UNIVERSITÀ
DEGLI STUDI
FIRENZE

DOTTORATO DI RICERCA TOSCANO IN NEUROSCIENZE

CICLO XXXIV

COORDINATORE Prof.ssa Maria Pia Amato

# CLINICO-PATHOLOGICAL VARIABILITY AND PROPOSAL OF NEW BIOMARKERS IN CENTRAL NERVOUS SYSTEM AND MUSCULAR SARCOIDOSIS

Settore Scientifico Disciplinare MED/26


**Dottorando**
Dott.ssa Silvia Bocci
*(firma)*

**Tutore**
Prof. Fabio Giannini
*(firma)*

**Coordinatore**
Prof.ssa Maria Pia Amato
*(firma)*


Anni 2018/2021

# TABLE OF CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| **$^{18}$F-FDG** | $^{18}$F-fluorodeoxyglucose |
| **6MWT** | Six-minute walk test |
| **ACCESS** | A Case Control Etiologic Study of Sarcoidosis |
| **ACE** | Angiotensin-converting enzyme |
| **ACR** | American College of Rheumatology |
| **ADA** | Adalimumab |
| **ALS** | Amyotrophic Lateral Sclerosis |
| **APCs** | Antigen presenting cells |
| **ATS** | American Thoracic Society |
| **AZA** | Azathioprine |
| **BAL** | Bronchoalveolar lavage |
| **BTS** | British Thoracic Society |
| **CK** | Creatine kinase |
| **CNS** | Central nervous system |
| **CS** | Corticosteroids |
| **CSF** | Cerebrospinal fluid |
| **CT** | Computed tomography |
| **CTO** | Chitotriosidase |
| **CYC** | Cyclophosphamide |
| **DLCO** | Carbon-monoxide diffusing lung capacity |
| **DM** | Dermatomyositis |
| **EBUS** | Endobronchial ultrasound |
| **EMG** | Electromyography |
| **EULAR** | European League Against Rheumatism |
| **FVC** | Forced vital capacity |
| **GBS** | Guillain-Barré syndrome |
| **HCQ** | Hydroxychloroquine |
| **HLA** | Human leucocyte antigens |
| **HRCT** | High-resolution computed tomography |
| **IBM** | Inclusion body myositis |

| | |
|---|---|
| **IENFD** | Intraepidermal nerve fiber density |
| **IFNγ** | Interferon-gamma |
| **IFX** | Infliximab |
| **IgG4-RD** | IgG4-Related Disease |
| **IIM** | Idiopathic inflammatory myopathies |
| **IL** | Interleukin |
| **ILD** | Interstitial lung disease |
| **IQR** | Interquartile range |
| **IVIg** | Intravenous Immunoglobulins |
| **KL-6** | Krebs von den Lungen-6 |
| **LEF** | Leflunomide |
| **LSZ** | Lysozyme |
| **MAA** | Myositis-associated antibodies |
| **MAC** | Membrane Attack Complex |
| **MGCs** | Multinucleated giant cells |
| **MHC** | Major Histocompatibility Complex |
| **MMF** | Mycophenolate mofetil |
| **MRC** | Medical Research Council |
| **MRI** | Magnetic resonance imaging |
| **mRS** | Modified Rankin Scale |
| **MS** | Multiple Sclerosis |
| **MSA** | Myositis-specific antibodies |
| **MTX** | Methotrexate |
| **NAM** | Necrotizing autoimmune myopathy |
| **NCCG** | Neurosarcoidosis Consortium Consensus Group |
| **NCS** | Nerve conduction studies |
| **NEWM** | Non-enhancing white matter |
| **NS** | Neurosarcoidosis |
| **PCR** | Polymerase chain reaction |
| **PET** | Positron emission tomography |
| **PFTs** | Pulmonary function tests |
| **PM** | Polymyositis |
| **PNS** | Peripheral nervous system |
| **RTX** | Rituximab |

| | |
|---|---|
| **SFN** | Small fiber neuropathy |
| **sIL-2R** | Soluble Interleukin-2 Receptor |
| **SPECT** | Single-photon emission computerized tomography |
| **TBNA** | Transbronchial needle aspiration |
| **TEM** | Transmission electron microscopy |
| **TNFα** | Tumor Necrosis Factor-alpha |
| **TNFR1-2** | Tumor Necrosis Factor-alpha receptors 1 and 2 |
| **TPMT** | Thiopurine s-methyltransferase |
| **WASOG** | World Association of Sarcoidosis and Other Granulomatous Disorders |

# CHAPTER 1: SARCOIDOSIS

Sarcoidosis is a multi-system granulomatous disease of unknown etiology characterized by the infiltration of various organs by non-necrotizing granulomas. The first clinical description, as *lupus pernio*, was reported by Besnier in 1889 *(Besnier, 1889)* while Boeck, ten years later, firstly described sarcoid granuloma pathology *(Boeck, 1899)*. Nowadays, although it remains a disease of unknow etiology, mechanisms underlying granuloma formation are better understood than in the past, including genetic susceptibility and environmental factors *(Sakthivel, 2017)*.

## 1.1 Epidemiology

Sarcoidosis is a ubiquitous disease, although the prevalence of it varies greatly in different populations, from 1-5/100.000 in South Korea, Taiwan and Japan *(Park, 2018; Wu, 2017; Pietinalho, 1995)* to 140-160/100.000 in Sweden and Canada *(Arkema, 2016; Fidler, 2019)*. The real prevalence and incidence of sarcoidosis is however difficult to establish, as asymptomatic individuals may elude epidemiological studies and the disease is almost asymptomatic in half of the patients. Moreover, a high prevalence of *Mycobacterium tuberculosis* in many regions may lead to a significant sarcoidosis underdiagnosis *(Lazarus, 2009)*.

The first population study performed in the US reported an age- and sex-adjusted incidence of 6,1 per 100.000 person-years, with similar values in males (5,9/100.000) and females (6,3/100.000) *(Henke, 1986)*. Different incidence rates were reported by later studies, varying from 0,73 to 71 cases per 100.000 person-year, with major differences due to ethnicity and race, being lower in Asians and higher in African Americans *(Rybicki, 1997; Cozier, 2011; Morimoto, 2008)*. A recent population study expanded the first one on the American cohort, extending the original observation period (1946-1975) up to 2013 and finding an annual incidence of sarcoidosis of 10,0 per 100.000 people (9,4 in males and 10,5 in females) *(Ungprasert, 2016a)*. These results are in line with those from other studies mainly including Caucasian populations *(Gribbin, 2006; Hillerdal, 1984; Fazzi 1992)*.

Sarcoidosis generally affects adult population, with mean age at diagnosis of 48,3

years in males and 42,8 years in females, with a progressive increase over the years *(Ungprasert, 2016a; Morimoto, 2008)*.

In Italy, sarcoidosis is the most prevalent interstitial lung disease, representing 33,7% of all diagnosis reported in the national register for rare diffuse infiltrative lung disorders. It has a slight predominance in females (54,9%) and a mean age at diagnosis of 52,4 years *(Tinelli, 2005)*.

Many studies investigated potential associations of sarcoidosis with different professional or environmental risk factors, supported by temporal and space-time clusters. Risk factors include exposure to musty odors, insecticides or to metal-processing industries while a decreased risk has been linked to cigarette smoking *(Laney, 2009; Newman, 2004)*. A high incidence of sarcoidosis was reported in fire fighters and other responders after the attacks on the World Trade Center in 2001 *(Crowley, 2011)*. Moreover, neighborhood socioeconomic position is associated with sarcoidosis severity *(Harper, 2020)* and high deprivation (implying psychological stress, decreased use of preventive healthcare, decreased exercise and more) was found to be associated with 20% increased odds of sarcoidosis in a study from Sweden *(Li, 2019)*. Lastly, several studies reported that obesity, which was previously considered as induced by systemic corticosteroid therapy, was associated with an increased incidence of sarcoidosis also when present at baseline and prior to treatment, mostly in females *(Cozier, 2015; Dumas, 2017; Ungprasert, 2016c)*. These reports support the possible role of obesity as a risk factor for sarcoidosis *(Arkema, 2020)*.

Sarcoidosis is usually sporadic, but is reported as familial in 2,9-9,6% of cases in different populations *(Pietinalho, 1999; Brennan, 1984)*. The 80-time increased risk in monozygotic twins *(Sverrild, 2008)* and the 3,7-fold increased risk in first degree relatives *(Rossides, 2018)* lend support to the idea that genetic factors may play a relevant role in disease susceptibility.

### 1.2 Etiology and pathogenesis

The exact cause of sarcoidosis is still unknown and probably both genetic susceptibility and environmental factors contribute to disease development.

Immunologically, sarcoidosis is an exaggerated immune response to hitherto unidentified antigens. Two major categories of agents are considered as potential cause

of sarcoidosis: microbial organisms and noninfectious environmental agents (organic or inorganic) *(Lazarus, 2009)*.

Many infectious agents have been proposed as possible cause of sarcoidosis. Similarities with tuberculosis induced wide investigation on possible involvement of *Mycobacterium tuberculosis complex*, without clear conclusions. Studies based on polymerase chain reaction (PCR) techniques identified mycobacterial DNA in over 26% of sarcoidosis biopsy specimens, 9- to 19-fold more frequently than in non-sarcoidosis tissue control samples *(Gupta, 2007)*. However, these results are not consistently reproducible, and due to other pieces of evidence, such as the observation that sarcoidosis patients treated with corticosteroids do not show reactivation of tuberculosis, a direct role of mycobacterial infection in sarcoidosis remains questionable.

Another well-studied infectious agent is *Propionibacterium acnes*, due to the detection of its DNA in lymph nodes and bronchoalveolar lavage cells from patients with sarcoidosis *(Eishi, 2002; Hiramatsu, 2003)* and to the efficacy of clarithromycin administration in some sarcoidosis patients *(Takemori, 2014)*. However, presence of *Propionibacterium acnes* DNA in control tissues and healthy individuals raises concern in attributing this organism as a potential agent *(Ishige, 2005)*.

Ultimately, there is no evidence that sarcoidosis is an infectious disease, but most likely it is an exaggerated immune response to pathogen-associated molecular patterns of killed and partly degraded mycobacteria and propionibacteria. These agents tend to persist in macrophage phagosomes due to their high lipid content in membranes, and many of their glycolipoproteins are not very soluble and resist degradation. Various studies detected mycobacterial and proprionibacterial pathogen-associated molecular patterns in tissues from patients with sarcoidosis substantially more often than in tissues from healthy individuals *(Chen, 2008; Ishige, 1999)*. Moreover, several different pathogen-associated molecular patterns from mycobacteria and lysates from heat-killed propionibacteria induce pulmonary granuloma in mice *(Swaisgood, 2011; McCaskill, 2006)*. Therefore, it is probable that a microbial-induced host response promotes the aggregation and persistence of the non-degradable antigens, forming a nidus for granuloma formation and causing sarcoid lesions through an exaggerated immune response from the close interaction between macrophages and T cells, which stimulate each other *(Zissel, 2013)*.

Regarding environmental agents, pathogenicity hypothesis are mostly based on

variability in prevalence and incidence in different occupational and social context. Among many environmental and occupational risks reported in the literature, only agriculture-related occupations, mold or musty odors at work, and exposure to pesticides reached an odds ratio of 1.5 in the ACCESS study *(Newman, 2004)*. Some of these contexts may represent a source of mycobacterial antigens, due to the ability of such agents to proliferate in humid and musty environments. Therefore, mechanisms underlying sarcoidosis pathogenesis would be similar to a granulomatous hypersensitivity pneumonitis *(Newman, 2012)*. Granulomatous inflammation can also be seen from exposure to inorganic agents, such as beryllium, zirconium, nickel, chromium, and synthetic mineral fibers *(Lazarus, 2009)*. However, both chronic beryllium lung disease and hypersensitivity pneumonitis, whose findings may resemble that of sarcoidosis, lack extra-thoracic manifestations. Moreover, it must be kept in mind that the development of real sarcoidosis requires not only an antigen, but also an inflammatory context favoring granuloma formation.

Genes regulating class I and II human leucocyte antigens (HLA) expression, play an important role in the immune response leading to sarcoidosis. Different class I and II alleles were reported as associated to increased or reduced risk of developing sarcoidosis and to different courses or organ involvement *(Lazarus, 2009)*. In addition, other genes outside HLA complex, such as BTNL2 and ANXA11, have shown an association to sarcoidosis susceptibility in genome-wide association studies, with mechanisms still to be completely understood *(Valentonyte, 2005; Hofmann, 2008)*.

The key pathologic hallmark of sarcoidosis is the compact epithelioid granuloma, and disease morbidity is strictly related to the mechanisms that govern granulomatous inflammation *(Figure 1)*.

Granulomas are tiny aggregates of highly differentiated immune cells with discrete lymphoid-like structures *(Sakthivel, 2017)*. When circulating monocytes encounter foreign antigens, they differentiate in antigen-presenting cells (APCs) such as macrophages and dendritic cells (*Rivera, 2016*). After phagocytosis and degradation of the antigens, processed proteins are presented to T-CD4+ lymphocytes, together with costimulatory signals. In sarcoidosis patients, an excessive T-CD4+ response in disease localizations (as demonstrated by increased CD4/CD8 ratio in bronchoalveolar lavage) induces macrophages activation and their transformation into epithelioid cells. This

process is mediated by IL-2 and IFNγ production, due to a predominant $T_H1$ response, at least during the initial stage of the disease (*Kumar, 2010*). Macrophages population increases also due to recruiting of circulating cells, and their enhanced production of TNFα and chemokines further promotes the activation of $T_H1$ lymphocytes, creating a self-maintaining loop. Other lymphocytes subtypes, such as $T_H17$, and cytokines, such as IL-12, are probably involved in the process *(Lazarus, 2009; Sakthivel, 2017)*. Under the influence of inflammatory signals, cell-cell fusion occurs between macrophages and monocytes/dendritic cells, creating multinucleated giant cells (MGCs) (*Okamoto, 2003*). In a minority of patients (less than 20%), sarcoidosis can evolve in pulmonary parenchyma fibrosis, responsible of major morbidity and mortality. Which factors promote fibrosis is still not fully understood, but a switch to at least partial $T_H2$ lymphocyte polarization can play a role in the process *(Chen, 2008)*.

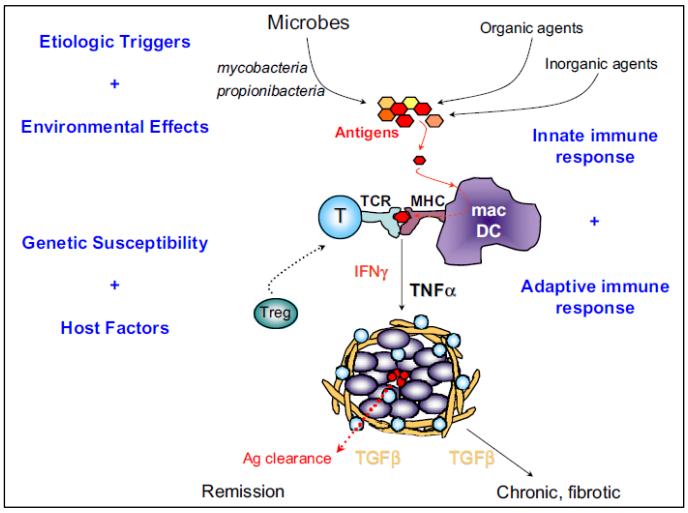



**Figure 1. Hypothetical model of the interactions between environmental, microbial, and immunologic factors that result in granulomatous inflammation in sarcoidosis** *(Chen, 2008)*

Ultimately, sarcoid epithelioid granuloma is a cellular attempt to isolate a non-soluble or poorly soluble antigen from surrounding tissues which could be damaged by its presence. The failure of antigen clearance is constantly fenced by persistent deregulated immune cells, preventing further antigen dissemination *(Sakthivel, 2017)*. Sarcoid granuloma structure is typically composed of two segments, core and crust. The core consists of tight clusters of macrophages, epithelioid cells and some MGCs, typically without any necrosis (except rare and scarce fibrinoid necrosis). The crust usually exhibits a high number of T lymphocytes and very few B lymphocytes and plasmacells *(Spector, 1976)*.

### 1.3 Clinical manifestations

Sarcoidosis can potentially affect any organ, with extremely variable clinical presentations and prognosis *(Figure 2)*. In older studies, up to half of the patients could have asymptomatic disease identified on chest X-ray performed for other reasons *(Reich, 2013)*. Incidental diagnosis is nowadays rare, reported in 8,4% of patients *(Mañá, 2017)*.

Presentation may be acute or chronic. Patients with acute disease generally have a good prognosis, with complete remission during the first 2 years *(Bargagli, 2018)*. *Löfgren's syndrome* is a clinically distinct phenotype of sarcoidosis with acute onset, characterized by fever, bilateral hilar lymphadenopathy, erythema nodosum and arthritis (mainly involving lower limbs) *(Rubio-Rivas, 2020)*. It usually occurs between 25 to 40 years, with a second peak around 40 to 60 years, and it is more prevalent in women (70%) *(Karakaya, 2017)*. Extrapulmonary manifestations are reported in only 12% of patients (mainly uveitis, parotitis, facial palsy, skin, liver or spleen involvement), and prognosis is positive, being self-remitting in the great majority of cases *(Mañá, 2017)*. Association of uveitis, parotitis and facial palsy, often associated with fever, is known as *Heerfordt-Waldenström's syndrome*, which is another acute presentation of sarcoidosis *(Bargagli, 2018)*.

Patients with subacute presentations of sarcoidosis often complain general symptoms, as fatigue (up to 50-70% of patients) *(Drent, 2012)*, concentration disturbances *(Elfferich, 2010)*, fever (usually of low grade, but sometimes up to 39-40°C), weight loss and night sweats *(Crouser, 2020)*.

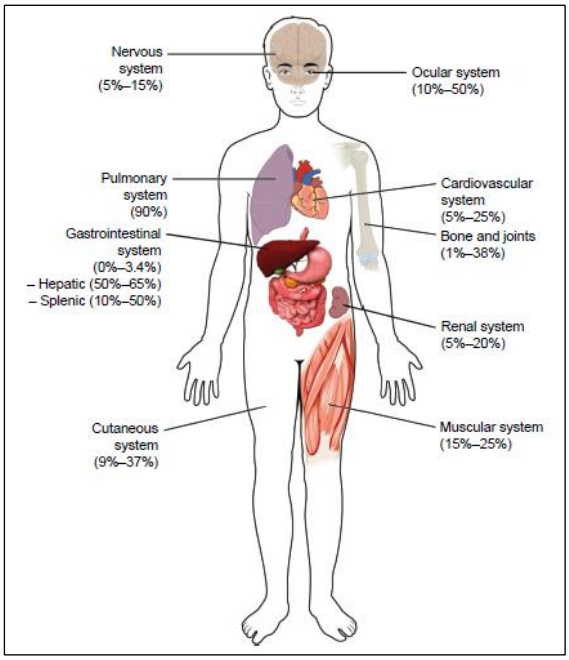



**Figure 2. Estimated frequencies of extrapulmonary organ involvement** *(Al-Kofahi, 2016)*

Respiratory symptoms are found at presentation in 30-53% of patients, mainly cough, dyspnea and chest pain *(Mañá, 2017; Judson, 2003)*. Thorax examination is often normal, except for scarce expiratory crackles. Bilateral perihilar lymphadenopathy and perilymphatic pulmonary micronodules predominant in upper lobes are the most typical imaging finding *(Jeny, 2020)*. Pulmonary fibrotic changes may be dominant in late stages, with architectural distortion, volume loss and bronchiectasis *(Sève, 2021)*. Pulmonary function tests (PFTs) usually show restriction of lung volumes, especially forced vital capacity (FVC), and decreased carbon-monoxide diffusing lung capacity (DLCO) *(Valeyre, 2014)*, although obstructive patterns can be seen in late stages, due to pulmonary fibrosis with bronchial distortion and stenosis or to diffuse bronchial granulomatosis or to bronchial compression by lymphadenopathy *(Abehsera, 2000; Lavergne, 1999; Naccache, 2008)*. Six-minute walk test (6MWT) distance is often reduced *(Baughman, 2007)*. Bronchoalveolar lavage (BAL) shows lymphocytic alveolitis in 80% of cases and T lymphocytes CD4/CD8 ratio over 3,5 in 50% of cases; neutrophil count may increase in advanced sarcoidosis due to pulmonary fibrosis, with unfavourable prognosis *(Costabel, 2010)*. A variable proportion of patients can also develop pulmonary arterial hypertension, due to granulomatous involvement of pulmonary vessels, parenchymal destruction or compressive mediastinal infiltration *(Boucly, 2017)*.

Symptomatic involvement of upper respiratory tract is reported in 6-15% of patients *(Rottoli, 2006; Mrówka-Kata, 2010)*. Sinonasal and laryngeal sarcoidosis are rare (1-4%) but usually severe *(Mrówka-Kata, 2010)*. Symptoms of sinonasal involvement include nasal obstruction, rhinorrhea, anosmia, crusting rhinitis, epistaxis and facial pain, and destructive forms are reported *(Aubart, 2006)*. Laryngeal sarcoidosis usually involves supraglottis and spares vocal cords, manifesting with hoarseness, inspiratory dyspnea and dysphagia *(Duchemann, 2014)*. Tracheal involvement is rare, while bronchial sarcoidosis can cause obstructive airway disease due to nodular granulomatous lesions in bronchial mucosa or to end-stage pulmonary fibrosis *(Morgenthau, 2011)*.

