectopically in such sarcoid lesions.

However, it is important to note that most of the studies in our meta-analysis evaluating the role of *P. acnes* in sarcoidosis were by Japanese groups testing Japanese patients, while only very limited data exist for African American or Caucasian patients [22, 24, 25]. The results

were conflicting in these three studies, but interestingly, the pooled OR was above unity and statistically significant (5.58; CI:1.13–27.42). Despite these surprising results, the ORs observed in studies with Japanese patients were far superior, and the results were more consistent and robust. Differences between these two groups may be due to the geographical, ethnic, or racial composition of the study population. Sarcoidosis in Japanese patients is characterized by a high rate of ocular, cutaneous, and cardiac involvement, while in Europe and the USA, this disease mainly affects the lungs.

In 2002, the first large, relevant study was published as a collaboration between several countries [35]. The results of this international study suggest an association between *P. acnes* and sarcoidosis in not only Japanese patients (positive signal rate of 89.2%), but also in Europeans (positive signal rate of 81.4%). However, more international corporative studies with quantitative PCR are needed to clarify the role of *P. acnes* in sarcoidosis and for better understanding of the phenotypic variability of this disease.

Recent years have witnessed substantial discussion among investigators about the role that mycobacteria may have in the pathogenesis of sarcoidosis, and the issue remains unsettled, if not controversial. With the emergence of new microbiological techniques, especially in the molecular biology area, several studies have been conducted in order to investigate this possible association more deeply.

In the present meta-analysis, we identified 36 studies assessing the presence of mycobacteria in a total of 1034 sarcoidosis patients and 1054 controls. The results suggest a strong association of sarcoidosis with NTM (OR 10.39; CI:5.25–20.56) and with MTBC (4.29; CI:2.60–7.08). However, to evaluate the possible relationship between mycobacteria and sarcoidosis, the current incidence of tuberculosis should be taken into account in general populations of the different countries where the studies of sarcoidosis were performed. In the sensitivity analyses, a significant association was also found (OR 4.33; CI:2.06–9.10) when we restricted the analysis to include only studies performed in countries with low prevalence of tuberculosis. This further confirms the robustness of the results and the relevance of this association worldwide.

Despite the heterogeneity of analyzed studies and the potential publication bias suggested by the mycobacteria funnel plots, most of the ORs derived from individual data were significantly above unity. Furthermore, sensitivity and subgroup analyses including only studies performed on lung samples or lymph nodes showed low heterogeneity. Therefore, it is important to account for the heterogeneity in sarcoidosis specimens (lung versus skin or lymph nodes). We found significant increased ORs in studies performed on lung or lymph node samples but not in skin specimens. Possible explanations for this include the following: 1) In the initial phase of the disease, systemic sarcoidosis primarily affects and spreads through the lymphatic system, following the lymphatic vessels to the hilar and mediastinal lymph nodes. 2) Lung and lymph node samples are obtained sterilely by endoscopy biopsies and thus avoid possible microorganism contamination, in contrast to skin biopsies. 3) The two studies performed on sarcoidosis skin samples were both retrospective [37, 40]. In such studies, there is a greater possibility of both contamination of the paraffin-embedded specimens and more DNA fragmentation. In contrast, several studies performed on lung and lymph node samples were prospective, and only fresh tissues were used. Additionally, when we conducted the subgroup analysis according to the type of study, it was found a low heterogeneity in the 10 prospective studies ($I^2 = 0\%$), contrasting with the moderate heterogeneity in retrospective studies ($I^2 = 57.1\%$).

The hypothesis that *B. burgdorferi* could be a possible causal infectious agent for sarcoidosis was first mentioned in 1989 in epidemiological studies [78]. Since then, several studies have been conducted using serological or molecular techniques in order to clarify the role of *Borrelia* in the pathogenesis of sarcoidosis. We identified six articles assessing the presence of *Borrelia* in sarcoidosis tissues using molecular techniques (251 cases and 1292 controls), and we did not find a significant association (OR 4.82; CI:0.98–23.81). On the other hand, the two studies that reported a significant association between *Borrelia* and sarcoidosis [4, 65] were both conducted in regions where Lyme disease is endemic, in contrast to the four other articles performed in non-endemic areas [66–69], where the results did not reach statistical significance.