Lymph nodes are a peculiar localization of sarcoidosis, not only for mediastinal perihilar lymphadenopathy: more than 20% of patients present peripheral lymphadenopathy involving cervical, axillary, inguinal and epitrochlear glands. Affected lymph nodes are usually moderately swollen and painless *(Bargagli, 2018; Judson, 2008)*.

The skin is the second or third most commonly affected organ, involved in up to one-third of patients *(Caplan, 2020)*. Cutaneous sarcoidosis is often the first presentation of the disease and can remain isolated in more than 30% of cases *(Marcoval, 2011)*. Cutaneous findings can be "specific" or "non-specific", based on the presence or absence of classic sarcoidosis granulomas on histological examination. Specific lesions include multiple erythematous macules, papules, plaques or subcutaneous nodules; they are usually asymptomatic but aesthetically relevant if localized to the face, as in classical lupus pernio, consisting in indolent violaceus indurated plaques usually affecting nose, cheeks, ear lobes and fingers *(Descamps, 2016)*. Erythema nodosum is the most common acute cutaneous manifestation of sarcoidosis, consisting in subcutaneous nodules with erythematous halo, mainly localized on the extensor surfaces of limbs *(Bargagli, 2018)*; nodules do not contain granulomas, therefore represent a non-specific manifestation *(Sève, 2021)*. Other reported manifestations include psoriasiform, lichenoid, verrucous or angiolupiod lesions and nail, scars or tattoos involvement *(Yanardağ, 2003; Haimovic, 2012; Antonovich, 2005*).

Ocular involvement has been reported in 10-50% of patients with sarcoidosis, with higher prevalence in African Americans and women, and can present in the absence of any apparent systemic involvement *(Sève, 2021)*. All ocular structures may be involved, but uveitis is the most frequent presentation (up to 20-30% of patients with sarcoidosis) *(Bodaghi, 2012)*. Acute anterior uveitis, typically presenting with pain, hyperemia and photophobia, can be associated to Löfgren's or Heerfordt-Waldenström's syndromes and usually recovers with mild or no visual impairment *(Rochepeau, 2017)*. Chronic iridocyclitis, instead, often bilateral, can lead to severe visual impairment due to the development of glaucoma, cataract or cystoid macular edema *(Birnbaum, 2015; Rochepeau, 2017)*. Other ocular presentations include lacrimal-gland enlargement with sicca syndrome, conjunctivitis and optic neuritis (which will be addressed throughout next chapters) *(Sève, 2021)*.

Cardiac involvement is reported in 3 to 39% of patients with systemic sarcoidosis, but autoptic series report a prevalence of cardiac granulomas in up to 46,9% of cases *(Sève, 2021)*. Any part of the heart can be affected, with a predilection for left ventricular wall, interventricular septum and conducting system *(Nunes, 2010)*. Patients are mostly asymptomatic, but may present chest pain, palpitations, dyspnea, congestive heart failure due to cardiomyopathy, pericardial effusion or arrhythmias and the most common abnormality is atrioventricular block *(Kandolin, 2015)*. The most typical

feature seen in transthoracic echocardiography, highly specific for diagnosis, is interventricular thinning, but overall sensitivity is poor (around 25%); other echocardiographic findings are increased myocardial wall thickness, ventricular aneurysms, ventricular diastolic and systolic disfunction and isolated wall movements abnormalities *(Kurmann, 2018)*. Cardiac magnetic resonance imaging (MRI) has both sensitivity and specificity over 90%, identifying areas of myocardial damage through late gadolinium enhancement, usually with multifocal and patchy distribution *(Kouranos, 2017)*. $^{18}$F-fluorodeoxyglucose Positron emission tomography/Computed tomography ($^{18}$F-FDG PET/CT) is useful in monitoring disease activity and response to treatment *(Birnie, 2020)*, while endomyocardial biopsy has a low sensitivity (≅30%) due to the patchy nature of cardiac sarcoidosis, and is an invasive procedure *(Ardehali, 2005)*.

Granulomatous interstitial nephritis is the typical renal presentation of sarcoidosis, reported in up to 13% of patients in autoptic studies, but clinically evident in only 0,7-4,3% of cases *(Mahévas, 2009; Sève, 2021)*. Impairment of renal function with or without abnormal urinalysis results is the most common clinical presentation, and renal biopsy also shows only signs of interstitial nephritis without granuloma *(Mahévas, 2009)*. Other renal diseases can be associated to sarcoidosis, such as nephrocalcinosis and nephrolithiasis secondary to hypercalcemia and hypercalciuria *(Bergner, 2003)*, while glomerular diseases and isolated tubular disfunction have been more rarely reported *(Bergner, 2018)*.

Hepatic involvement is reported in up to 80% of cases in autoptic series *(Tadros, 2013)*, but the most common presentation of sarcoidosis is asymptomatic elevation in liver function tests (mainly alkaline phosphatase), seen in about one-third of patients *(Deutsch-Link, 2018)*. Clinical symptoms may include hepatomegaly, fatigue, abdominal pain, pruritus, jaundice, and weight loss *(Deutsch-Link, 2018)*. Liver nodules can be detected by computed tomography (CT) scan, ultrasonography, MRI or $^{18}$F-FDG PET/CT, but a definite diagnosis requires detection of non-caseating granulomas by hepatic biopsy *(Sève, 2021)*.

Splenic involvement is most often detected by imaging rather than by symptoms or laboratory abnormalities, and it is reported with similar incidence as hepatic involvement *(Sève, 2021)*. Marked splenomegaly is quite rare, and CT scan usually shows single or multiple hypodense nodules, sometimes with calcifications *(Folz, 1995)*.

Gastrointestinal tract is rarely involved, with a reported incidence between 0,1% and 1,6% *(Morimoto, 2008)*. Symptoms include abdominal pain, weight loss, nausea, vomiting, diarrhea, and digestive bleeding, and on endoscopy macroscopic lesion can be observed in the esophagus (9%), stomach (78%), duodenum (9%), colon (25%), and rectum (19%) *(Ghrenassia, 2016)*.

Bone sarcoidosis is reported in 0,5% to 30% of patients, depending on the sensitivity of imaging procedures, as studies with $^{18}$F-FDG PET/CT show a higher rate of bone involvement detection than does radiography *(Bechman, 2018; Mostard, 2012)*. Bone lesions are asymptomatic in half of the cases, and usually located bilaterally in hands and feet phalanges; skull, long bones, ribs, pelvis and vertebrae may also be affected. Lesions may be sclerotic, osteolytic, cystic or punched-out *(Sève, 2021)*.

Sarcoid arthropathy, aside from acute forms of Löfgren's syndrome, is relatively rare and usually presents as chronic symmetrical oligo- or polyarthritis involving medium and large joints (mostly ankles) *(Bechman, 2018; Visser, 2002)*.

Central and peripheral nervous system and skeletal muscle involvement will be addressed extensively throughout next chapters.

### 1.4 Diagnosis

The diagnosis of sarcoidosis in based on three major criteria: a compatible clinical presentation, demonstration of non-necrotizing granulomatous inflammation in one or more tissue samples and the exclusion of alternative causes of granulomatous disease *(Crouser, 2020)*.

In order to define uniform standards to assess the probability of organ involvement in sarcoidosis, consensus criteria were established in 1999 *(Judson, 1999)* and then updated in 2014 *(Judson, 2014)* by the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG). Clinical manifestations were defined as "*highly probable*" (likelihood of sarcoidosis of at least 90%, i.e. uveitis, bilateral hilar adenopathy), "*probable*" (likelihood of sarcoidosis of 50-90%, i.e. lachrymal gland swelling, upper lobe or diffuse infiltrates) and "*possible*" (likelihood of sarcoidosis of less than 50%, i.e. arthralgias, localized infiltrate on chest radiography) *(Judson, 2014)*. Moreover, in 2018, Bickett et al. proposed a Sarcoidosis Diagnostic Score, based on WASOG criteria, which might accurately differentiate sarcoidosis from other granulomatous diseases *(Bickett, 2018)*.

Recently, two clinical statements covering sarcoidosis diagnosis have been published, respectively from American Thoracic Society (ATS) *(Crouser, 2020)* and British Thoracic Society (BTS) *(Thillai, 2021)*. Both papers address the question of whether lymph node sampling is required or not in patients with high clinical suspicion. Historically, histological confirmation was considered mandatory, but both these recent consensus papers stated that in patients with highly specific presentations, such as Löfgren's and Heerfordt's syndromes or lupus pernio, lymph node sampling might be spared and replaced by close clinical follow-up *(Crouser, 2020; Thillai, 2021)*. In all the other scenarios, histologic confirmation should instead be pursued before any treatment is introduced, and tissue should be obtained from the most accessible site, such as skin lesions or peripheral lymph nodes, if abnormal findings are present *(Thillai, 2021)*. If no peripheral site is available, intrathoracic sampling is required, and endobronchial ultrasound (EBUS)-guided transbronchial needle aspiration (EBUS-TBNA) should be preferred as initial sampling procedure, being less invasive and better tolerated than mediastinoscopy and also allowing association of transbronchial parenchymal sampling in case of concomitant parenchymal disease *(Figure 3)*. If conventional bronchoscopic biopsies are non-diagnostic, mediastinoscopy should then be taken into account *(Crouser, 2020; Thillai, 2021)*.

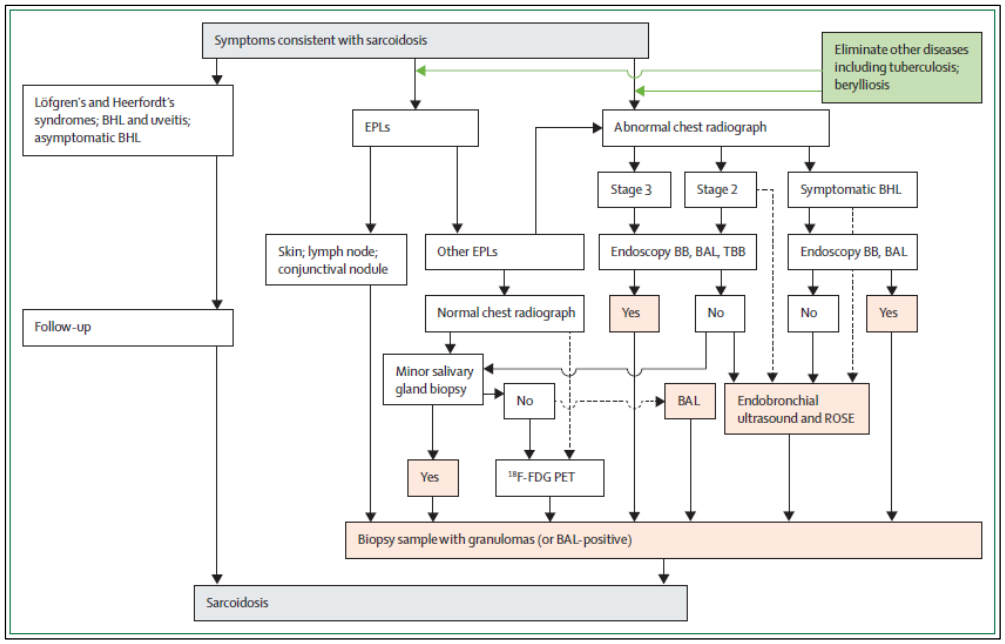



**Figure 3. Diagnosis of sarcoidosis** (*Valeyre, 2014*)
Solid line indicates usual practice, dotted line indicates alternative practice.
Abbreviations: BHL = bilateral hilar lymphadenopathy; EPL = extrapulmonary localizations; BB = bronchial biopsy; BAL: bronchoalveolar lavage; TBB = transbronchial biopsy; ROSE = rapid on-site cytological examination; $^{18}$F-FDG = $^{18}$F-fluorodeoxyglucose.

Respiratory examination is often normal and PFTs can also be unremarkable or show restrictive or obstructive alteration or isolated reduction in gas transfer; patients with fibrotic pulmonary disease show obviously more prominent and frequent abnormalities *(Thillai, 2021)*.

From a radiological point of view, sarcoidosis has been historically staged using *Scadding scale*, based on standard chest X-ray findings. It identifies five stages: stage 0 represents normal findings, stage I bilateral hilar lymphadenopathy, stage II bilateral hilar lymphadenopathy plus parenchymal infiltrates, stage III parenchymal infiltrates without hilar lymphadenopathy and stage IV pulmonary fibrosis *(Scadding, 1961)*.

Extensive use of chest high-resolution computed tomography (HRCT) has markedly increased diagnostic sensitivity, allowing the detection of subtle parenchymal involvement and thus providing a comprehensive overview of anatomical details and abnormalities of lung structures *(Silva, 2015)*. Sarcoidosis features detectable by HRCT include nodules (usually small, bilateral, with regular margins, scattered on bronchial walls and clefts or subpleurally), hilar-lobar and mediastinal adenopathy, pseudoplaques, roundish opacities resulting from converging nodules, symmetrical bilateral parenchymal thickenings with a "butterfly wing" pattern, and areas of air trapping *(Bargagli, 2018)*.

Abdominal organs and superficial lymph nodes can be visualized with ultrasonography or CT scan, while MRI is more suitable for the detection and follow-up of cardiac and central nervous system involvement *(Prasse, 2016)*. Moreover, recent whole-body MRI techniques can also be used to search for organ involvement *(Hostettler, 2014)*. In addition, $^{18}$F-FDG PET/CT may show suggestive sarcoid-like uptake pattern, with hypermetabolic mediastinal and hilar lymph nodes combined or not with lung parenchymal active disease, supporting sarcoidosis diagnosis, or sometimes reveal smoldering superficial localizations, such as cervical lymph nodes, more easily accessible to biopsy *(Keijsers, 2020)*.

BAL analysis can be very helpful for diagnosis, usually showing mildly elevated total cell count with a predominance of lymphocytes, a normal percentage of eosinophils and neutrophils, and lack of plasma cells and ''foamy'' alveolar macrophages *(Drent, 2007)*. These findings, indicating lymphocytic alveolitis, are often associated with increased CD4/CD8 ratio in patients with active disease and a ratio of above 3,5 has a high specificity for sarcoidosis *(Welker, 2004)*. On the other hand, peripheral analysis of

lymphocyte subpopulations can demonstrate a decrease in CD4+/CD8+ ratio due to the recruitment of CD4+ cells in affected organs *(Bargagli, 2018)*.

Routine laboratory analyses are recommended for both displaying general alterations, as hypercalcemia and hypercalciuria or hematological abnormalities, and screening for extrapulmonary sarcoidosis in asymptomatic patients, including renal or hepatic involvement *(Crouser, 2020; Thillai, 2021)*.

An important and debated role in sarcoidosis diagnosis is played by serum biomarkers. The first and most known is angiotensin-converting enzyme (ACE), an acid glycoprotein converting angiotensin I into angiotensin II and playing a key role in regulating blood pressure and electrolyte balance *(Coates, 2003)*. ACE is mainly produced by epithelioid cells in sarcoid granulomas, is an important modulator of granuloma formation and correlates with the granuloma burden of the disease *(Sheffield, 1997; Costabel, 1997)*. Elevated serum ACE levels in sarcoidosis were firstly described in 1975 *(Lieberman, 1975)* and since then it is the most frequently used laboratory test in sarcoidosis. Studies published over the last 20 years showed increased serum ACE level in 40% to 86% of sarcoidosis patients, with sensitivity ranging from 22 to 86% and specificity ranging from 54% to 99% *(Ramos-Casals, 2019)*. This wide variability is probably due to differences in enzymatic assays, cut-off values and timing of measurement after diagnosis, and to the effect of ongoing therapies in different patients, including not only anti-sarcoidosis treatments, but also possible ACE inhibitor drugs that significantly lower serum ACE levels *(Chopra, 2016; Krasowski, 2015)*. Furthermore, ACE concentrations are genetically influenced, since an insertion (I) or deletion (D) polymorphism in the ACE gene can determine significant variations in serum levels, with homozygous carriers of the deletion (DD) or insertion (II) expressing the highest and lowest ACE levels respectively, whereas heterozygous (ID) individuals express intermediate ACE levels *(Floe, 2014)*. Unfortunately, the use of a genotype-specific reference range slightly increases the diagnostic sensitivity of the standard serum ACE measurement but lowers the specificity of the test *(Kruit, 2007; Stokes, 1999)*. Elevated serum ACE levels have also been reported in several other conditions, including infectious granulomatous diseases (tuberculosis, leprosy), non-infectious granulomatous diseases (silicosis, berylliosis, Gaucher's disease) and non-granulomatous diseases (hyperthyroidism, psoriasis, Hodgkin's lymphoma) *(Chopra, 2016)*. Given all these confounding factors, it is problematic to accurately calculate sensitivity or specificity of serum ACE for the diagnosis of sarcoidosis and overall, the

specificity of this measurement appears inadequate to base the diagnosis of sarcoidosis exclusively on this biomarker. In order to have a conservative approach, literature recommends to consider as strongly suggestive of sarcoidosis only serum ACE levels greater than 2 times over upper normal limits, whereas all other results should be integrated with other clinical and diagnostic elements *(Chopra, 2016)*.

Lysozyme (LSZ) is a bacteriolytic enzyme that hydrolyses glycosidic bonds in bacterial cell walls, and in sarcoidosis is produced by macrophages and epithelioid cells forming granulomas, with an increase of its serum levels *(Majcherczyk, 1999; Silverstein, 1977)*. However, elevated levels of serum LSZ are reported also in pulmonary tuberculosis, silicosis, asbestosis and berylliosis, significantly lowering its specificity as a diagnostic biomarker *(Ramos-Casals, 2019)*. However, LSZ levels have been shown to correlate with chest radiographic findings, which makes LSZ more suitable as a prognostic biomarker of disease activity *(Turton, 1979; Tomita, 1999)*.

Soluble Interleukin-2 Receptor (sIL-2R) is the circulating form of membrane IL-2R, which is usually upregulated in activated Th1 cells; increased sIL-2R levels are therefore considered a marker of Th1 cells activation in the formation and perpetuation of granuloma *(Rubin, 1990; Sakthivel, 2017)*. Elevated sIL-2R levels in serum of sarcoidosis patients have been described since 1983 *(Hunninghake, 1983)*, and further studies reported a wide range of frequencies of such increase, ranging from 30% to 100% of patients *(Ramos-Casals, 2019)*. In studies differentiating between healthy controls and unselected cased of sarcoidosis, sensitivity and specificity ranged from 63% to 82% and from 57% to 100%, respectively *(Ramos-Casals, 2019)*. Unfortunately, sIL-2R levels can be elevated in several infectious (HIV, tuberculosis, leprosy), lymphoproliferative (lymphoma and leukemia) and inflammatory (idiopathic pulmonary fibrosis, scleroderma) conditions *(Chopra, 2016)*. Therefore, sIL-R levels can be a useful diagnostic tool only when combined with other clinical and imaging biomarkers. On the other hand, sIL-R levels showed to correlate with various measures of disease activity, including radiographic stage of disease *(Keicho, 1990)* and serum ACE levels *(Bargagli, 2008)*, and higher levels of sIL-2R have been found in extraparenchymal sarcoidosis compared to isolated pulmonary sarcoidosis *(Grutters, 2003)*. Based on these data, serum sIL-2R levels may be useful as a prognostic biomarker in selected sarcoidosis patients, as they seem also to predict progression or relapse after treatment discontinuation *(Vorselaars, 2014)*.

Chitotriosidase (CTO) is an enzyme of the chitinase family which degrades chitin, a

polymer secreted by fungi and parasites; it is secreted by neutrophils and macrophages under stimulation of IFNγ and TNFα *(van Ejik, 2005)*. CTO is indeed a specific marker of macrophagic activation, and the main biochemical marker of Gaucher's disease, although raised levels have been reported also in atherosclerosis, malaria, multiple sclerosis and tuberculosis *(Vellodi, 2005; Michelakakis, 2004)*. However, significantly raised CTO levels have been reported in patients with sarcoidosis in comparison with patients with tuberculosis, asbestosis, idiopathic pulmonary fibrosis or systemic sclerosis, with sensitivity and specificity of 88,79% and 92,86%, respectively, for the lowest cut-off (48,8 nmol/ml/h, used by Bargagli et al.) and of 82,5% and 70%, respectively, for a higher cut-off (100 nmolml/h, used by Popević et al.) *(Bargagli, 2013; Popević, 2016)*. Therefore, CTO seems to have higher sensitivity and specificity in differentiating sarcoidosis from other diseases with respect to other known biomarkers *(Bargagli, 2007; Bargagli, 2013)*. Moreover, CTO showed also good sensitivity in sarcoidosis patients under systemic treatment and seemed to correlate with extrapulmonary involvement *(Bergantini, 2019)*.

Krebs von den Lungen-6 (KL-6) is a mucin-like high-molecular weight glycoprotein derived from type II pneumocytes and respiratory bronchiolar epithelial cells *(Kohno, 1989)*. Elevated KL-6 concentrations in serum and BAL reflect alveolar epithelial cell damage and progressive interstitial thickening, thus are typical of interstitial lung diseases (ILD) with fibrotic evolution *(Ohnishi, 2002; D'Alessandro, 2020)*; in serum, a cut-off value of 465 U/ml has been proposed to distinguish ILD patients from healthy subjects and patients with other nonfibrotic lung diseases *(Ohnishi, 2002)*. Several studies showed higher serum KL-6 levels in patients with sarcoidosis than in healthy controls *(Janssen, 2003; Kitaichi, 2003)*. Despite not being a specific biomarker, specific phenotypes, clinical presentations and localizations of sarcoidosis have been associated with different KL-6 levels, showing for instance higher levels in the fibrotic stage *(Bergantini, 2019)*. Moreover, a close correlation with radiological and laboratory markers of pulmonary involvement has been extensively described *(Kobayashi, 1996; Honda, 2011; Miyoshi, 2010)*.

Increased levels of the aforementioned biomarkers can also be found in BAL *(Meyer, 2012)*.

Differential diagnosis to consider while assessing patients with suspect sarcoidosis is mainly with granulomatous disorders of infectious and non-infectious cause. The first group includes primarily tuberculosis and atypical mycobacterial infections, but also

leprosy, brucellosis, leishmaniasis and fungal infections as histoplasmosis or aspergillosis. The second group includes pneumoconiosis as chronic berylliosis, hypersensitivity pneumonitis, malignancies as lymphomas and lymphomatoid granulomatosis or sarcoid-like reactions to tumors, granulomatous reactions to immune-modulating drugs, and other autoimmune conditions as ANCA-associated vasculitides or IgG4-related disease (IgG4-RD) *(Crouser, 2020)*.

### 1.5 Treatment

As sarcoidosis may be a self-limiting disease, without any negative impact on the quality of life or prognosis, the decision whether to start or not a pharmacological treatment should be based on a careful evaluation of pros and cons performed by the physician, and the decision should be carefully discussed and shared with the patient. Key criteria to consider are the potential danger of a fatal outcome or permanent disability or an unacceptable loss of the quality of life *(Thillai, 2021)*. Commonly agreed indications for systemic treatment in sarcoidosis are the presence of symptoms impairing quality of life, persistent lung infiltrates or decline in lung function at follow-up, presence of hypercalcemia or hypercalciuria resistant to vitamin D and calcium restrictions, severe skin involvement, ocular involvement not responding to topical therapy, cardiac or neurological involvement and any other end-organ failure *(Melani, 2021)*.

Topical treatment with corticosteroids may be effective in managing many cases of skin, joint and eye sarcoidosis, while no evidence exists on the efficacy of inhaled corticosteroids in pulmonary sarcoidosis *(Milman, 1994; du Bois, 1999)*.

Drugs used in sarcoidosis management are listed in *Figure 4*.

Corticosteroids (CS) are also the first choice when systemic treatment is required. Despite only few randomized studies have shown their benefits *(James, 1967; Gibson, 1996)*, various case series have documented their effectiveness since 1951 *(Sones, 1951)*. Treatment initiation should be considered when there is significant reduction in pulmonary function tests, but also in patients with progressive breathlessness due to pulmonary disease *(Thillai, 2021)*. Most experts recommend oral prednisone 20-40 mg or equivalent daily in a single administration for naïve patients, while aggressive therapy with intravenous methylprednisolone 1 mg/kg/day or 500-1000 mg/day for three days may sometimes be required in order to obtain rapid remission in severe or

life-threatening presentations, often due to extrapulmonary disease *(Rahaghi, 2020)*. Induction dose is usually maintained for 6-8 weeks, followed by slow tapering (i.e. 5 mg every 2 weeks) to a low maintenance dose, usually 5-10 mg per day. In the longer term, such dosage can be acceptable, but possible side effects may require individualization of the dose and however, attempts at withdrawing CS should be made every 6-12 months, if feasible. Continued treatment is instead required if withdrawal or dose reduction is associated with a relapse, which can require a brief return to a higher dose in case of major relapse *(Thillai, 2021)*. Due to relevant side effects of chronic treatment (including weight gain and fluid retention, osteoporosis, hyperglycemia and diabetes, hypertension, gastritis, myopathy, opportunistic infections, psychosis and mood swings, insomnia, cataract and glaucoma), patients who do not respond to CS, who cannot be controlled with less than 10 mg/day of prednisone or those who develop intolerance, are candidates for treatment with alternative drugs *(Khan, 2017; Schutt, 2010)*.

Second-line treatment involves antimetabolite immunosuppressants, which come all with significant risks of toxicity, including myelosuppression, hepatotoxicity, opportunistic infections and teratogenicity. For this reason, all these agents require assessment of full blood profile, renal and liver function, and serology for HIV, hepatitis B and C and interferon-gamma release assay for tuberculosis prior to treatment, followed by serial blood test monitoring during administration *(Ledingham, 2017; Thillai, 2021)*. Moreover, tapering CS dosage is not recommended for at least 2 to 3 months after the addition of an immunosuppressant, as the latter takes several weeks to reach its maximum therapeutics effect *(Melani, 2021)*.

Methotrexate (MTX) is the most widely used second-line agent. It is administered orally, subcutaneously or intramuscularly on a weekly basis, with an initial dose of 5 to 10 mg per week and incrementing every two weeks to a target dose of 15 to 20 mg per week as tolerated; supplementation with folic acid 5 mg per week is recommended *(Cremers, 2013)*. It is contraindicated in patients with advanced chronic kidney disease, liver disease, active infections and during pregnancy and breastfeeding; adverse effects include gastrointestinal, hepatic and hematologic toxicities and a rare but serious hypersensitivity pneumonitis *(Cremers, 2013)*.

Azathioprine (AZA) is the first alternative to MTX, showing similar efficacy, but with a higher rate of infections *(Vorselaars, 2013)*. Usual starting dose is 50 mg per day orally, increased by 25 mg every 2 weeks to a maximum of 200 mg per day. Most common

side effects are gastrointestinal, and thiopurine S-methyltransferase (TPMT) serum levels should be assessed before initiation, because carriers of TPMT mutations, which leads to a poor metabolization of AZA, have increased risk of life-threatening bone-marrow toxicity *(Schaeffeler, 2019)*. AZA can be administered during pregnancy, but not during breastfeeding *(Götestam Skorpen, 2016)*.

Leflunomide (LEF), administered orally 10 to 30 mg per day, has similar effect to MTX, but different toxicity profile, showing less gastrointestinal side effects. However, silent liver fibrosis and peripheral neuropathy have been reported and suggest caution *(Sahoo, 2011)*.

Mycophenolate mofetil (MMF) seems well tolerated, but data regarding its use in sarcoidosis are limited, and it should not be considered before MTX or AZA *(Hamzeh, 2014; Thillai, 2021)*. The usual oral starting dose is 500 mg twice a day, increased by 250 mg every few weeks to a maximum of 1500 mg twice a day *(Melani, 2021)*. The most frequent side effects are gastrointestinal, and it has potential teratogenicity in women of childbearing age *(Coscia, 2015)*.

Cyclophosphamide (CYC), administered intravenously 500-1000 mg per week or every other week, can be considered as a short-term rescue option in very severe forms of sarcoidosis, not controlled by MTX or AZA *(Pande, 2020)*. Due to its toxicity profile, which includes bone marrow, cutaneous and gastrointestinal side effects and also hemorrhagic cystitis, is rarely used as steroid-sparing agent *(Thillai, 2021)*.

Hydroxychloroquine (HCQ) is an antimalarial drug used to treat joint and skin sarcoidosis and in the management of sarcoidosis-related hypercalcemia and fatigue *(Rahaghi, 2020)*. The usual dose is 200 mg once or twice per day, orally. It is not associated with increased risk of infections, but the main toxicities are gastrointestinal and ocular, with the risk of irreversible retinopathy which increases with cumulative dose and is a contraindication to prolonged treatment *(Schrezenmeier, 2020)*. An ophthalmic examination is recommended at the time of treatment initiation, and patients should also perform a baseline EKG to exclude long QT interval *(Thillai, 2021)*.

Biological agents are considered third-line therapeutics agents, to be initiated only after a failure of second-line treatment in the context of a specialist tertiary center. The most relevant target is TNFα, a pro-inflammatory cytokine secreted by macrophages with a key role in the maintenance of granuloma formation *(Korsten, 2016)*.

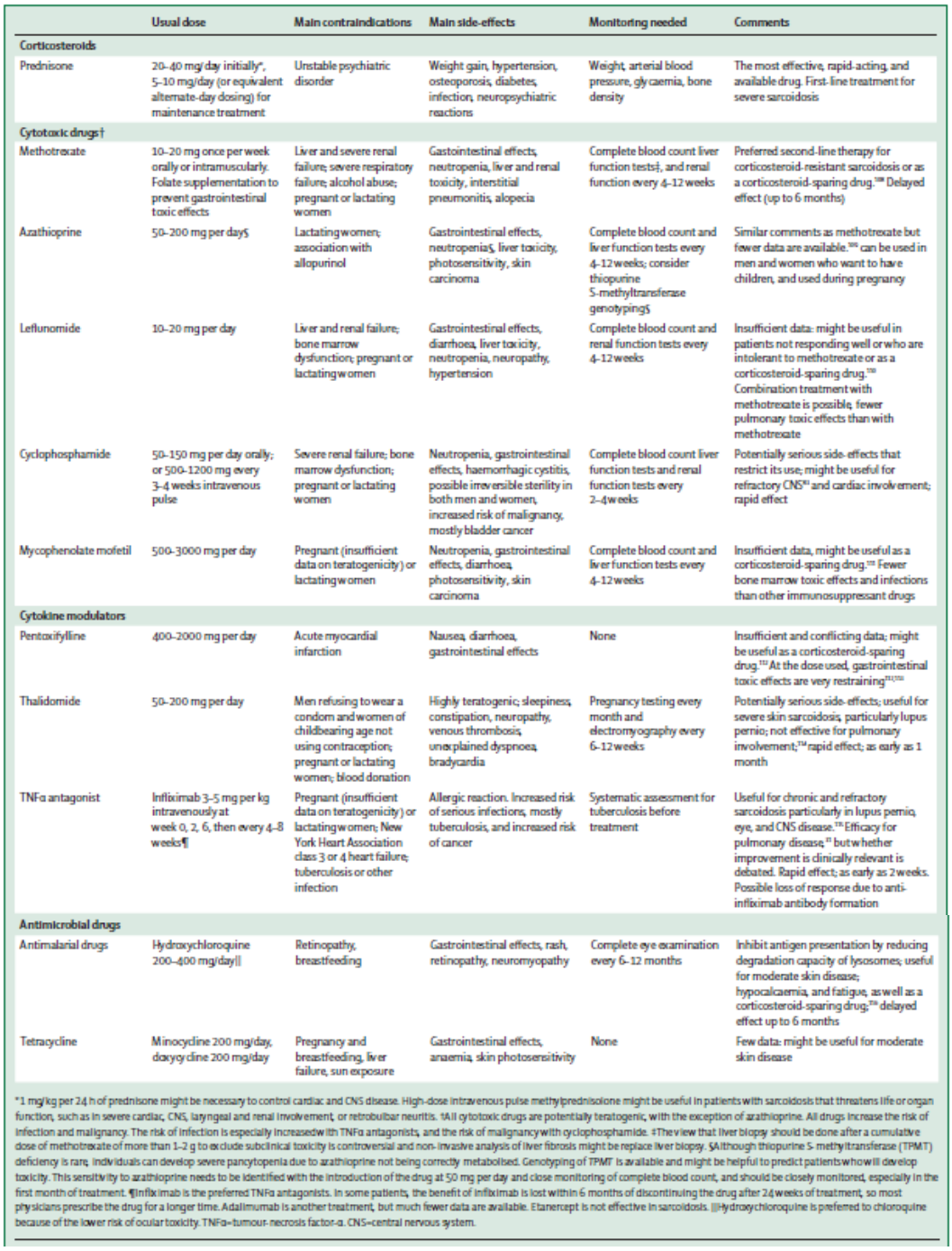
Infliximab (IFX), a chimeric monoclonal antibody directed against TNFα, is the most used biological agent in sarcoidosis and has shown to be effective in severe and

refractory pulmonary and extrapulmonary sarcoidosis *(Baughman, 2006; Jamilloux, 2017; Gelfand, 2017)*. The usual induction regimen is 5 mg/kg intravenously at weeks 0, 2 and 6, then the frequency of maintenance is every 4 to 8 weeks, based on patient response. Optimal duration of treatment is unclear, but the high frequency of relapses after suspension, mainly during first year of treatment *(Panselinas, 2009; Vorselaars, 2014)*, suggests prolonged regimens with reduction of dose and increase of administration interval *(Drent, 2014)*. Side effects include allergic reactions against the antibody itself, the possibility of developing autoantibodies that reduce drug efficacy and, mostly, increased susceptibility to infections, mainly mycobacterial and fungal; therefore, patients must be screened for tuberculosis prior to starting treatment with IFX *(Nordgaard-Lassen, 2012)*.

Adalimumab (ADA), a fully human anti-TNFα monoclonal antibody, showed lower risk of allergic reaction with respect to IFX and is administered subcutaneously at a dose of 40 mg every 2 weeks, making self-administration possible *(Crommelin, 2016)*.

Interestingly, etanercept, a TNFα receptor antagonist, did not show the same efficacy as the aforementioned monoclonal antibodies *(Ehlers, 2005)* and paradoxically, the development of non-caseating granulomas consistent with sarcoidosis has been reported during anti-TNFα therapy for other diseases *(Skoie, 2012; Vigne, 2013)*.

Several case reports and series suggested the efficacy of rituximab (RTX), a monoclonal antibody directed against CD-20+ cells, in treating refractory extrapulmonary sarcoidosis *(Sweiss, 2014; Cinetto, 2015)*. It is administered intravenously, and usually proposed therapeutic schemes are 1000 mg repeated twice with 2-week interval or 375 $mg/m^2$ weekly for 4 consecutive weeks *(Cinetto, 2016)*. Infusion-related reactions are common and premedication with paracetamol and corticosteroids is recommended; moreover, side effects include increased risk of bacterial, fungal or viral infections, hypogammaglobulinemia and neutropenia, requiring careful serologic assessment prior to initiation of treatment *(Lower, 2020)*.In addition to immunomodulating treatment, sarcoidosis-associated fatigue as well as complications including pulmonary fibrosis and pulmonary hypertension should be considered and addressed by physicians *(Thillai, 2021; Melani 2021)*.



| | Usual dose | Main contraindications | Main side-effects | Monitoring needed | Comments |
|---|---|---|---|---|---|
| **Corticosteroids** | | | | | |
| Prednisone | 20–40 mg/day initially*, 5–10 mg/day (or equivalent alternate-day dosing) for maintenance treatment | Unstable psychiatric disorder | Weight gain, hypertension, osteoporosis, diabetes, infection, neuropsychiatric reactions | Weight, arterial blood pressure, glycaemia, bone density | The most effective, rapid-acting, and available drug. First-line treatment for severe sarcoidosis |
| **Cytotoxic drugs†** | | | | | |
| Methotrexate | 10–20 mg once per week orally or intramuscularly. Folate supplementation to prevent gastrointestinal toxic effects | Liver and severe renal failure; severe respiratory failure; alcohol abuse; pregnant or lactating women | Gastrointestinal effects, neutropenia, liver and renal toxicity, interstitial pneumonitis, alopecia | Complete blood count liver function tests‡, and renal function every 4–12 weeks | Preferred second-line therapy for corticosteroid-resistant sarcoidosis or as a corticosteroid-sparing drug.[108] Delayed effect (up to 6 months) |
| Azathioprine | 50–200 mg per day§ | Lactating women; association with allopurinol | Gastrointestinal effects, neutropenia§, liver toxicity, photosensitivity, skin carcinoma | Complete blood count and liver function tests every 4–12 weeks; consider thiopurine S-methyltransferase genotyping§ | Similar comments as methotrexate but fewer data are available.[109] can be used in men and women who want to have children, and used during pregnancy |
| Leflunomide | 10–20 mg per day | Liver and renal failure; bone marrow dysfunction; pregnant or lactating women | Gastrointestinal effects, diarrhoea, liver toxicity, neutropenia, neuropathy, hypertension | Complete blood count and renal function tests every 4–12 weeks | Insufficient data: might be useful in patients not responding well or who are intolerant to methotrexate or as a corticosteroid-sparing drug.[110] Combination treatment with methotrexate is possible; fewer pulmonary toxic effects than with methotrexate |
| Cyclophosphamide | 50–150 mg per day orally; or 500–1200 mg every 3–4 weeks intravenous pulse | Severe renal failure; bone marrow dysfunction; pregnant or lactating women | Neutropenia, gastrointestinal effects, haemorrhagic cystitis, possible irreversible sterility in both men and women, increased risk of malignancy, mostly bladder cancer | Complete blood count liver function tests and renal function tests every 2–4 weeks | Potentially serious side-effects that restrict its use; might be useful for refractory CNS[111] and cardiac involvement; rapid effect |
| Mycophenolate mofetil | 500–3000 mg per day | Pregnant (insufficient data on teratogenicity) or lactating women | Neutropenia, gastrointestinal effects, diarrhoea, photosensitivity, skin carcinoma | Complete blood count and liver function tests every 4–12 weeks | Insufficient data, might be useful as a corticosteroid-sparing drug.[112] Fewer bone marrow toxic effects and infections than other immunosuppressant drugs |
| **Cytokine modulators** | | | | | |
| Pentoxifylline | 400–2000 mg per day | Acute myocardial infarction | Nausea, diarrhoea, gastrointestinal effects | None | Insufficient and conflicting data; might be useful as a corticosteroid-sparing drug.[113] At the dose used, gastrointestinal toxic effects are very restraining[113][114] |
| Thalidomide | 50–200 mg per day | Men refusing to wear a condom and women of childbearing age not using contraception; pregnant or lactating women; blood donation | Highly teratogenic; sleepiness, constipation, neuropathy, venous thrombosis, unexplained dyspnoea, bradycardia | Pregnancy testing every month and electromyography every 6–12 weeks | Potentially serious side-effects; useful for severe skin sarcoidosis, particularly lupus pernio; not effective for pulmonary involvement;[114] rapid effect; as early as 1 month |
| TNFα antagonist | Infliximab 3–5 mg per kg intravenously at week 0, 2, 6, then every 4–8 weeks¶ | Pregnant (insufficient data on teratogenicity) or lactating women; New York Heart Association class 3 or 4 heart failure; tuberculosis or other infection | Allergic reaction. Increased risk of serious infections, mostly tuberculosis, and increased risk of cancer | Systematic assessment for tuberculosis before treatment | Useful for chronic and refractory sarcoidosis particularly in lupus pernio, eye, and CNS disease.[115] Efficacy for pulmonary disease,[11] but whether improvement is clinically relevant is debated. Rapid effect; as early as 2 weeks. Possible loss of response due to anti-infliximab antibody formation |
| **Antimicrobial drugs** | | | | | |
| Antimalarial drugs | Hydroxychloroquine 200–400 mg/day‖ | Retinopathy, breastfeeding | Gastrointestinal effects, rash, retinopathy, neuromyopathy | Complete eye examination every 6–12 months | Inhibit antigen presentation by reducing degradation capacity of lysosomes; useful for moderate skin disease; hypocalcaemia, and fatigue, as well as a corticosteroid-sparing drug;[116] delayed effect up to 6 months |
| Tetracycline | Minocycline 200 mg/day, doxycycline 200 mg/day | Pregnancy and breastfeeding, liver failure, sun exposure | Gastrointestinal effects, anaemia, skin photosensitivity | None | Few data: might be useful for moderate skin disease |

*1 mg/kg per 24 h of prednisone might be necessary to control cardiac and CNS disease. High-dose intravenous pulse methylprednisolone might be useful in patients with sarcoidosis that threatens life or organ function, such as in severe cardiac, CNS, laryngeal and renal involvement, or retrobulbar neuritis. †All cytotoxic drugs are potentially teratogenic, with the exception of azathioprine. All drugs increase the risk of infection and malignancy. The risk of infection is especially increased with TNFα antagonists, and the risk of malignancy with cyclophosphamide. ‡The view that liver biopsy should be done after a cumulative dose of methotrexate of more than 1–2 g to exclude subclinical toxicity is controversial and non-invasive analysis of liver fibrosis might be replace liver biopsy. §Although thiopurine S-methyltransferase (TPMT) deficiency is rare, individuals can develop severe pancytopenia due to azathioprine not being correctly metabolised. Genotyping of TPMT is available and might be helpful to predict patients who will develop toxicity. This sensitivity to azathioprine needs to be identified with the introduction of the drug at 50 mg per day and close monitoring of complete blood count, and should be closely monitored, especially in the first month of treatment. ¶Infliximab is the preferred TNFα antagonists. In some patients, the benefit of infliximab is lost within 6 months of discontinuing the drug after 24 weeks of treatment, so most physicians prescribe the drug for a longer time. Adalimumab is another treatment, but much fewer data are available. Etanercept is not effective in sarcoidosis. ‖Hydroxychloroquine is preferred to chloroquine because of the lower risk of ocular toxicity. TNFα=tumour-necrosis factor-α. CNS=central nervous system.