It is important to note that the frequency of exposure to *Borrelia. spirochete* is different between patients living in regions where the disease is endemic and those in regions where it is not. Thus, in countries with elevated *B. burgdorferi* prevalence, a protective immunity against this microorganism has to be assumed in the general population. T-helper lymphocyte activity to this microorganism might be a trigger for the development of sarcoidosis in endemic regions, which could explain the positive results in studies published in Austria and Japan [4, 65]. Apart from these two studies, the fact that significant positive PCR results could not be found argues against the hypothesis of a connection between *B. burgdorferi* infection and sarcoidosis. However, more studies are needed to clarify the possible association, especially in endemic areas.

There are several clinical implications of this study. Currently, immune suppression remains the primary

treatment modality for sarcoidosis. Given our meta-analysis, it is worth exploring whether certain antibacterial or antimycobacterial drugs might alter the course of sarcoidosis. In the past, some clinical trials have been published with conflicting results using classical antituberculous drugs, such as isoniazid, amino-salicylic acid, and streptomycin [79–82]. Recently, Drake et al [83] conducted a double-blind, placebo-controlled study to investigate the efficacy of oral antimycobacterial therapy (levofloxacin, ethambutol, azithromycin, and rifampin) in patients with cutaneous sarcoidosis. The results were promising, with significant reductions in cutaneous lesion size. The same authors also conducted an open-label investigation using the same therapy regimen in pulmonary sarcoidosis patients, and the results were again very interesting with significant improvements in forced vital capacity from baseline to completion of therapy [84].

Other antimicrobial agents such as minocycline and doxycycline have been shown to be quite effective in treating cutaneous sarcoidosis in some series [85, 86]. However, the exact mechanism of action of these drugs it is not fully understood [87].

Currently, other clinical trials are being done (NCT02024555 and NCT01245036) to clarify the role that antimicrobial agents might have in the treatment of sarcoidosis.

Several limitations in our study should be recognized. First, one of the main potential limitations relates to the variability and heterogeneity of the results analyzed. It is important to consider that the majority of these studies were assessed retrospectively and that data were obtained from different databases and hospitals. This could lead to different types of bias in the included studies and to variability in the results. Second, the risk of contamination or DNA fragmentation in PCR techniques can lead to false positive or false negative results. In addition, PCR does not discriminate between living and dead microorganisms. Third, the patients had varied clinical manifestations of sarcoidosis; moreover, the non-sarcoidosis controls were comprised of different types of subjects across the studies, which may cause misclassification bias and heterogeneity.

Conclusion

The present meta-analysis, involving more than 6000 patients from various countries worldwide, suggests a significant association between sarcoidosis and some infectious agents, taking into account the marked difference in the percentage of microbial DNA-positive samples in sarcoidosis patients versus controls, especially mycobacteria (OR 6.8) and *P. acnes* (OR 18.80). Furthermore, our study also suggests caution regarding a putative association between sarcoidosis and *B. burgdorferi*.

What seems clear is that more than one infectious agent might be implicated in the pathogenesis of sarcoidosis; probably the patient's geographical location might dictate which microorganisms are more involved.

More studies and clinical trials are needed to extend this evidence to a more global level.

Additional files

Additional file 1: PRISMA Checklist (PDF 545 kb)

Additional file 2: Search strategy of MEDLINE via OVID. (DOC 22 kb)

Additional file 3: Flow diagram of the current meta-analysis. (DOC 1550 kb)

Abbreviations

B. burgdorferi: Borrelia burgdorferi; CI: Confidence interval; HHV-8: Human herpesvirus 8; MTBC: Mycobacterium tuberculosis complex; NTM: Nontuberculous mycobacteria; OR: Odds ratio; *P. acnes*: Propionibacterium acnes; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Acknowledgements

We thank Miguel Angel Descalzo, responsible for the research unit of the Spanish Academy of Dermatology and Venereology (AEDV), for his assistance with statistical analysis and Rubina Alves, M.D., for reviewing an earlier version of the manuscript.

Funding

None.

Availability of data and materials

We declare that the data supporting the conclusions of this article are fully described in the article.

Authors' contribution

TE and VGP contributed equally to the study. TE, GA and VGP conceived the study and designed the systematic review and meta-analysis. TE, GA and VGP contributed to the data extraction, performed the analysis and interpreted the results. TE and VGP wrote the first draft; TE, GA and VGP contributed to the revision of the final report. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Since this study was a literature review and meta-analysis of previously reported studies, ethical approval or additional consent from participants was not required.

Prior presentation

This data has not been published elsewhere.

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Received: 17 May 2016 Accepted: 22 November 2016
Published online: 28 November 2016

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