**Figure 4. Drugs used in sarcoidosis** (*Valeyre, 2014*)

# CHAPTER 2: NEUROSARCOIDOSIS

## 2.1 History, epidemiology and risk factors

First reports on neurological involvement were published as soon as sarcoidosis was recognized as a multisystem disease, starting with the description made by Heerfordt in 1909 of three patients with "*subchronic uveo-parotid fever*", later known as the aforementioned *Heerfordt-Waldenström's syndrome (Heerfordt, 1909)*. In 1948 a review article found 118 published case reports of nervous system involvement in sarcoidosis, with facial and optic neuropathies as the most common presentations *(Colover, 1948)*. Since then, many case reports and series expanded the spectrum of possible manifestations of neurosarcoidosis, which can virtually involve any part of central and peripheral nervous system.

Symptomatic nervous system involvement was historically reported to occur in 3-16% of patients with sarcoidosis *(Baughman, 2001; Morimoto, 2008; Ungprasert, 2016a)*, although this number could reflect a sampling bias from pulmonary sarcoidosis-focused cohorts. Few autoptic studies found signs of granulomatous inflammation in nervous system in 25-27% of sarcoidosis patients *(Iannuzzi, 2007; Manz, 1983)* and another study showed that only half of the patients with autoptic diagnosis of neurosarcoidosis were already diagnosed *ante-mortem (Iwai, 1993)*. These numbers suggest that a significant proportion of neurosarcoidosis patients might be asymptomatic, underdiagnosed or misdiagnosed. Moreover, a recent cohort study identified neurosarcoidosis in 234 out of 620 (34,9%) patients with systemic sarcoidosis *(Joubert, 2017)*.

Isolated neurosarcoidosis (occurring with no other organ involvement) is rare, representing less than 10% of neurosarcoidosis patients and only 1% of all sarcoidosis patients *(Delaney, 1977a; Smith, 2004)*. On the other hand, about 90% of patients show at onset or will develop over time the involvement of other organs, mainly lungs (67%), eye (25%), skin or joints (21% both) *(Pawate, 2009; Fritz, 2016)*. One retrospective study showed that over 80% of patients with initial diagnosis of isolated neurosarcoidosis successively developed extra-neurologic involvement during an average follow-up of 9,6 years *(Joseph, 2009)*.

However, neurologic manifestations are the presenting syndrome in more than 50% of

patients with neurosarcoidosis *(Delaney, 1977a; Fritz, 2016)*, whereas about 75% of the remaining patients develop neurologic involvement in the context of systemic sarcoidosis during the first two years from onset *(Krumholz, 2014)*.

However, it is important to underline that the presence of neurological symptoms in sarcoidosis patients does not necessarily demonstrate a neurosarcoidosis. Indeed, in a retrospective study on 649 sarcoidosis patients, the authors found that only 45% of those presenting neurological symptoms (i.e., 33 out of 74) actually had neurosacoidosis as a cause of such symptoms *(Stern, 1985)*.

Similarly to sarcoidosis, also neurosarcoidosis has increased incidence in African Americans *(Judson, 2012)* and affects women more than men (55% vs 45%), with a mean age at onset (43 years) slightly higher with respect to systemic disease *(Baughman 2001; Fritz, 2016)*. Pediatric cases are rare, with a mean age at onset of approximately 12 years *(Rao, 2016)*. A recent study comparing neurosarcoidosis patients with and without African American ethnicity, showed no differences in presentation, management and outcomes *(Affan, 2020)*.

In the literature, there are no conclusive data regarding predisposing or triggering factors, nor regarding specific demographic features particularly associated with neurosarcoidosis. Although haplotypes that increase the risk of specific sarcoidosis phenotypes, such as *Löfgren syndrome (Moller, 2017)*, were reported, there seems also to be little or no concordance in phenotypic patterns and outcomes across affected siblings with sarcoidosis *(Judson, 2006)*.

## 2.2 Pathophysiology

Similarly to systemic sarcoidosis, neurosarcoidosis is characterized pathologically by the development of non-necrotizing granulomas, formed by macrophages, derived epithelioid cells, $T_H1$ and $T_H17$ lymphocytes. In central nervous system (CNS) granulomas are generally located in proximity of blood vessels and are associated with non-granulomatous mononuclear and lymphocytic perivascular infiltration *(Bagnato, 2015)*. Similarly, in peripheral nervous system (PNS) granulomas usually occur around blood vessels and may be associated with lymphocytic necrotizing vasculitis affecting epineurium and perineurium, with variable degrees of nerve fascicle damage, mediated not only by an inflammatory process but also by an ischemic mechanism *(Said, 2013)*.

Tissue damage in neurosarcoidosis might indeed result from different mechanisms,

among which the formation of intra-axial granulomatous masses resulting in mechanical compression is only the first and most predictable one. A second mechanism of damage is due to leptomeningeal involvement, possibly leading to hydrocephalus *(Bagnato, 2015)*. Starting from leptomeningeal localizations, granulomatous inflammation can further invade brain and spinal cord parenchyma via Virchow-Robin perivascular spaces, which are connected with the cervical lymphatics and probably act as an elective interface between immune and nervous system *(Mirfakhraee, 1986)*. Their greater size at the base of the brain, alongside cerebrospinal fluid (CSF) flow dynamics may explain the preferential involvement of the basal meninges, cranial nerves, and midline neuroendocrine structures seen in neurosarcoidosis *(Tavee, 2015)*.

Neurosarcoidosis is also known to create a procoagulant state, favoring both ischemic and hemorrhagic CNS lesions. Ischemia can result from either small or large vessel vasculitis, sometimes with granulomas invading blood vessel walls and causing stenosis or occlusions *(Bagnato, 2015)*. Intracranial hemorrhage due to neurosarcoidosis have been occasionally reported with variable localizations, being mainly supratentorial (62%), but also infratentorial (31%) and subarachnoid (7%). In these patients, inflammation of the walls of small veins might disrupt their integrity and lead to blood leakage at low pressure *(O'Dwyer, 2013)*.

Regarding PNS, non-necrotizing granuloma formation and/or vasculitis account only for a proportion of patients with sarcoidosis-associated neuropathy, as shown by pathologic cohorts *(Vital, 2008)*. Moreover, in patients with small fiber neuropathy there is no evidence of granulomatous inflammation within the nerves, and damage is probably mediated by the high concentrations of circulating cytokines, mainly TNFα *(Heij, 2012b)*.

Granulomatous involvement of skeletal muscle is also commonly reported, although patients found to have myopathic involvement of neurosarcoidosis are often asymptomatic. Granulomas are usually located in the perimysium and sometimes in the endomysium *(Stjernberg, 1981)*.

### 2.3 Clinical manifestations

As already stated, neurosarcoidosis can virtually involve every part of CNS and PNS with a variety of pathophysiological mechanisms.

A large metanalysis reviewed 1088 neurosarcoidosis patients described in 29 studies,

between 1965 and 2015 *(Fritz, 2016)*. This research confirmed the huge clinical heterogeneity of the disease, showing as the three most common presentations were cranial neuropathy (55%), headache (32%) and sensory abnormalities (29%). Spinal cord involvement was present in 18% of patients, peripheral neuropathy in 17%, aseptic meningitis in 16%, myopathy in 15% and neuro-endocrine dysfunction in 9%.

Moreover, the majority of patients seems to present at onset with more than one neurological symptom and up to half of them develops additional neurologic symptoms during the course of the disease *(Leonhard, 2016)*.

### *2.3.1 Cranial nerves*

Cranial neuropathies are the most common presentations of neurosarcoidosis *(Fritz, 2016)*. Involvement of all cranial nerves is reported, but the most commonly affected are VII, II, VIII and V cranial nerves, sometimes with concomitant involvement of more than one nerve *(Carlson, 2015; Fritz, 2016)*. Isolated cranial neuropathies are usually acute, while cranial multineuropathies (concurrent or serial) present more often a chronic course *(Gullapalli, 2004; Bradshaw, 2 021)*.

An older cohort suggested that facial nerve palsy accounted for two thirds of neurosarcoidosis cases *(Stern, 1985)*, but more recent studies report rates between 11% and 25% of neurosarcoidosis patients. About one-third of facial nerve palsies are bilateral and could be either concurrent or sequential *(Ungprasert, 2017b)*. Facial nerve palsies were formerly thought to be a consequence of sarcoidosis-associated inflammation of the parotid gland as classically described by Heerfordt, whereas more recent studies failed to demonstrate a relationship between these two conditions. Indeed, one study reported that only 20% of patients with facial nerve palsy had an associated parotitis (*Stern, 1985*), while another study reported no facial nerve palsy in 7 patients with parotitis due to sarcoidosis (*Ungprasert, 2016b*). Nowadays, epineural inflammation, perineural inflammation, and external compression by granulomatous mass in leptomeninges are more commonly accepted as the cause of cranial neuropathies in sarcoidosis, including facial nerve palsy *(Carlson, 2015)*. Imaging is normal in half of the cases, but in others may show nerve enhancement and sometimes more widespread changes in the absence of other clinical features *(Kidd, 2018)*. Prognosis is usually good, with complete recovery in about 85% of patients after corticosteroid treatment; some patients might also spontaneously recover *(Tavee, 2015)*.

Optic neuritis accounts for 7% to 35% of neurosarcoidosis cases, and bilateral

involvement is slightly more frequent than unilateral *(Ungprasert, 2017b)*. Typical presentation includes subacute visual loss, retrobulbar pain and papilledema or signs of optic atrophy at fundus examination *(Nozaki, 2012)*. A minority of patients can present with concomitant uveitis *(Joseph, 2009)*. Optic chiasm may be involved, often in association with sellar involvement due to anatomic proximity *(Koczman, 2008)*. Prognosis is usually poor, with residual visual impairment in a high number of cases, mostly in patients with bilateral involvement *(Pawate, 2009)*.

Involvement of vestibulocochlear nerve can present with intermittent or persistent sensorineural hearing loss or vestibular dysfunction and is reported in 3% to 17% of patients with neurosarcoidosis *(Ungprasert, 2017b; Carlson, 2015)*. Alterations can be unilateral or bilateral and patients typically recover at least partially with corticosteroid treatment, although significant chronic hearing loss is not unusual with bilateral involvement (*Tavee, 2014*). It is commonly thought to be a consequence of granulomatous meningitis *(Kane, 1976)*.

Trigeminal involvement, reported in about 12% of patients, seem to present mainly as paresthesia and hyperesthesia, while a real trigeminal neuralgia is rare *(Braksick, 2013; Fritz, 2016)*.

Ultimately, cases of oculomotor or bulbar palsies and anosmia or hyposmia are also reported *(Colover, 1948; MacLean, 2015)*. The latter can result not only from olfactory nerve involvement due to basal meningitis, but also from granulomatous inflammation of nasal mucosa (*Delaney, 1977b*).

### *2.3.2 Meninges*

Meningeal involvement accounts for 10% to 20% of cases of neurosarcoidosis, although the frequency of subclinical alterations detected on imaging studies is much higher *(Ungprasert, 2017b)*. Both pachymeninges and leptomeninges can be affected, with a clear predominance of basal skull localization, which may extend to spinal cord meninges *(Smith, 2004; Bradshaw, 2021)*.

Symptomatic patients typically present with subacute or chronic headache, constitutional symptoms and meningeal signs. Cranial nerves impairment is common, mostly when basal skull meninges are involved, sometimes in association with neuro-endocrine dysfunction. In case of a more diffuse meningeal involvement, gait dysfunction, cognitive changes and seizures can also be present, suggesting a possible concomitant parenchymal damage (*Tavee, 2015*). Cavernous sinus involvement has

been occasionally reported *(Rosini, 2017)*.

Radiologic and laboratory findings are not clearly specific, showing thickening and post-contrast enhancement on MRI and active inflammation in CSF analysis with negative cultures. Non-necrotizing granulomas are found in about two thirds of meningeal biopsies *(Smith, 2004; Joseph, 2009; Pawate, 2009)*.

Prognosis is usually positive, because these patients tend to dramatically respond to corticosteroid therapy, although relapses after tapering or withdrawal of treatment may occur *(Plotkin, 1986; Nozaki, 2012)*.

Hydrocephalus is a serious and potentially lethal complication, reported in 9% of patients *(Fritz, 2016)*. It can be both communicating or noncommunicating and can result from impaired arachnoid villi absorption, ependymal inflammation or obstruction due to granulomatous masses *(Benzagmout, 2007; Tabuchi, 2013; Yoshitomi, 2015)*.

### *2.3.3 Brain parenchyma*

Patients with brain parenchymal disease may present with seizure, headache, cognitive or behavioral problems, focal neurological signs, or symptoms related to increased intracranial pressure *(Stern, 1985; Stern, 2010)*.

Seizures may be focal o generalized, and are usually associated with intracranial mass, diffuse encephalopathy or vasculopathy, leptomeningitis with cortical irritation or hydrocephalus *(Krumholz, 1991)*. They are reported in 14% of patients *(Fritz, 2016)* and are often difficult to control, as treatment requires both antiepileptic drugs and glucocorticoids to address the underlying abnormality *(Krumholz, 1991)*. Sometimes seizures may occur in the absence of underlying structural abnormalities with clearly better prognosis, suggesting that the real negative prognostic indicators are the underlying structural lesions instead of seizure itself *(Krumholz, 2014)*.

Depression is reported in over 60% of patients with sarcoidosis *(Chang, 2001)* and other cognitive or behavioral symptoms including delirium, personality changes, psychosis and memory disturbances develop in up to 20% of patients with neurosarcoidosis *(Joseph, 2009)*. The cause of these manifestations is likely multifactorial, including encephalopathy from neurosarcoidosis itself, use of glucocorticoids, and the emotional burden of living with a chronic disease

*(Ungprasert, 2017b)*.

The pathogenesis of neurosarcoidosis-associated encephalopathy remains poorly understood. Findings consistent with diffuse parenchymal inflammation are detectable

on imaging studies and intracranial granulomatous mass lesions, presenting as solitary mass or multiple nodules can be seen in any part of the CNS *(Stern, 1985; Joseph, 2009)*. Nonspecific white matter changes without gadolinium enhancement can also be found occasionally in asymptomatic patients and their relationship with neurosarcoidosis is uncertain, as they may also reflect comorbid pathology *(Bradshaw, 2021)*. In symptomatic patients, however, they can cause problems in differential diagnosis with vasculitis or multiple sclerosis, also due to their frequent periventricular and subcortical localization *(MacLean, 2015; Smith 2004)*.

### *2.3.4 Cerebrovascular disease*

Despite numerous pathological studies showing granulomatous involvement of cerebral vessels in sarcoidosis *(Meyer, 1953; Herring, 1969; Manz, 1983)*, clinical signs of CNS vasculitis and in particular stroke events related to sarcoidosis seem to be quite rare. Moreover, sarcoidosis-related cerebral vasculitis seems to result more commonly in a slowly progressive encephalopathy rather than in an ischemic stroke *(Brown, 1989)*.

However, a recent population-based study demonstrated that the risk of cerebrovascular accidents in patients with sarcoidosis is 3 times higher than in the general population, together with a similar increased risk of other atherosclerotic diseases, such as myocardial infarction and peripheral arterial disease, suggesting a specific mechanism for stroke and cardiovascular diseases in sarcoidosis *(Ungprasert, 2017a)*.

Cerebrovascular events in patients with sarcoidosis have variable presentations, ranging from ischemic transient or permanent strokes (69%), to hemorrhagic stroke (31%) occasionally due to central venous thrombosis. Stroke can be the first sign of sarcoidosis in up to 40% of cases, but most of the patients show several signs of disease activity at the time of stroke, including other neurological manifestations *(Jachiet, 2018)*.

Ischemic insults probably result from a combination of small-vessel vasculitis, large-vessel inflammation or cardioembolic phenomena secondary to sarcoid cardiomyopathy. Perforating arteries are the most frequently involved, causing small profound lacunar infarcts, while large vessels stroke are exceptionally rare *(Bathla, 2018)*. Hemorrhagic lesions are mainly intraparenchymal, usually small or microhemorragic with a supratentorial and lobar predilection, but subarachnoid hemorrhage is also rarely reported *(Bathla, 2018)*.

Sarcoidosis-related stroke also seems to be associated with an increased risk of

mortality (23%) and a high risk of permanent neurological impairment (up to 50% of patients) *(Jachiet, 2018)*.

### *2.3.5 Spinal cord*

In the past, spinal cord localizations of sarcoidosis were thought to be extremely rare, accounting for less than 5% of cases, but more recent studies showed a higher incidence, up to 20% of patients with neurosarcoidosis *(Stern, 1985; Fritz, 2016)*. Isolated spinal cord sarcoidosis, without any other organ involvement, has also been occasionally reported *(Duhon, 2012)*.

The most common presentation is subacute to chronic onset of paresthesia and weakness below the affected spinal cord level, with progressive paraparesis developing over months *(Durel, 2016)*. Other manifestations include back pain, radicular pain, proprioceptive disturbance, sphincter dysfunction, and cauda equina syndrome (C*ohen-Aubart, 2010; Bradley, 2006*).

MRI findings are often disproportionate in relation to clinical presentation. Indeed, despite extensive cord lesions involving multiple vertebral segments, patients often report only mild imbalance, walking difficulty, and leg numbness *(Tavee, 2015)*. In a series from 1993, MRI findings were classified in 4 stages according to clinical progression: leptomeningeal enhancement, fusiform spinal cord enlargement, focal or diffuse intramedullary disease, and spinal cord atrophy *(Junger, 1993)*. A more recent study described instead 4 main patterns of sarcoidosis myelitis: longitudinally extensive myelitis (45%), short tumefactive myelitis (23%), meningitis/meningoradiculitis (23%) and anterior myelitis adjacent to disc degeneration (10%) *(Murphy, 2020)*. Abnormal gadolinium enhancement with dorsal cord subpial pattern longer than 2 spinal segments and persistence of enhancement for more than 2 months despite treatment seem to be clues towards a diagnosis of sarcoidosis in longitudinally extensive myelitis *(Flanagan, 2016)*.

Granulomatous inflammation can be not only intramedullary, but also intradural extramedullary (presenting as aracnoiditis), o extradural, and seems to mostly involve cervical ant thoracic spine *(Cohen-Aubart, 2010; Bradley, 2006)*. By contrast, multi-radicular involvement is more frequent in lumbosacral segments, often presenting with gadolinium enhancement of conus medullaris and/or cauda equina *(Koffmann, 1999)*.

Historically, the outcome of these patients was unfavorable, with high incidence of permanent neurologic deficits *(Stern, 1985)*. However, recent series demonstrated

instead a more favorable functional outcome, possibly because of a more prompt recognition and aggressive glucocorticoid and immunosuppressive therapy *(Bradley, 2006; Durel 2016)*.

### *2.3.6 Neuro-endocrine system*

Hypotalamic and/or pituitary involvement with consequent neuro-endrocrine dysfunction is reported between 2% and 9% of patients with neurosarcoidosis *(Pawate, 2009; Fritz, 2016)*.

Endocrine dysfunction most often includes anterior hypopituitarism (LH/FSH 88,8%, TSH 67,4%, GH 50% and ACTH 48,8%), hyperprolactinemia (48,8%), and diabetes insipidus (65,2%) *(Anthony, 2016)*. Other hypothalamus-pituitary hormonal axes, including thyroid hormone, growth hormone, and cortisol, may be affected as well *(Langrand, 2012)*. Rare manifestations include inappropriate secretion of ADH, hyperphagia and obesity due to satiety center involvement, alteration of body temperature regulator, insomnia, and personality changes *(Kirkland, 1983; Vanhoof, 1992; Ungprasert, 2017b)*.

MRI findings include thickening and contrast enhancement of the pituitary gland or stalk sometimes with extension into the hypothalamus and often multifocal *(Langrand, 2012)*. Radiologic outcome is often good, with improvement or disappearance of abnormalities after corticosteroid treatment, but most endocrine defects persist due to an early and irreversible damage to secreting cells, requiring life-long replacement therapy (*Langrand 2012*).

### *2.3.7 Peripheral nerves*

Peripheral neuropathy involving large fibers in patients with neurosarcoidosis is reported in 2% to 17% of cases *(Pawate, 2009; Fritz, 2016)*. Despite its rarity, a wide spectrum of presentations have been described, including distal symmetrical polyneuropathy, which can be pure sensory, pure motor or mixed, single or multiple mononeuropathy and more rarely Guillain–Barré syndrome (GBS) and multifocal sensory-motor neuropathy with conduction block *(Said, 2013)*. Unlike cranial neuropathies, peripheral neuropathies tend to have more often a slowly progressive onset and a chronic course.

Electrophysiological studies usually show axonal alterations, but signs of demyelination are occasionally reported in GBS-like or multifocal neuropathies with conduction block,

suggesting that multifocal demyelination can be an early stage of nerve pathology induced by sarcoid granulomas *(Said, 2013)*.

Large series of sarcoid peripheral neuropathies with histological confirmation are rare in the literature *(Said, 2002; Burns, 2006)*. Pathology shows epineural, perineural and occasionally endoneural granulomas with perineurial inflammatory infiltrates and variable and patchy involvement of nerve fascicles and axon loss. Granulomas are usually located around blood vessels and often associated with lymphocytic necrotizing vasculitis, likely contributing to nerve damage by ischemic mechanism *(Said, 2013)*. Signs of granulomatous inflammation are almost always found also in muscle biopsies of these patients, being useful to differentiate sarcoid granulomas from leprous granulomas, which do not affect muscles. Therefore, muscle biopsy should always be performed in association with nerve biopsy in these patients *(Said, 2002)*.

Prognosis of peripheral neuropathy appears to be more benign than other types of neurosarcoidosis because most patients respond favorably to glucocorticoids and immunomodulatory treatment *(Ungprasert, 2017)*.

Small fiber neuropathy (SFN) seems to be a much more frequent finding, occurring in up to 40% to 60% of sarcoidosis patients *(Drent, 2015)*. Moreover, a recent web-based survey involving more than 1000 patients with sarcoidosis suggested even higher prevalence rates, as 81% of participants reported symptoms consistent with SFN *(Voortman, 2019a)*.

Clinical manifestations include pain, numbness, burning dysesthesias, intolerance to bed sheets, and vibrating or electric shock–like sensations that might be migratory and intermittent at onset, but tend to become constant and can assume both a length-dependent and non-length-dependent distribution *(Tavee, 2011)*. Over 50% of patients also present symptoms of dysautonomia, with orthostatic hypotension and palpitations being the most common, followed by gastrointestinal disturbances and sweating dysfunction *(Tavee, 2017)*. Symptoms are often disabling for patients, even when systemic disease is under control, and have an important impact on their quality of life.

Conventional electrophysiological studies are usually unremarkable, unless a large fiber neuropathy is associated. The only accurate diagnostic test for demonstrating SFN is skin biopsy for the assessment of intraepidermal nerve fiber density (IENFD), which shows a reduction of small nerve fiber density at dermo-epidermal junction *(Hoitsma, 2002; Tavee, 2011)*. However, other etiologies of SFN should also be considered and

ruled out, including B12 deficiency and diabetes, before concluding that SFN in patients with sarcoidosis is inevitably related to sarcoidosis *(Tavee, 2017)*.

The pathogenesis of SFN in sarcoidosis is still not completely understood. Unlike large fiber neuropathy, which as already mentioned is granulomatous in nature, SFN falls within the group of so-called *non-organ manifestations* of sarcoidosis, namely clinical symptoms that are non-granulomatous in nature *(Drent, 2015)*. This group also includes fatigue, depression, cognitive changes and other constitutional symptoms and has also been defined as *parasarcoidosis* or *paraneurosarcoidosis (Judson, 2014; Tavee, 2014; Datema, 2015)*. Pathogenesis is thought to be related to high concentrations of circulating cytokines and chemokines, which might induce hyperexcitability within fibers, calcium influx and oxidative stress leading to axonal degeneration *(Kidd, 2020)*. This hypothesis is supported both by the recurrence of SFN in several immune-mediated inflammatory diseases and by the increasing reports of clinical improvement of these patients after treatment with intravenous immunoglobulins (IVIg) and TNFα inhibitors *(Hoitsma, 2006; Parambil, 2011; Tavee, 2017)*. Clinical improvement has also been reported after treatment with ARA 290, a nonhematopoietic erythropoietin analogue with potent anti-inflammatory and tissue protective properties, acting at the innate repair receptor *(Heij, 2012a; Dahan, 2013)*.

### *2.3.8 Skeletal muscle*

Asymptomatic involvement of skeletal muscles is reported in 25% to 75% of patients with sarcoidosis, while symptomatic involvement occurs in only 0.5% to 5% *(Kobak, 2015)*. However, sarcoidosis patients report fatigue and myalgia more commonly than healthy controls *(Hinz, 2011)* and a recent study showed that the presence of muscular involvement, even if asymptomatic, represents a negative prognostic factor for the outcome of sarcoidosis patients, also being often associated to a more widespread extrapulmonary involvement *(Yanardağ, 2018)*.

Symptomatic sarcoid myopathy has historically been classified in three peculiar clinical patterns (acute myopathy, chronic myopathy, and nodular myopathy) *(Silverstein, 1969; Zisman, 2002)*, all characterized by the presence of non-caseating granulomas in skeletal muscles. A detailed review of the available literature will be outlined in chapter 6.1, as the introduction of part of our research.

## 2.4 Diagnostic criteria

Three main sets of diagnostic criteria have been proposed in the literature to define neurosarcoidosis.

The first and historically most used criteria are those proposed by Zajicek and colleagues in 1999, which distinguish three different grades of clinical certainty *(Zajicek, 1999)*. In the presence of a clinical presentation suggestive of neurosarcoidosis and after excluding alternative causes, a *definite* diagnosis requires positive nervous system histology. For a *probable* diagnosis, confirmation of systemic sarcoidosis together with laboratory support for CNS inflammation (namely pleocytosis, hyperproteinorrachia and/or presence of oligoclonal bands in CSF analysis or a suggestive MRI) is sufficient. In the absence of such requirements, only a *possible* diagnosis is allowed. For the confirmation of systemic sarcoidosis, original criteria required either a positive organ histology or a positive Kveim test, or at least two indirect indicators from Gallium-67 scan, chest X-ray and serum ACE *(Table 1)*. Further reviews of these criteria proposed to exclude Kveim test (no longer used in clinical practice), replace chest X-ray with chest HRCT, replace serum ACE with CD4/CD8 ratio >3,5 in BAL or >5 in CSF and add to Gallium-67 scan also $^{18}$F-FDG PET/CT scan *(Marangoni, 2006)*. Elevated CSF IgG index was also added to the signs supporting CNS inflammation *(Tavee, 2014)*.

**Table 1. Proposed criteria for the diagnosis of neurosarcoidosis**
(*Zajicek, 1999 and subsequent modifications: Marangoni, 2006; Tavee, 2014*)

| | | |
|---|---|---|
| • **Clinical presentation suggestive of neurosarcoidosis**<br>• **Exclusion of other possible diagnosis** | | |
| **Definite** | Positive nervous system histology | |
| **Probable** | Laboratory signs of CNS inflammation | • Compatible MRI findings **or**<br>• Inflammatory CSF alterations<br>▪ ↑ proteins<br>▪ ↑ cells<br>▪ ↑ IgG index<br>▪ Oligoclonal bands<br>▪ CD4/CD8 > 5 |
| | Evidence for systemic sarcoidosis | • Positive histology in other organ **or**<br>• At least 2 positive indirect indicators<br>▪ $^{18}$F-FDG PET/CT scan<br>▪ Gallium-67 scan<br>▪ Chest HRCT<br>▪ Serum ACE<br>▪ BAL CD4/CD8 > 3.5 |
| **Possible** | Above criteria not met | |

Abbreviations: CNS = central nervous system; MRI = magnetic resonance imaging; 18-FDG = 18F-fluorodeoxyglucose; PET/CT = positron emission tomography/computed tomography; HRCT = high-resolution computed tomography; ACE = angiotensin-converting enzyme; BAL = bronchoalveolar lavage; CSF = cerebrospinal fluid.

In 2014, WASOG revised the criteria to assess organ involvement in sarcoidosis, updating those defined during ACCESS study in 1999 *(Judson, 1999; Judson, 2014)*. These criteria are based on the consensus of groups of experts which evaluated, according to scientific evidence and personal experience, a list of common clinical conditions that could be considered as representing organ involvement of sarcoidosis. Consensus was reached with at least 70% agreement among the experts. Clinical conditions were graded in three categories based on the likelihood of sarcoidosis causing that manifestation. Manifestations highly specific for sarcoidosis, including cases of positive organ histology, were defined as *highly probable*, replacing the "*definite*" denomination. Manifestations considered as fairly specific for sarcoidosis, enough not to require histologic confirmation, were defined as *at least probable*, while manifestations considered as consistent but not specific for sarcoidosis were defined as *possible*. For some of the proposed manifestations, consensus was not reached, therefore it is unclear if such clinical conditions were adequate or not to represent organ involvement of sarcoidosis. Two criteria were required to be fulfilled in order to apply

this assessment: histologic evidence of granulomatous inflammation of unknown cause needed to be demonstrated in at least one other organ and all alternative causes for the clinical manifestations assessed had to be reasonably excluded *(Table 2)*.

**Table 2. WASOG criteria for assessing nervous system involvement in sarcoidosis**
(*Judson, 2014*)

| | | |
|---|---|---|
| • **Histologic evidence of granulomatous inflammation in at least one organ**<br>• **Exclusion of alternative causes** | | |
| **Highly probable** | Clinical syndrome consistent with granulomatous inflammation of the meninges, brain, ventricular (CSF) system, cranial nerves, pituitary gland, spinal cord, cerebral vasculature or nerve roots | **Plus one** of the following:<br>• Abnormal MRI characteristic of neurosarcoidosis → abnormal enhancement after gadolinium administration<br>• Inflammation in CSF analysis |
| **At least probable** | Isolated facial palsy with negative MRI | |
| | Clinical syndrome consistent with granulomatous inflammation of the meninges, brain, ventricular (CSF) system, cranial nerves, pituitary gland, spinal cord, cerebral vasculature or nerve roots without MRI or CSF findings | |
| **Possible** | Seizures with negative MRI | |
| | Cognitive decline with negative MRI | |
| **No consensus** | Peripheral neuropathy involving large fibers (axonal and demyelinating polyneuropathies and multiple mononeuropathies) | |
| | Cranial nerve palsies other than facial with negative MRI | |
| | CSF pleocytosis | |
| | Low CSF glucose | |

Abbreviations: CSF = cerebrospinal fluid; MRI = magnetic resonance imaging.

In 2018 the Neurosarcoidosis Consortium Consensus Group (NCCG), a panel formed by 10 neurologists and 4 pneumologists experienced in the management of patients with sarcoidosis and neurosarcoidosis, developed new consensus criteria for the diagnosis of neurosarcoidosis *(Stern, 2018)*. The aim was to enhance the clinical care of patients with suspected neurosarcoidosis and to encourage standardization of research initiatives addressing the disease. These criteria focus mainly on the presence of clinical manifestations and diagnostic findings (including MRI, CSF and/or EMG/NCS findings) suggestive of granulomatous inflammation of the nervous system and on the rigorous exclusion of other causes, especially infections and malignant neoplasms. Supplementary material also includes detailed differential diagnostic considerations and recommended tests for each presentation and suspect. In view of this, the degree of diagnostic certainty is determined by the presence of pathology findings consistent with

sarcoidosis in the nervous system for *definite* neurosarcoidosis, or in other organs for *probable* neurosarcoidosis; in the absence of pathologic confirmation of granulomatous disease, neurosarcoidosis is only considered *possible (Table 3)*. However, authors underline that even pathologic identification of granulomas cannot be considered as 100% definitive and emphasize the need to reassess diagnosis on the basis of the patient clinical course and response to treatment, albeit they decided not to include treatment response as a formal component of the criteria because many other diseases may transiently respond to immunosuppressive treatment.

**Table 3. NCCG proposed diagnostic criteria for CNS and PNS neurosarcoidosis**
(*Stern, 2018*)

- **Clinical presentation and diagnostic evaluation suggesting neurosarcoidosis**
  - Clinical manifestations
  - MRI, CSF and/or EMG/NCS findings typical of granulomatous inflammation of the nervous system
- **Rigorous exclusion of other causes**

| | | |
|---|---|---|
| **Definite** | Nervous system pathology consistent with neurosarcoidosis | |
| | **Type a.** | **Type b.** |
| | Extraneural sarcoidosis is evident | No extraneural sarcoidosis is evident (*isolated neurosarcoidosis*) |
| **Probable** | Pathologic confirmation of systemic granulomatous disease consistent with sarcoidosis | |
| **Possible** | No pathologic confirmation of granulomatous disease | |

Abbreviations: MRI = magnetic resonance imaging; CSF = cerebrospinal fluid; EMG/NCS = electromyography/nerve conduction study.

## 2.5 Diagnostic tests

As defined in the aforementioned sets of criteria, the highest grade of diagnostic certainty for neurosarcoidosis is reached with a CNS or PNS histology positive for sarcoid granulomas, in the presence of a suggestive (or at least consistent) clinical manifestation and after the exclusion of alternative diagnosis *(Zajicek, 1999; Judson, 2014; Stern, 2018)*. However, due to the invasive and possibly destructive nature of nervous system biopsy, often it is not possible to obtain histological confirmation of the diagnosis. Moreover, false negative results are not infrequent and up to 40% of cerebral and meningeal biopsies turn out to be inconclusive *(Stern, 2010)*. Therefore, the diagnostic process for confirming neurosarcoidosis should focus on two main

objectives: confirming a neuro-inflammatory basis of disease and investigating systemic sarcoidosis *(Ibitoye, 2017)*.

### *2.5.1 Histology*

Despite histology of neurosarcoidosis has been less diffusely studied than other organ involvement, pathologic studies performed on biopsies and autoptic material demonstrated that typical sarcoid lesions in nervous system show the same characteristics found in other organs.

Lesions consist of epithelioid granulomas, which may be either scattered or confluent in larger masses (up to 1-2 cm); in nervous system they tend to be typically located closer to vessels, be smaller, and contain giant cells less commonly compared to other organs *(van Dellen, 2013; Jefferson, 1958)*. In CNS, the most common localization of granulomas is meningeal, which usually occurs at the skull base and involves the leptomeninges but can extend from the subarachnoid space into the superficial brain parenchyma along the Virchow-Robin spaces. The most common sites of parenchymal involvement are hypothalamus and pituitary gland, while the spinal cord sites preferentially involved are the cervical or thoracic cord. In the peripheral nerves, the granulomas primarily involve epineurium, perineurium and the vasa nervorum *(Tana, 2015)*.

Microscopically, neurosarcoidosis granulomas are 150-400 μm diameter round compact collections of epithelioid histiocytes interspersed with lymphocytes and surrounded by a rim of sparse lymphoid cells and variable amount of fibrosis and collagen deposition, depending on their "age" *(Figure 5)*. Granulomas, in fact, become fibrotic starting from the periphery toward the center, with eventual replacement of the whole lesion by a hyalinized nodular fibrous scar. When located within the CNS parenchyma, granulomas are often surrounded by a dense reactive gliosis *(Tana, 2015)*. Small foci of necrosis (granular, fibrinoid, or eosinophilic) may occasionally be present in the center of sarcoid granulomas, in a rare form of 'necrotizing' neurosarcoidosis which results in increased complexity of differential diagnosis towards granulomatous infectious diseases *(Tobias, 2002)*. Neurosarcoidosis may also show associated vasculitis, with infiltration of the adventitia and media of small arteries or veins by granulomas, giant cells, or lymphocytes, which can be responsible for foci of parenchymal ischemic necrosis *(Tana, 2015)*.

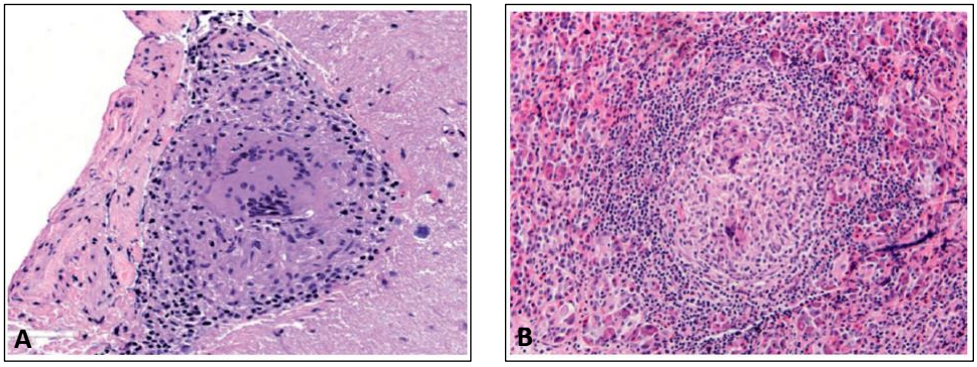



**Figure 5. Sarcoid granulomas in subarachnoid space (A) and in the pituitary gland (B)** (H&E stain, original magnification x200) *(Tana, 2015)*

Epithelioid and multinucleated giant cells composing granulomas stain for histiocytic markers (CD68, HAM-56, and CD163) and may stain weakly for CD4. CD4 also stains the T-cells within the granulomas, while lymphocytes rimming the granulomas are usually T-cells staining for CD8, possibly mixed with rare CD20 staining B-cells, CD138-staining plasma cells and rare tryptase and CD117-staining mast cells. However, immunohistochemistry is rarely needed for diagnostic purposes, since granulomas are usually easily identified on routine hematoxylin-eosin stained sections *(Tana, 2015)*.

### *2.5.2 Neuroimaging*

Contrast-enhanced MRI of the brain and/or the spine is the most sensitive imaging modality for assessing CNS involvement in sarcoidosis and is currently considered the standard of care, although its sensitivity decreases in case of concomitant or previous steroid therapy *(Pawate, 2009; Ungprasert, 2017)*.

Non-enhancing white matter (NEWM) lesions, T2-hyperintense, are often described as the most common imaging abnormality and are reported in more than half of the patients in some cohorts *(Bathla, 2020)*. They variably involve periventricular, deep and subcortical white matter and are mostly scattered or early confluent, mimicking multiple sclerosis. Their pathogenesis is unclear, but small infarcts due to granulomatous angiopathy or extension of a subependymal granulomatous process may contribute. However, NEWM lesions are often asymptomatic and usually do not improve with therapy and are therefore considered fairly nonspecific *(Bathla, 2016)*.

Small parenchymal granulomas may be seen only after gadolinium administration, while larger masses are often isointense on T1-weighted images and show T2 prolongation *(Bathla, 2016)*; they usually present with diffuse or rim enhancement, although non-enhancing lesions may rarely occur and coexist with enhancing ones

*(Lury, 2004; Bathla, 2020)*. Supratentorial localization seems to be the most common, followed by combined supra- and infra-tentorial localization *(Bathla, 2020)*. Necrosis and parenchymal calcifications are rare *(Shah, 2009)*.

Hypothalamic and pituitary gland involvement usually presents as nonspecific thickening and enhancement of the pituitary-infundibulum-hypothalamus, which may extend into the surrounding meninges *(Bathla, 2016)*.

Leptomeningeal involvement is reported in 18% to 71% of patients, and some authors describe it as the most common finding *(Bathla, 2020)*. It usually manifests as smooth or nodular abnormal meningeal enhancement, has often a basal or suprasellar localization and may be associated with perivascular enhancement due to spread of the inflammatory process along the Virchow-Robin spaces *(Bathla, 2016)*.

Pachymeningeal involvement is reported in about 30% of patients, mostly involving posterior fossa. Lesions may be focal or plaque-like, are often hypontense on T2-weighted images and uniformly enhance after gadolinium administration *(Bathla, 2016; Smith, 2004)*. Interestingly, dural and leptomeningeal involvement are rarely present together in the same region, a finding attributed to the arachnoid barrier cells, which prevent spread of disease through the arachnoid membrane *(Smith, 2004)*.

Hydrocephalus is reported in 4% to 38% of patients and may be either communicating (probably due to impaired CSF reabsorption) or obstructive (usually due to retractions caused by leptomeningeal inflammation) *(Bathla, 2020; Bathla, 2016)*.

Involved cranial nerves may appear thickened and show smooth or nodular gadolinium enhancement, which may occur isolated or over a background of leptomeningeal involvement *(Bathla, 2016; Shah, 2009)*. The most common involved nerve on imaging is usually the II, followed by the VII and the V, and multiple concomitant alterations are frequent *(Bathla, 2020)*. However, clinical signs and symptoms of cranial neuropathy have a poor correlation with MRI abnormalities because some patients have abnormal MRI without any clinical symptoms whereas others have clinical symptoms with normal MRI *(Ungprasert, 2017)*.

Cerebrovascular manifestations were considered rare but have increasingly been described in the last few years, probably because they often manifest clinically with progressive encephalopathy rather than a distinct stroke-like syndrome *(Jachiet, 2018)*. Ischemic strokes commonly involve the basal ganglia, thalamus, and brain stem, due to preferential involvement of small perforating arteries, while large-vessel

involvement is exceptionally rare. They are often small and may be most apparent on diffusion-weighted images as foci of restricted diffusion, while T2-weighted images may not be very useful due to the possible presence of surrounding parenchymal edema or white matter lesions which tend to make them even more inconspicuous. Occasionally the infarcts may be multiple, recurrent, or of varying ages and rarely they may be more superficial and subcortical, due to involvement of the cortical vessels from surrounding meningeal inflammation *(Bathla, 2018)*. Symptomatic hemorrhagic lesions are rare, usually intraparenchymal and mainly supratentorial, but their distribution is variable, probably due to pathologically demonstrated "mixed" venous and arterial involvement *(Bathla, 2018)*. However, asymptomatic microhemorrhages, demonstrated with SWI sequences, seem to occur in approximately 30% of patients *(Zamora, 2018)*. Because the underlying etiology is vasculitis, these patients may present changes of both ischemia and hemorrhage at different time points *(Bathla, 2018)*. Rare vascular manifestations, including dural sinus thrombosis *(Byrne, 1983)*, cavernous sinus involvement *(Rosini, 2017)*, subarachnoid hemorrhage *(Berek, 1993)* and Moyamoya-like vasculopathy *(Ko, 2009)* have also been occasionally reported. A recent study analyzed a small cohort of neurosacoidosis patients with high resolution vessel-wall imaging, showing that 69% of patients had some evidence of vascular involvement, including dilatation or tortuosity of lenticulostriate perforators and/or medullary veins, perivascular enhancement, large vessels involvement and microhemorrhages or chronic infarcts *(Bathla, 2021)*. These features, although non-specific, should however drive the diagnostic suspect towards neurosarcoidosis, especially when combined with other suggestive imaging abnormalities, such as leptomeningeal or cranial nerve involvement.

In the spinal cord, the most frequent MRI finding is intramedullary involvement, mostly in the cervical or upper thoracic spine. Lesions often extend over multiple segments and show T1/T2 prolongation and patchy gadolinium enhancement, which is often peripheral *(Bathla, 2016; Shah, 2009)*. There is often fusiform enlargement of the affected cord segment and overlying leptomeningeal involvement, which is thought to be a precursor of intramedullary lesions *(Lury, 2004; Junger, 1993)*. Spinal nerves may be involved, presenting as non-specific leptomeningeal enhancement, while dural lesions are rare and often show T2 prolongation, unlike intracranial dural lesions *(Bathla, 2016; Lury, 2004)*.

MRI can sometimes show also sarcoid bone lesions, often asymptomatic. Skull and vertebral column involvement is rare, but usually affects lower thoracic and upper lumbar spine *(Smith, 2004)*. Lesions are often T1 isointense and may show T2 prolongation or shortening based on whether they are predominantly lytic or sclerotic, may be multiple and enhance after gadolinium administration *(Bathla, 2016)*.

However, all the aforementioned findings are compatible with neurosacoidosis but clearly nonspecific, raising relevant differential diagnosis issues. Therefore, although sensitive, MRI is not sufficient for diagnosing neurosarcoidosis with reasonable confidence and needs integration with other clinical and laboratory findings.

Other neuroimaging techniques have more limited applications. Contrast-enhanced CT scan may be helpful for patients with contraindications to MRI, but show a much lower sensitivity *(Stjepanović, 2014)*. Conventional angiography can demonstrate vasculitis, but carries a high risk of false negatives, due to the frequent selective involvement of smaller perforating vessels and veins *(Bathla, 2018)*. $^{18}$F-FDG PET/CT scan has a limited role in the assessment of neurosarcoidosis lesions in CNS, because it can show both accelerated metabolism due to sarcoid inflammation, or delayed metabolism due to neuron dysfunction and decreased metabolic demand with respect to the usual high metabolic needs of the brain (*Stjepanović, 2014*). On the other hand, $^{18}$F-FDG PET/CT scan may play a major role in demonstrating systemic sarcoidosis abnormalities and identifying potential biopsy localizations outside CNS *(Fritz, 2020a)*.

### *2.5.3 CSF analysis*

CSF findings in neurosarcoidosis are not specific, but lumbar puncture should however be performed in these patients, in order to establish the presence of intrathecal inflammation and rule out other possible etiologies of the clinical picture.

Usual CSF findings are elevated proteins, lymphocyte predominant pleocytosis and hypoglycorrhachia *(Pawate, 2009; Leonhard, 2016)*, especially in patients with leptomeningeal involvement *(Wengert, 2013)*. Oligoclonal bands (sometimes matched with serum) and elevated IgG index may be seen in 20% to 40% of patients *(Pawate, 2009; Leonhard, 2016)*.

However, CSF analysis is sometimes normal, especially in patients with isolated cranial

neuropathy, clinically inactive or already on steroid treatment, therefore a negative finding does not always rule out diagnosis (*Ungprasert, 2017; Stern, 1985)*.
For differential diagnosis purpose, viral PCRs with associated serologies, mycobacterial PCR and bacterial, acid-fast bacterial and fungal cultures should be performed, along with cytology and flow-cytometry to rule out malignancies *(Stern, 2018)*.
Several studies investigated potential CSF biomarkers for neurosarcoidosis, with contradictory results. A detailed review of the available literature will be outlined in chapter 5.1, as the introduction of part of our research.

### *2.5.4 Other investigations*

Electrophysiological studies are useful for diagnosis and follow-up of patients with suspected peripheral nerve or muscular involvement and can help determine localization, demyelinating versus axonal pathophysiology, degree of severity, and chronicity of the lesion, but their findings are nonspecific *(Tavee, 2015)*. Musculoskeletal MRI may also demonstrate nodular lesions within the muscle suggestive of sarcoidosis involvement, and histologic confirmation may be obtained through nerve or muscle biopsy *(Prieto-González, 2014)*. In patients with SFN, quantitative sudomotor axon reflex testing, tilt table testing and confocal corneal microscopy may be useful, but skin biopsy for IENFD evaluation is required for confirming the diagnosis *(Tavee, 2011*).
Visual, somatosensory and brainstem evoked potential have shown to be abnormal often also in asymptomatic patients, being able to detect subclinical CNS involvement *(Oksanen, 1986b; Gott, 1997)*.
Different presentations may also require more focused testing. Indeed, an electroencephalogram should be obtained in patients presenting with seizures and a neuroendocrine evaluation may be needed in patients presenting with symptoms of hypothalamic-pituitary dysfunction *(Tavee, 2015)*.

Finally, it is important to repeat that in patients without prior history of systemic sarcoidosis it is mandatory to search for other potential organ involvement, both for diagnostic confirmation and for accurate prognostic evaluation. Investigation should include serum biomarkers testing, evaluation of lungs with chest HRCT (eventually extended to abdomen and pelvis to search for lymphadenopathies), broncho-alveolar

lavage, pulmonary function tests and ophthalmologist evaluation. Whenever possible, histologic confirmation of sarcoidosis should be pursued in at least one organ. If aforementioned tests fail to show evidence of systemic disease, further evaluation with a whole body $^{18}$F-FDG PET/CT scan should be considered. *(Tavee, 2014; Krumholtz, 2014).*

### 2.6 Differential diagnosis

Among granulomatous diseases, the most important differential diagnosis to consider is *tuberculosis* involving the nervous system, especially in areas where it is endemic and in patients who have a history of exposure. Nervous system involvement by tuberculosis has a similar radiological and histopathological distribution, with basilar meningeal involvement and extension to the parenchyma with military granulomas or larger tuberculomas. Although classic tubercular granulomas are necrotizing, tuberculosis may occasionally show only non-necrotizing granulomas on biopsy *(Chokoeva, 2014)*. Therefore, every effort should be made to rule out mycobacterial infection, including acid-fast stains, immunostains for mycobacteria, PCR-based molecular tests and cultures *(Tana, 2015).*

Other granulomatous infections which may occasionally present with only non-necrotizing granulomas are histoplasmosis, aspergillosis, and cryptococcosis and serologic testing, special stains, and molecular diagnostic methods should be employed to rule them out. In general, the presence of more than focal necrosis strongly suggests an infectious process, as does the presence of more than occasional non-lymphocytic cells within the center or periphery of granulomas (i.e. plasma cells in *syphilis*, neutrophils in *suppurative granulomas* or eosinophils in *parasitic granulomas*) *(Kosjerina, 2012).*

Among non-infectious diseases, granulomatous vasculitides should be considered in neurosarcoidosis differential diagnosis. *Granulomatosis with polyangiitis*, formerly known as *Wegener's granulomatosis*, can frequently involve CNS affecting meninges, optic nerve or other cranial nerves and the pituitary gland. It usually involves small- and medium-sized vessels with granulomatous inflammation and necrosis, in patients with ANCA-positive serology *(Seror, 2006)*. *Primary central nervous system vasculitis* may present granulomatous aspects in 50% of patients; in such cases differential diagnosis with neurosarcoidosis is complex and should be based on the presence of necrotizing

vasculitis, the absence of meningeal, cranial nerve or spinal cord involvement and the absence of systemic disease *(Hajj-Ali, 2013; Saygin, 2020)*.

A number of neoplastic conditions may present with granulomas. These include *lymphomatoid granulomatosis*, a B-cell lymphoma that may present with extensive infiltration of the meninges, blood vessels, and brain by lymphoid cells which show at least focally atypical features *(Lucantoni, 2009)*. Moreover, *pineal germinomas* may be associated with sarcoid-like reactions composed of numerous small, non-necrotizing granulomas that may obscure the presence of the neoplastic germ cells *(Tana, 2015)*.

Another multi-system immune-mediated disease to consider in differential diagnosis, although not exactly granulomatous, is the so-called *IgG4-related disease* (IgG4-RD) which can affect CNS with a chronic pachymeningitis often involving cranial nerves and pituitary gland *(Stone, 2012)*. Although 2019 ACR/EULAR criteria comprise the presence of primary granulomatous inflammation among exclusion criteria *(Wallace, 2020)*, at least two patients with lymphadenopathy due to IgG4-RD presenting granulomas at histologic examination were reported *(Bateman, 2015)*. Such findings underline that this disease should at least be considered in differential diagnosis of neurosarcoidosis.

Lastly, it should be reminded that, unexpectedly, *anti-TNFα drugs* can induce sarcoid reactions, potentially mimicking neurosarcoidosis *(Berrios, 2015; Hunter, 2016)*.

Main differential diagnosis of neurosarcoidosis sorted by predominant clinical syndromes are reported in *Figure 6*.

| Cranial neuropathies | Leptomeningeal disease | Parenchymal brain disease |
|---|---|---|
| Lyme disease | Tuberculosis | Multiple sclerosis |
| Multiple sclerosis | Cryptococcal meningitis | CNS Lymphoma |
| Neuromyelitis optica | Lyme disease | Gliomas |
| Neurosyphilis | HIV | Craniopharyngioma |
| HIV | Leptomeningeal metastasis | Germ cell tumors |
| Varicella zoster virus | Vogt-Koyanagi-Harada disease | Primary CNS angiitis |
| Optic nerve gliomas | Brucellosis | Lymphocytic hypophysitis |
| Optic nerve meningiomas | Behçet's disease | Toxoplasmosis |
| Sjögren's syndrome | | Whipple's disease |
| Systemic lupus erythematosus | **Pachymeningeal disease** | Behçet's disease |
| Infiltrative neoplasm | ANCA-associated vasculitis | |
| Lymphoma | IgG4 related pachymeningitis | **Myelopathy** |
| | Meningiomas | Multiple sclerosis |
| **Neuropathy** | Idiopathic pachymeningitis | Neuromyelitis optica |
| Vasculitic neuropathies | Rosai-Dorfman disease | Degenerative disc disease |
| Guillain-Barré syndrome | Dural metastasis | Tuberculosis myelitis |
| Gluten-related disorders | Lymphoma | VZV myelitis |
| Fibromyalgia | Intracranial hypotension | HTLV-1 associated myelopathy |
| | | Sjögren's syndrome |
| | | Systemic lupus erythematosus |
| | | CMV myeloradiculitis |
| | | Schistosomal myeloradiculopathy |

**Figure 6. Differential diagnosis of neurosarcoidosis by predominant clinical syndrome** *(Agnihotri, 2014)*
Abbreviations: ANCA = antineutrophilic cytoplasmic antibodies; CNS = central nervous system; CMV = cytomegalovirus; HIV = human immunodeficiency virus; VZV = varicella zoster virus.

Among non-granulomatous disorders, the most important differential diagnosis of neurosarcoidosis is certainly *multiple sclerosis* (MS). Clinically isolated neurosarcoidosis can indeed be almost indistinguishable from multiple sclerosis, mimicking symptoms, relapsing-remitting course and temporo-spatial dissemination of lesions in MRI. However, some clinical features can suggest neurosarcoidosis over multiple sclerosis *(Figure 7)*: for instance, severe bilateral and progressive optic neuropathy, co-occurrence of neuroendocrine and optic nerve dysfunction or a facial palsy which is bilateral or unilateral but associated to vestibulocochlear dysfunction are way more characteristic of neurosarcoidosis. Similarly, a persistent steroid sensitivity with relapses upon steroid tapering or suspension, or the coexistence of central and neuromuscular symptoms are not typical for multiple sclerosis *(MacLean, 2015)*. Regarding neuroimaging, the association of parenchymal and meningeal lesions strongly directs towards neurosarcoidosis. Lastly, spinal cord presentations of neurosarcoidosis are usually more severe and disabling and MRI lesions are more likely to be longitudinally extensive and show a more heterogeneous and patchy enhancement after gadolinium administration *(MacLean, 2015; Junger, 1993)*.

| | CNS sarcoidosis | MS |
|---|---|---|
| **Clinical manifestations** | | |
| Relapsing/remitting course | Common | Very common |
| Diverse neurological findings | Common | Very common |
| Extraneural involvement | Highly suggestive | Possible (esp. rheumatologic) |
| Visual apparatus | Anterior segment > Optic nerve | Optic nerve > Anterior segment |
| Cranial neuropathy | Highly suggestive | Possible, but not common |
| **MR findings** | | |
| Leptomeningeal enhancement | Highly suggestive | Rare |
| Dural enhancement | Highly suggestive | Rare |
| Enhancing mass adjacent to meninges | Highly suggestive | Rare |
| Enhancement of parenchymal lesions | Persistent (more than a few weeks) | Transient (within a few weeks) |
| Hydrocephalus | Highly suggestive | Rare |
| Involvement of hypothalamus/pituitary | Highly suggestive | Rare |
| Non-enhancing periventricular WM lesions | Common | Common |
| Spinal cord | Intradural extramedullary > Intramedullary | Intramedullary |
| **CSF findings** | | |
| Lymphocytosis, Elevated protein level | Common | Common |
| Hypoglycorrhachia | Suggestive | Rare |
| Elevated ACE level | Suggestive | Possible |
| Oligoclonal bands | Possible | Highly suggestive |

**Figure 7. Differentiating features of central nervous system sarcoidosis from multiple sclerosis** *(Nozaki, 2012)*
Abbreviations: ACE = angiotensin converting enzyme; CNS = central nervous system; CSF = cerebrospinal fluid; MR = magnetic resonance; MS = multiple sclerosis; WM = white matter.

## 2.7 Treatment

Unlike pulmonary sarcoidosis, spontaneous resolution of neurosarcoidosis is exceptionally rare, limited to sporadic cases with isolated V or VII cranial nerve presentations *(Arun, 2021)*. Therefore, treatment of neurosarcoidosis is almost always warranted, in order to minimize morbidity and mortality *(Voortman, 2019b)*. Unfortunately, due to the rarity of the condition, no randomized clinical trials have been performed so far, and currently available treatment recommendations are only based on expert opinions and retrospective studies *(Figure 8)*.

| Drug | Dosage | Adverse Effects | Monitoring |
|---|---|---|---|
| Prednisone | 5–40 mg daily | Diabetes, hypertension, weight gain, cataracts, glaucoma steroid myopathy | Blood pressure, weight, glucose if clinically indicated, osteoporosis and bone density checks |
| Hydroxychloroquine | 200–400 mg daily | Ocular, hepatic, cutaneous | Eye examination every 6–12 mo |
| Methotrexate | 5–20 mg weekly | Hematologic, hepatoxic | CBC, hepatic every 1–3 mo |
| Azathioprine | 50–200 mg daily | Hematologic, gastrointestinal | CBC, hepatic every 1–3 mo |
| Mycophenylate | 500–1500 mg daily twice daily | Hematologic, gastrointestinal | CBC, hepatic every 1–3 mo |
| Infliximab | 3–5 mg/kg initially, 2 wk later, then every 4–8 wk | Allergic reactions, increased risk for infections, especially tuberculosis, worsening congestive heart failure, possible increased risk for malignancy, PML | PPD before initiating therapy, hold drug in face of active infection |
| Adalimumab | 40–80 mg SQ every 1–2 wk | Allergic reactions, increased risk for infections, especially tuberculosis, worsening congestive heart failure, possible increased risk for malignancy, PML | PPD before initiating therapy, hold drug in face of active infection |
| Rituximab | 1000 mg IV, 2 doses, 2 wk apart | Hematologic, allergic reactions increased risk for infections, especially HBV, PML, renal | HBV screening before initiating therapy, hold drug in face of active infection, CBC, renal function every 1–3 mo |

**Figure 8. Therapies for neurosarcoidosis** *(Tavee, 2015)*
Abbreviations: CBC = complete blood count; HBV = hepatitis B virus; IV = intravenous; PML = progressive multifocal leukoencephalopathy; PPD = purified protein derivative, skin test to diagnose tuberculosis; SQ = subcutaneous.

As in pulmonary and systemic sarcoidosis, CS are the first line of treatment, although they seem to be effective, when administered in monotherapy and at low dosage, in a lower percentage of patients, usually with milder presentations as unilateral or bilateral facial nerve palsies, isolated leptomeningeal involvement or pituitary disease *(Voortman, 2019b; Arun, 2021)*. Reported dosages vary, ranging from 0,5-1 mg/kg/day of oral prednisone to courses of 3-5 days of intravenous methylprednisolone 1 g/day for severe and potentially life-threatening presentations. In general, patients with neurosarcoidosis often require a prolonged course of corticosteroid therapy (at least 6-12 months) and then, once adequate clinical and radiological response is achieved, treatment can be gradually tapered *(Ungprasert, 2017)*.

Second-line immunosuppressive treatment is recommended in case of severe disease at presentation, refractory or relapsing disease during CS treatment or tapering, when prolonged treatment with high doses of CS is required or a primary contraindication for corticosteroids exists *(Voortman, 2019b)*. It is important to underline that most second-line therapies need 3 to 6 months before a clinical response might be expected, therefore

the use of combination therapies with CS and immunosuppressant *ab initio* should be considered in severe presentations *(Fritz, 2017; Ungprasert, 2017)*.

MTX is the most frequently used second-line agent *(Fritz, 2016)*. One retrospective study reported a response rate of 61% in patients treated with MTX and corticosteroids, after corticosteroids monotherapy failed *(Lower, 1997)* and in another study neurosarcoidosis patients treated with MTX were able to taper prednisone without relapses in about half of cases *(Bitoun, 2016)*. Reported dosages range from 10 to 25 mg per week and adverse effects do not occur frequently and can be lessened by the use of folic acid *(Ungprasert, 2017)*.

AZA is the second most used immunosuppressant after MTX *(Fritz, 2016)*, but most of the data about its efficacy come from pulmonary sarcoidosis studies, showing similar treatment response and adverse events compared to MTX, except for a higher rate of infections *(Vorselaars, 2013)*. Usual dosage is 2 mg/kg/day orally.

MMF (2 g/day orally) has occasionally been reported as effective in neurosarcoidosis *(Androdias, 2011)*, but a comparative study showed a significantly higher relapse rate when prednisone was withdrawn with respect to MTX-treated patients *(Bitoun, 2016)*.

Few case reports and series report the use of intravenous CYC 500-1000 mg every 2 or 3 weeks, with a response rate of around 50% *(Lower, 1997; Doty, 2003)*. However, adverse effects are more frequent and serious than other immunosuppressants, therefore it is considered a third-line treatment option in severe disease, refractory to other cytotoxic agents *(Fritz, 2017)*.

Third-line treatment consists of TNFα inhibitors, mainly IFX. Few recent studies reported on relatively large cohorts of neurosarcoidosis patients treated with IFX *(Cohen-Aubart, 2017; Gelfand, 2017; Fritz, 2020b)*, showing favourable long-term outcomes also in patients who failed to respond to previous immunosuppressant therapies. However, studies also demonstrated a high rate of relapses after tapering or withdrawal and frequent occurrence of infectious complications, underlining that a strict follow-up remains essential.

The use of ADA is mainly reported in patients who did not respond or showed adverse events after MTX or IFX *(Hutto, 2021)*. The limited data available show efficacy in a variety of neurologic phenotypes, with a lower risk of developing anti-drug antibodies with respect to IFX.

Few case reports and a small case series have reported efficacy of RTX administration in a limited number of refractory neurosarcoidosis patients *(Bomprezzi, 2010; Zella,*

*2018; Earle, 2019).*

Patients with painful SFN due to sarcoidosis have shown poor response to corticosteroid treatment, whereas clinical improvement was reported after immune-modulating treatment with IVIg, TNFα inhibitors and ARA 290 *(Parambil, 2011; Hoitsma, 2006; Heij, 2012a; Dahan, 2013).*

# CHAPTER 3: AIMS OF THE RESEARCH STUDY

CNS and neuromuscular involvement are both rare presentations of sarcoidosis, representing a diagnostic challenge for neurologists mainly due to their polymorphic clinical presentations and the lack of established sensitive and specific diagnostic biomarkers of the disease.

Thanks to the cooperation with the *Sarcoidosis Regional Referral Center*, managed by *Respiratory Diseases and Lung Transplantation Unit* of the University Hospital of Siena, we were able to evaluate and follow a large number of sarcoidosis patients with suspected or confirmed neurologic involvement.

Therefore, we decided to critically analyze the data derived from clinical experience and to perform focused clinico-pathologic studies, in order to gain more insight on the disease and its complications and to propose new potential diagnostic tools.

Specifically, our research study was developed in three main parts:

- A retrospective cohort study on a population of CNS neurosarcoidosis patients, performed with the aims of identifying recurrent and peculiar clinical patterns and evaluating the possible use of current diagnostic criteria in clinical practice;
- A controlled study on serum and CSF biomarkers of CNS neurosarcoidosis including CTO and KL-6, which were never systematically investigated in this localization of the disease;
- A retrospective/prospective cohort study on sarcoidosis patients undergoing muscular biopsy, performed in order to define every clinico-pathological presentation and investigate for the first time the role of TNFα in sarcoid myopathy.

# CHAPTER 4: CNS NEUROSARCOIDOSIS, EXPERIENCE FROM A SARCOIDOSIS REFERRAL CENTER

## 4.1 Materials and methods

The current study was performed at University Hospital of Siena and originated from the cooperation between the two Neurology Units (*Neurology and Clinical Neurophysiology Unit* and *Clinical Neurology and Neurometabolic Diseases Unit*) and the *Sarcoidosis Regional Referral Center*, managed by *Respiratory Diseases and Lung Transplantation Unit*.

This is a retrospective cohort study on neurosarcoidosis patients followed between January 1st 2011 and December 31st 2020 by the *Sarcoidosis Regional Referral Center*, which enrolls about 50 newly diagnosed sarcoidosis patients every year.

The retrospective review of medical records involved:

- Sarcoidosis patients followed by *Sarcoidosis Regional Referral Center* who underwent at least one neurologic evaluation in the suspect of neurosarcoidosis;
- Inpatients and outpatients from *Neurology and Clinical Neurophysiology Unit* and *Clinical Neurology and Neurometabolic Diseases Unit* who were lately diagnosed with sarcoidosis.

All patients who fulfilled at least a *possible* diagnosis according to at least one of the three major neurosarcoidosis diagnostic criteria proposed in the literature *(Zajicek, 1999; Judson, 2014; Stern, 2018)* were included. Charts with at least one missing piece of information required for an adequate diagnostic evaluation according to the aforementioned criteria, were excluded. Patients with peripheral nerve or skeletal muscle involvement were excluded from this cohort, in order to focus on CNS manifestations, and will be the object of a further study presented in the next chapters.

Demographics, medical history, clinical presentation, laboratory data, radiological and histopathological results, treatment details and response to treatment at follow-up were collected for each included patient and were entered into an electronic database.

The following tables outline detailed information about the study population.

**Table 4. Demographics and past clinical history**

| Pt | Sex | Age at NS onset | Ethnicity | Immune-related comorbidities | Previous S | Time S-NS |
|---|---|---|---|---|---|---|
| **01 – DD** | M | 44 yrs | Caucasian | – | Yes | 87 mos |
| **02 – ME** | F | 68 yrs | Caucasian | Bronchial asthma | Yes | 72 mos |
| **03 – VF** | M | 47 yrs | Caucasian | IgA nephropathy, psoriasis | No | – |
| **04 – DG** | M | 39 yrs | Caucasian | Drug allergy | No | – |
| **05 – MV** | F | 16 yrs | Caucasian | – | No | – |
| **06 – GM** | F | 45 yrs | Southern-American | – | No | – |
| **07 – LM** | M | 49 yrs | African | – | Yes | 40 mos |
| **08 – VC** | F | 47 yrs | Caucasian | Sjögren syndrome | No | – |
| **09 – PP** | F | 46 yrs | Caucasian | – | No | – |
| **10 – PA** | F | 47 yrs | Caucasian | – | No | – |
| **11 – DA** | M | 61 yrs | Caucasian | Drug allergy | No | – |
| **12 – PC** | F | 27 yrs | Caucasian | – | No | – |
| **13 – TG** | M | 53 yrs | Caucasian | GBS | No | – |
| **14 – CL** | F | 56 yrs | Caucasian | Bronchial asthma, drug allergy, MS | No | – |
| **15 – GP** | M | 37 yrs | Caucasian | – | No | – |
| **16 – SP** | M | 52 yrs | Caucasian | – | Yes | 130 mos |
| **17 – SM** | F | 48 yrs | Caucasian | – | Yes | 30 mos |
| **18 – AM** | M | 56 yrs | Caucasian | – | Yes | 36 mos |
| **19 – SP** | M | 45 yrs | Caucasian | Allergic oculorhinitis | Yes | 31 mos |
| **20 – SD** | M | 31 yrs | Caucasian | – | No | – |
| **21 – MG** | M | 42 yrs | Caucasian | – | Yes | 144 mos |
| **22 – MC** | M | 52 yrs | Caucasian | Drug allergy | Yes | 34 mos |

Abbreviations: Pt = patient; NS = neurosarcoidosis; S = sarcoidosis; time S-NS = time (months) from sarcoidosis diagnosis to onset of neurological symptoms; yrs = years; GBS = Guillain-Barré syndrome; MS = multiple sclerosis; mos = months.

**Table 5. Pulmonary and systemic sarcoidosis**

| Pt | Pathology | Chest HRCT (Scadding 0-IV) | BAL | $^{18}$F-FDG PET/CT | Serum ACE (IU/l) | Serum LSZ (mg/l) | Serum CTO (nmol/ml/h) | Extra-thoracic localizations |
|---|---|---|---|---|---|---|---|---|
| **01 – DD** | **+ LN** | I | n.a. | n.d. | 68 | 6,6 | **87** | LN, skeletal muscle |
| **02 – ME** | **+ TBNA** | III | n.a. | n.d. | 13 | 6,2 | **160** | – |
| **03 – VF** | **+ TBNA** | II | CD4/CD8 2<br>L 7% | n.d. | 2 | **8,2** | n.d. | – |
| **04 – DG** | **+ LN** | II | CD4/CD8 1<br>L 4% | n.d. | 52 | 4 | n.d. | – |
| **05 – MV** | **+ TBNA** | III | **CD4/CD8 3,98**<br>**L 46,5%** | n.d. | **112,2** | 4,4 | **64** | – |
| **06 – GM** | **+ LN** | II | n.d. | **+** | **141,4** | **11** | **98** | LN, bones |
| **07 – LM** | **+ LN and TBNA** | II | **CD4/CD8 3,8**<br>**L 63%** | n.d. | **139** | **13** | **369,4** | LN |
| **08 – VC** | n.d. | II | **CD4/CD8 8,6**<br>**L 53%** | n.d. | **107** | 6,3 | **303,2** | – |
| **09 – PP** | **+ LN** | II | n.d. | n.d. | **91** | 7 | **268,9** | LN |
| **10 – PA** | n.d. | II | n.d. | **+** | 60 | 4,8 | **52** | – |
| **11 – DA** | **+ CNS** | 0 | n.d. | n.d. | **156,6** | 3,9 | **109** | – |
| **12 – PC** | - TBNA | I | CD4/CD8 2,17<br>**L 37%** | n.d. | **91** | 6,2 | **352,5** | – |
| **13 – TG** | n.d. | III | CD4/CD8 1,95<br>**L 30%** | - | **102,9** | 4,8 | **133** | – |
| **14 – CL** | **+ LN** | II | **CD4/CD8 3,55**<br>L 2% | **+** | 79,5 | 7 | 43,3 | LN |

| Pt | Pathology | Chest HRCT (Scadding 0-IV) | BAL | $^{18}$F-FDG PET/CT | Serum ACE (IU/l) | Serum LSZ (mg/l) | Serum CTO (nmol/ml/h) | Extra-thoracic localizations |
|---|---|---|---|---|---|---|---|---|
| **15 – GP** | **+ LN** | II | **CD4/CD8 6,2**<br>**L 23%** | n.d. | 67 | 4,1 | **275,3** | – |
| **16 – SP** | **+ LN** | I | n.a. | n.d. | 64 | 5,2 | **91,6** | LN |
| **17 – SM** | **+ orbit and kidney** | II | CD4/CD8 2,35<br>L 5% | **+** | 76,9 | 3,3 | **260** | Orbit, kidney |
| **18 – AM** | **+ pleura** | II | CD4/CD8 3,04<br>L 15% | n.d. | **99** | 7,3 | **155** | Skin |
| **19 – SP** | **+ TBNA** | II | CD4/CD8 2,53<br>**L 49%** | **+** | n.m. | **11** | **96,5** | – |
| **20 – SD** | n.d. | 0 | **CD4/CD8 3,58**<br>**L 34%** | - | 66,9 | 3,8 | **60** | – |
| **21 – MG** | **+ TBNA** | II | n.a. | - | 43 | 6 | **99** | – |
| **22 – MC** | **+TBNA** | IV | CD4/CD8 0,37<br>**L 63%** | **+** | **112,8** | 4,8 | **101** | – |

Abbreviations: Pt = patient; HRCT = high-resolution computed tomography; BAL = bronchoalveolar lavage; 18F-FDG PET/CT = 18F-fluorodeoxyglucose positron emission tomography/computed tomography; ACE = angiotensin-converting enzyme; LSZ = lysozyme; CTO = chitotriosidase; LN = lymph-nodes; TBNA = transbronchial needle aspiration; CNS = central nervous system; n.a. = not available; CD4/CD8 = CD4 lymphocytes / CD8 lymphocytes ratio; L = lymphocytes; n.d. = not done; n.m. = not measurable.
Reference values: CD4/CD8 BAL: < 3,5; L BAL: < 20%; serum ACE: 30-80 IU/l; serum LSZ: < 8 mg/l; serum CTO: 20,4-51 nmol/ml/h.
Abnormal values in bold.

**Table 6. Neurologic involvement**

| Pt | Clinical presentation | Localization | MRI | CSF analysis | | | |
|---|---|---|---|---|---|---|---|
| **01 – DD** | Central panhypopituitarism | Hypothalamus / pituitary gland | T2-hyperintensity and Gd-enhancement of infundibular recess and pituitary gland and stalk | n.d. | | | |
| **02 – ME** | Right trigeminal neuralgia and tactile/painful hypoesthesia (I and II branches) | Cranial nerve (V) | T2-hyperintense and Gd-enhancing lesion in the right brainstem (medulla oblongata/pons) | n.d. | | | |
| **03 – VF** | Diabetes insipidus | Hypothalamus / pituitary gland | Thickening and T2-hyperintensity of pituitary stalk (Gd: n.d.) | n.d. | | | |
| **04 – DG** | Left eye visual loss, lipothymia | Cranial nerve (II) | T2-hyperintensity and enlargement of left optic nerve and papilla | n.a. | | | |
| **05 – MV** | Right blepharoptosis, diplopia, orbital pain, headache, complete III cranial nerve palsy | Brain meninges | Thickening, T2-hyperintensity and Gd-enhancement of right cavernous sinus, superior orbital fissure and foramen ovale | P 20 mg/dl | G 53 mg/dl | 4 cells/mm$^3$ | |
| | | | | Alb 8,6 mg/dl | IgG 1 mg/dl | Alb index 0,002 | IgG index 0,71 |
| | | | | OCB - | | **ACE 13,07 IU/l** | |
| **06 – GM** | Headache with visual blurring, nausea and speech arrest, postural unsteadiness, hyperprolactinemia | Brain and spinal meninges and parenchyma, hypothalamus / pituitary gland | Diffuse and T2-hyperintense lesions in the brain hemispheres, pons and pituitary stalk with nodular Gd-enhancement of brain leptomeninges, right statoacoustic nerve and ganglion, ependyma, and pituitary stalk; thickening and Gd- enhancement of spinal meninges at C1-D2 and D10-L1 levels, parenchymal nodules at D11-D12, D12-11 and L1 levels and nodular enhancement of cauda equina roots | **P 251 mg/dl** | G n.d. | 3 cells/mm$^3$ | |
| | | | | **Alb 149 mg/dl** | **IgG 52 mg/dl** | **Alb index 0,0349** | **IgG index 0,85** |
| | | | | **OCB +** | | **ACE 27,84 IU/l** | |

| Pt | Clinical presentation | Localization | MRI | CSF analysis | | | |
|---|---|---|---|---|---|---|---|
| **07 – LM** | Diabetes insipidus, central hypothyroidism and hypogonadotropic hypogonadism | Brain and spinal meninges, hypothalamus / pituitary gland | Thickening, T2-hyperintensity and Gd-enhancement of pituitary stalk and infundibular recess; Gd-enhancement of meninges in left frontal operculum, pontomedullary junction and medulla oblongata | n.d. | | | |
| **08 – VC** | Left arm paresthesia plus burning dysaesthesia in neck and left hemithorax | Brain and spinal cord parenchyma | Diffuse T2-hyperintense lesions in the right cerebellar hemisphere, biemispheric WM, pons, corpus callosum and hippocampus with Gd-enhancement; multiple T2-hyperintense and Gd-enhancing lesions in the cervico-thoracic spinal cord | P 31,6 mg/dl | G 60 mg/dl | 1 cell/mm$^3$ | |
| | | | | Alb 22,8 mg/dl | **IgG 6,7 mg/dl** | Alb index 0,006 | **IgG index 0,74** |
| | | | | OCB - | | S biomarkers: n.d. | |
| **09 – PP** | Headache plus partial epileptic seizures (cacosmia, dysgraphia) | Brain meninges and parenchyma | Leptomeningeal and cortical T2-hyperintensity and Gd-enhancement in bilateral temporal lobes, left basal frontal lobe, bilateral sylvian cistern, left nucleobasal region and infundibular recess | **P 105 mg/dl** | G 41 mg/dl | **94 cells/mm$^3$ 97% M, 3% PM** | |
| | | | | **Alb 75,5 mg/dl** | **IgG 17,3 mg/dl** | **Alb index 0,015** | **IgG index 0,99** |
| | | | | **OCB +** | | S biomarkers: n.d. | |
| **10 – PA** | Right eye visual loss | Cranial nerve (II) | Reduced caliber of right optic nerve sheath in intraorbital and prechiasmatic tract and of right optic chiasma with Gd-enhancement | n.a. | | | |
| **11 – DA** | Asymmetrical spastic paraplegia, lower limbs paresthesia and ataxia | Spinal cord parenchyma | Parenchymal T2 hyperintensity at C5-D2 levels with swelling and Gd-enhancement | P 43 mg/dl | G 64 mg/dl | **27 cells/mm$^3$ 74% M, 26% PM** | |
| | | | | Alb 31,3 mg/dl | IgG 3,7 mg/dl | **Alb index 0,0084** | IgG index 0,52 |
| | | | | OCB - | | **ACE 14,3 IU/l** | |

| Pt | Clinical presentation | Localization | MRI | CSF analysis | | | |
|---|---|---|---|---|---|---|---|
| **12 – PC** | Right eye visual loss | Cranial nerve (II) | Thickening, T2-hyperintensity and Gd-enhancement of prechiasmatic right optic nerve | n.d. | | | |
| **13 – TG** | Paroxysmal Dysarthria, ataxia, nystagmus | Brain and spinal cord parenchyma | Focal T2-hyperintense lesion with patchy Gd-enhancement in central midbrain; multiple T2-hyperintense nodules with patchy Gd-enhancement at C2-C4, C6, D4, D8-D11 levels | **P 67,9 mg/dl** | G 51 mg/dl | **16 cells/mm$^3$ 25% M, 75% PM** | |
| | | | | **Alb 49,2 mg/dl** | **IgG 5,8 mg/dl** | **Alb index 0,011** | IgG index 0,55 |
| | | | | OCB - | | **ACE 12 IU/l** | **LSZ 0,8 mg/l** |
| **14 – CL** | Asymmetrical spastic paraplegia, headache, transient speech arrest | Brain and spinal meninges, brain parenchyma | Multiple T2-hyperintense lesions in the subcortical and paraventricular regions and in the pons and corpus callosum, micronodular Gd-enhancement of brain leptomeninges, infundibular recess, pituitary stalk, trigeminal and optic nerves; nodular Gd-enhancement of cervico-dorso-lumbar spinal cord leptomeninges and cauda equina roots | **P 103,3 mg/dl** | **G 28 mg/dl** | **19 cells/mm$^3$ 84% M, 16% PM** | |
| | | | | **Alb 65,4 mg/dl** | **IgG 20,4 mg/dl** | **Alb index 0,015** | **IgG index 1,35** |
| | | | | **OCB + (few in serum)** | | ACE n.m. | **LSZ 5 mg/l** |
| **15 – GP** | Right arm weakness and pain | Spinal cord parenchyma | Parenchymal T2-hyperintensity from C2 to C7 with swelling of spinal cord from C3 to C7 and Gd-enhancement from C3 to C5 | **P 50,9 mg/dl** | G 48 mg/dl | 0 cells/mm$^3$ | |
| | | | | **Alb 37 mg/dl** | IgG 3,3 mg/dl | **Alb index 0,0081** | IgG index 0,48 |
| | | | | **OCB + (serum and CSF)** | | S biomarkers: n.d. | |

| Pt | Clinical presentation | Localization | MRI | CSF analysis | | | |
|---|---|---|---|---|---|---|---|
| **16 – SP** | Behavioural changes, short-term memory impairment, complex visual hallucinations, postural unsteadiness | Brain meninges, cerebrovascular | Thickening and Gd-enhancement of the right temporal leptomeninges, focal right periventricular, peritrigonal and temporo-polar ischemic lesions with reduced blood flow in the M1-M2-M3 tracts of right MCA | **P 77,2 mg/dl** | G 72 mg/dl | 2 cells/mm$^3$ | |
| | | | | **Alb 41,2 mg/dl** | **IgG 8,1 mg/dl** | **Alb index 0,094** | IgG index 0,52 |
| | | | | OCB - | | S biomarkers: n.d. | |
| **17 – SM** | Right hypoacusia, vertigo, postural unsteadiness, headache, ataxia, low back pain | Cauda equina roots | "Ring" Gd-enhancement of the spinal conus and cauda equina roots | **P 257,3 mg/dl** | G 41 mg/dl | **81 cells/mm$^3$ 97,5% M, 2,5% PM** | |
| | | | | **Alb 228 mg/dl** | **IgG 43,4 mg/dl** | **Alb index 0,056** | **IgG index 0,96** |
| | | | | OCB 1+ | | **ACE 3,2 IU/l** | **LSZ 7,1 mg/l** |
| **18 – AM** | Right "peripheral" facial nerve palsy | Cranial nerve (VII) | Normal | n.d. | | | |
| **19 – SP** | Mild asymmetrical spastic paraplegia, unsteadiness, unilateral sciatic pain, neurogenic bladder | Spinal cord meninges | Arachnoid-web in D4-D5 tract with focal contact between spinal cord and posterior wall of vertebral canal, angled shape and thinning of the spinal cord, centromedullar T2-hyperintense lesion without Gd-enhancement | P 25 mg/dl | G 63 mg/dl | 2 cells/mm$^3$ | |
| | | | | Alb 18,1 mg/dl | IgG 1,9 mg/dl | Alb index 0,0039 | IgG index 0,57 |
| | | | | OCB - | | **ACE 6,97 IU/l** | |

| Pt | Clinical presentation | Localization | MRI | CSF analysis | | | |
|---|---|---|---|---|---|---|---|
| **20 – SD** | Headache, fever | Brain meninges and parenchyma | T2-hyperintensity of the left middle cerebellar peduncle, sigmoid sinus and cerebellar sulci with linear and nodular Gd-enhancement of cerebellar leptomeninges | **P 102,9 mg/dl** | G 46 mg/dl | **65 cells/mm$^3$ 95% M, 5% PM** | |
| | | | | **Alb 89,2 mg/dl** | **IgG 17,1 mg/dl** | **Alb index 0,018** | **IgG index 0,84** |
| | | | | OCB - | | ACE n.m. | **LSZ 1,3 mg/dl** |
| **21 – MG** | Isolated epileptic seizures | Brain parenchyma | Normal | n.d. | | | |
| **22 – MC** | Headache, visual blurring, postural unsteadiness, lower limb weakness | Cerebrovascular | Ischemic lesions in the left pons and pontomedullary junction, thalamus and right internal capsule, basilar artery occlusion | **P 519,9 mg/dl** | **G 15 mg/dl** | **118 cells/mm$^3$ 97% M, 3% PM** | |
| | | | | **Alb 335 mg/dl** | **IgG 148 mg/dl** | **Alb index 0,081** | **IgG index 1,72** |
| | | | | **OCB +** | | **ACE 6,5 IU/l** | **LSZ 7 mg/l** |

Abbreviations: Pt = patient; MRI = magnetic resonance imaging; CSF = cerebro-spinal fluid; Gd = gadolinium; CRF = chronic renal failure; WM = white matter; MCA = middle cerebral artery; P = total proteins; G = glucose; M = mononuclear cells; PM = polymorphonuclear cells; Alb = albumin; OCB = oligoclonal bands; ACE = angiotensin-converting enzyme; LSZ = lysozyme; S = sarcoidosis; n.d. = not done; n.a. = not available; n.m. = not measurable.
Reference values: CSF proteins: 20-45 mg/dl; CSF glucose: 40-70 mg/dl; CSF cells: < 10 cells/mm$^3$; CSF albumin: 10-32 mg/dl; CSF IgG: 0-4 mg/dl; albumin index: < 0,0063; IgG index: < 0,71.
Abnormal values in bold.

**Table 7. Diagnostic classification, treatment and follow-up**

| Pt | Zajicek, 1999 | WASOG, 2014 | NCCG, 2018 | Treatment | Follow up | Outcome |
|---|---|---|---|---|---|---|
| **01 – DD** | Probable | Highly probable | Probable | Oral CS, AZA | 297 mos | Clinical stability, MRI improvement |
| **02 – ME** | Probable | Highly probable | Probable | Oral CS | 101 mos | Clinical and MRI improvement |
| **03 – VF** | Probable | Highly probable | Probable | Oral CS | 189 mos | Clinical recovery, MRI improvement |
| **04 – DG** | Probable | At least probable | Probable | I.v. and oral CS, AZA, MTX | 89 mos | Clinical and MRI improvement |
| **05 – MV** | Probable | Highly probable | Probable | I.v. and oral CS | 84 mos | Clinical and MRI recovery |
| **06 – GM** | Probable | Highly probable | Probable | I.v. and oral CS | 14 mos | Clinical recovery, MRI improvement |
| **07 – LM** | Probable | Highly probable | Probable | Oral CS, MTX | 85 mos | Clinical stability, MRI recovery, CS side effects |
| **08 – VC** | Probable | n.a. | Possible | I.v. and oral CS, CYC, MTX | 58 mos | Clinical and MRI stability |
| **09 – PP** | Probable | Highly probable | Probable | I.v. an oral CS | 93 mos | Clinical improvement, MRI recovery |
| **10 – PA** | Probable | n.a. | Possible | Oral CS, AZA, MTX | 46 mos | Clinical and MRI stability, CS side effects |
| **11 – DA** | Definite | Highly probable | Definite – type b | Oral CS, MTX | 81 mos | Clinical and MRI improvement, CS side effects |
| **12 – PC** | Probable | n.a. | Possible | I.v. and oral CS, MTX | 38 mos | Clinical stability, MRI improvement |
| **13 – TG** | Probable | n.a. | Possible | I.v. and oral CS | 49 mos | Clinical recovery, MRI improvement |
| **14 – CL** | Probable | Highly probable | Probable | I.v. and oral CS, MTX | 67 mos | Clinical and MRI improvement, CS side effects |
| **15 – GP** | Probable | Highly probable | Probable | I.v. and oral CS, MTX | 52 mos | Clinical recovery, MRI improvement |
| **16 – SP** | Probable | Highly probable | Probable | Oral CS, MTX | 61 mos | Clinical and MRI improvement |
| **17 – SM** | Probable | Highly probable | Probable | Oral CS | 12 mos | Clinical and MRI improvement, CS side effects |
| **18 – AM** | Possible | At least probable | Probable | Oral CS, MTX | 12 mos | Clinical recovery, MRI stability (normal) |

| Pt | Zajicek, 1999 | WASOG, 2014 | NCCG, 2018 | Treatment | Follow up | Outcome |
|---|---|---|---|---|---|---|
| **19 – SP** | Probable | At least probable | Probable | MTX | 47 mos | Clinical improvement, MRI stability |
| **20 – SD** | Possible | n.a. | Possible | I.v. and oral CS, (RTX) | 45 mos | Clinical recovery, MRI improvement, relapse at CS decalage |
| **21 – MG** | Possible | Possible | Probable | Oral CS | 8 mos | Clinical recovery, MRI stability (normal) |
| **22 – MC** | Probable | Highly probable | Probable | Oral CS, MTX, RTX | 10 mos | Clinical improvement, MRI stability |

Abbreviations: Pt = patient; n.a. = not applicable (lack of histologic confirmation); I.v. = intravenous; CS = corticosteroids; AZA = azathioprine; MTX = methotrexate; CYC = cyclophosphamide; RTX = rituximab; mos = months; MRI = magnetic resonance imaging.

## 4.2 Results

### *4.2.1 Demographics and clinical history*

Twenty-two patients met the inclusion criteria of the study. Data regarding demographics and relevant clinical history of the population are summarized in *Table 4*.
The population included 13 males (59,1%) and 9 females (40,9%). Mean age at neurological onset was 45 years (range: 16-68). All patients were Caucasian except for patient n. 6 (Southern-American) and patient n. 7 (African). Interestingly, 9 patients (40,9%) had one or more immune-related comorbidities, notably allergic (drug allergies, bronchial asthma, allergic oculorhinitis), immune-mediated (GBS) or autoimmune (Sjögren syndrome, multiple sclerosis, psoriasis, IgA nephropathy).
At neurological onset, only 9 patients (49,9%) had a previous diagnosis of extra-neurologic sarcoidosis. In those patients, the mean interval between sarcoidosis diagnosis and occurrence of neurological symptoms was 67 months (range: 30-144). In the remaining 13 patients (59,1%) neurological symptoms were the first clinical manifestations of sarcoidosis.

### *4.2.2 Pulmonary and systemic sarcoidosis*

Data regarding pulmonary and systemic sarcoidosis involvement are summarized in *Table 5*.
In 17 patients (77,3%), diagnosis of sarcoidosis was confirmed by pathological findings. In 4 patients biopsy was not performed and in one patient (n. 12) TBNA resulted negative for granulomatous inflammation, but chest imaging and laboratory findings allowed however to make the diagnosis. One patient (n. 11) underwent CNS biopsy, providing a diagnosis of *definite neurosarcoidosis*. The other pathological confirmations came from TBNA, lymph node, kidney, orbital and pleural biopsies.
Chest HRCT was performed in all patients and presented abnormalities consistent with sarcoidosis in 20 out of 22 patients (90,9%). Scadding radiologic stage consisted of *stage I* in 3 patients (13,6%), *stage II* in 13 patients (59,1%), *stage III* in 3 patients (13,6%) and *stage IV* in 1 patient (4,5%). The 2 patients with negative HRCT (*stage 0*) were diagnosed with *isolated neurosarcoidosis* (9,1%).
Bronchoalveolar lavage data were available for 14 patients and 10 of them (71,4%) showed alterations consistent with sarcoidosis, specifically CD4/CD8 ratio > 3,5 in 6 patients (42,8%) and lymphocytes percentage > 20% in 9 patients (64,2%).

Only 9 patients underwent a total body $^{18}$F-FDG PET/CT scan. Among them, 6 patients (66,7%) presented abnormalities suggestive for sarcoidosis, and in 1 patient (n. 10) such finding was crucial for a diagnosis of sarcoidosis.

Serum ACE and LSZ were assessed in all patients, resulting increased only in 10 (45,5%) and 4 (18,2%) patients, respectively. Serum CTO was assessed in 20 patients and was increased in all patients but one (95%).

Eight patients (36,4%) presented also one or more extra-pulmonary localization of sarcoidosis, specifically extra-thoracic lymph nodes, skin, bones, kidney, orbit and skeletal muscle.

### *4.2.3 Neurologic involvement*

Neurological symptoms, localization of lesions in CNS and MRI and CSF findings are reported in *Table 6*.

The most frequent CNS localization was meningeal, found in 8 patients (36,4%) and involving only cranial meninges in 4, only spinal meninges in 1 and both cranial and spinal meninges in 3. The other localizations, sorted by frequency, were brain parenchyma in 7 patients (31,8%), spinal cord in 6 patients (27,3%) (specifically 5 patients with parenchymal involvement and 1 patient with involvement of cauda equina roots), cranial nerves in 5 patients (22,7%) (specifically II cranial nerve in 3 patients, VII cranial nerve in 1 patient and V cranial nerve in 1 patient), hypothalamus and pituitary gland in 4 patients (18,2%) and cerebrovascular involvement in 2 patients (9,1%). Eight patients (36,4%) presented more than one concomitant CNS localization.

Patient n. 13 presented with peculiar symptoms, deserving a detailed description. He suffered from three months of multiple daily brief and sudden episodes of cerebellar dysarthria and bilateral gait and limbs ataxia with frequent falls, without clear triggers and lasting a few dozens of seconds. His neurologic examination out of the paroxysmal episodes was unremarkable except for a multi-directional gaze-evoked nystagmus. His brain and spinal MRI examinations revealed non-homogeneously gadolinium-enhancing lesions, one in central midbrain and multiple in cervical and thoracic spinal cord cordonal white matter, none of which longer than two vertebral bodies *(Figure 9 A-D)*. Brain $^{18}$F-FDG PET/CT scan showed brainstem and right posterior parietal lobe hypometabolism *(Figure 9 E)*. This complex clinical and imaging picture has been previously reported as *paroxysmal dysarthria-ataxia*, first described by Harry Lee

Parker in 1946 *(Parker, 1946)* and later reported as rare presentation of demyelinating diseases (mainly multiple sclerosis but also neuro-Behçet, systemic lupus erythematosus, Bickerstaff's encephalitis) or occasionally in mesencephalic stroke *(Osterman, 1975; Piffer, 2014; Akman-Demir,1995; Matsui, 2004)*. To our knowledge, this presentation has never been previously reported in neurosarcoidosis. This syndrome is a manifestation of mesencephalic dysfunction, and its pathogenesis is probably due to ephaptic transverse activation of partially demyelinated axons interrupting cerebello-thalamo-cortical pathways. The right posterior parietal lobe hypometabolism on brain $^{18}$F-FDG PET/CT scan, namely *parietal diaschisis*, is similar to the left parietal lobe hypoperfusion described with single-photon emission computerized tomography (SPECT) imaging of a patient presenting with paroxysmal dysarthria-ataxia due to mesencephalic ischemic stroke *(Matsui, 2004)*. The proposed explanation of this finding is that mesencephalic ephapsis, by interrupting cerebello-thalamo-cortical pathway, causes a reduction in parietal cortex activation by cerebellar projections, consequently causing hypometabolism and hypoperfusion. The patient underwent symptomatic treatment with oral oxcarbazepine, with rapid and complete remission of paroxysmal episodes, and immune-modulating treatment with intravenous and oral corticosteroids, with clear improvement of MRI findings.

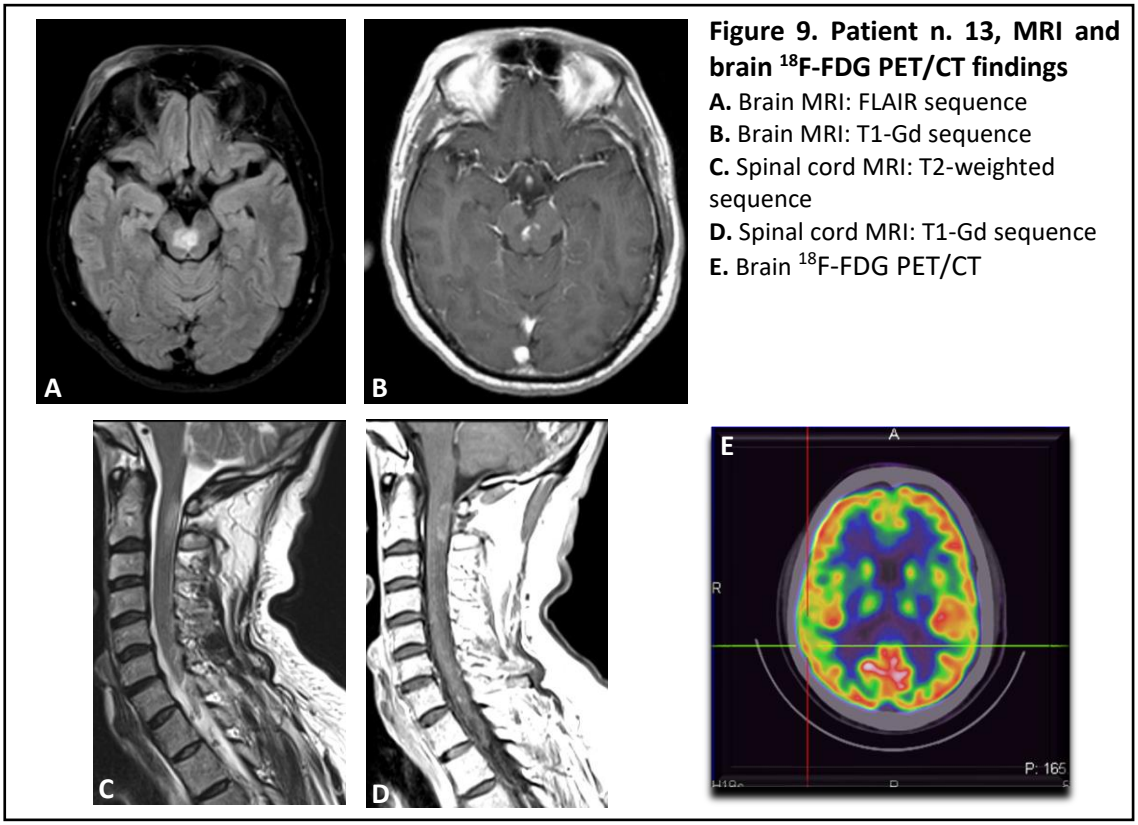



**Figure 9. Patient n. 13, MRI and brain $^{18}$F-FDG PET/CT findings**
**A.** Brain MRI: FLAIR sequence
**B.** Brain MRI: T1-Gd sequence
**C.** Spinal cord MRI: T2-weighted sequence
**D.** Spinal cord MRI: T1-Gd sequence
**E.** Brain $^{18}$F-FDG PET/CT

Brain and/or spinal cord MRI were performed in all patients, showing abnormal findings in 20 out of 22 (90,9%) and gadolinium-enhancing lesions in 16 out of 20 (80%). In one patient (n. 3) gadolinium was not administered due to concomitant chronic renal failure. The two patients with negative MRI presented, respectively, with isolated facial nerve palsy (n. 18) and isolated seizures (n. 21). Examples of MRI findings are reported in *Figure 10*.

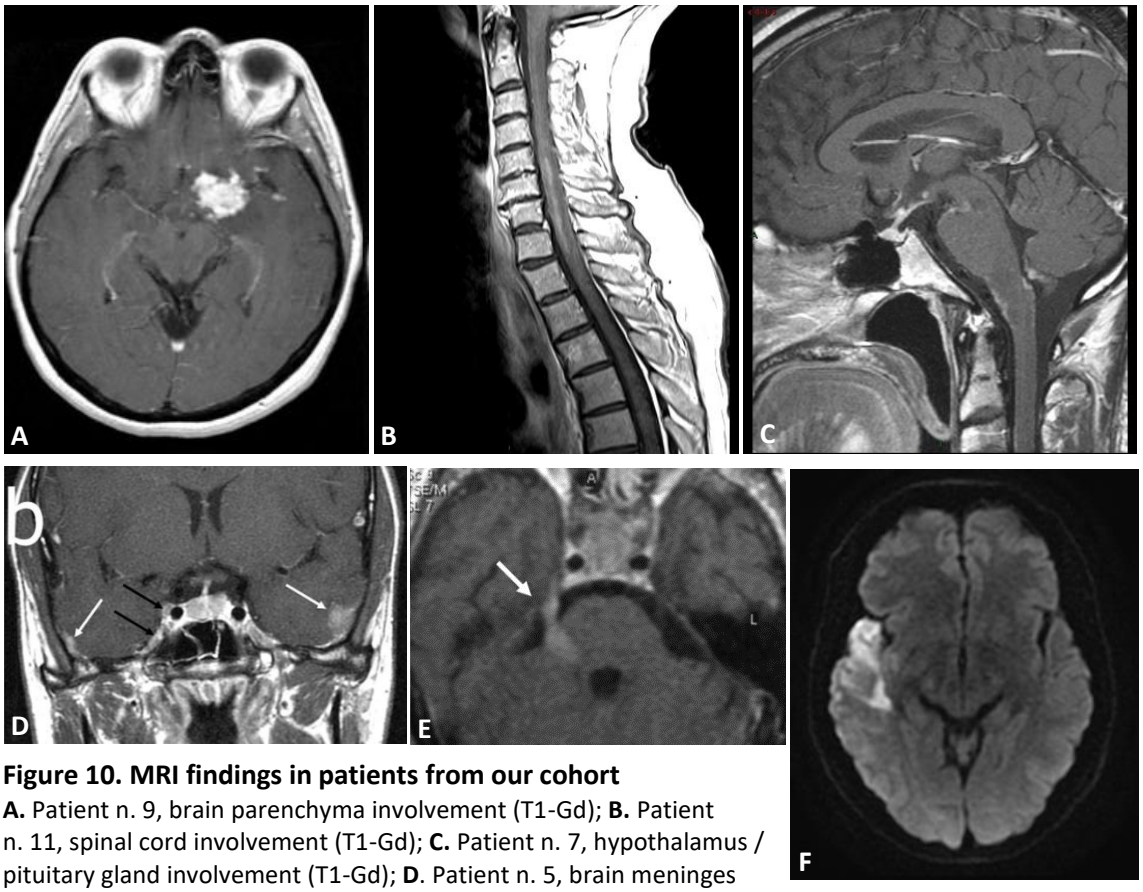



**Figure 10. MRI findings in patients from our cohort**
**A.** Patient n. 9, brain parenchyma involvement (T1-Gd); **B.** Patient n. 11, spinal cord involvement (T1-Gd); **C.** Patient n. 7, hypothalamus / pituitary gland involvement (T1-Gd); **D**. Patient n. 5, brain meninges Involvement (T1-Gd); **E.** Patient n. 2, V cranial nerve involvement (T1-Gd); **F:** Patient n. 16, cerebrovascular involvement (DWI).

Lumbar puncture was performed in 15 patients, but data regarding CSF analysis were available only for 13 patients. CSF analysis showed at least one abnormal finding in 11 patients (84,6%). Specifically, increased proteins were found in 9 patients (69,2%), increased cells in 7 patients (53,8%) with clear lymphocytic predominance in all but one, increased albumin in 9 patients (69,2%), increased IgG in 9 patients (69,2%), increased albumin index in 10 patients (76,9%) and increased IgG index in 7 patients (53,8%). Multiple oligoclonal bands were found in 5 patients (38,5%), and in 2 of them also in the serum.

CSF ACE was analyzed in 9 patients, resulting not measurable in 2 of them and giving results between 3,2 IU/l and 27,8 IU/l in the other 7 patients. CSF LSZ was analyzed in 4 patients, with measured values between 0,8 mg/l and 7,1 mg/l.

#### *4.2.4 Diagnostic classification*

Classifications of patients according to different proposed criteria are reported in *Table 7*.

Applying Zajicek's criteria (with subsequent modifications), 1 patient was classified as *definite* (4,5%), 18 patients were classified as *probable* (81,8%) and 3 patients were classified as *possible* (13,6%).

WASOG 2014 criteria, requiring pathological evidence of granulomatous inflammation in at least one "other" organ, were applicable only for 17 patients, because 5 patients lacked that condition. Among these 17 patients, 13 were classified as *highly probable* (76,5%), 3 were classified as *at least probable* (17,6%) and 1 was classified as *possible* (5,9%).

Applying NCCG criteria, 1 patient was classified as *definite (type b)* (4,5%), 16 patients were classified as *probable* (72,2%) and 5 patients were classified as *possible* (22,7%).

#### *4.2.5 Treatment and follow-up*

Treatment ad follow-up information are summarized in *Table 7*.

All patients except for one were treated with corticosteroids (95,4%), and 10 out of 21 (47,6%) were treated with high dose intravenous corticosteroids due to their severe presentation. 13 out of 21 patients (59,1%) required a second-line immunosuppressive therapy. First-choice agent was MTX in 8 patients, AZA in 3 patients and CYC in 1 patient; one of the MTX-treated patients switched to RTX due to inefficacy, while 2 of the AZA-treated patients switched to MTX due to gastrointestinal side effects; the CYC-treated patient switched to MTX due to poor extra-neurologic response to treatment. One patient did not undergo corticosteroid treatment due to morbid obesity and was treated with MTX as fist line.

Mean follow-up duration was 70 months (range: 8-297). From a clinical point of view, 8 patients (36,4%) experienced complete clinical recovery, 9 patients (40,9%) showed clinical improvement and 5 patients (22,7%) remained stable. From a radiologic point of view, MRI findings completely recovered in 3 patients (13,6%), improved in 13 patients (59,1%) and remained unchanged in 6 patients (27,3%) (2 of them having

negative MRI at presentation). Patient n. 20, after a complete clinical recovery and improvement of MRI findings with oral corticosteroids, experienced a clinical and radiological relapse during corticosteroid decalage, therefore is going to be treated with RTX as second-line therapy.
Five patients out of 21 presented side effects related to corticosteroid treatment, specifically osteoporosis in 4 patients with vertebral fractures in 3 and femoral head osteonecrosis in 1, alopecia and fluid retention in 1 patient.

### 4.3 Discussion

Although an analysis of incidence and prevalence of neurosarcoidosis in our region falls beyond the scope of this study, the 22 included subjects represent approximately 4,4% of all newly diagnosed sarcoidosis patients from *Sarcoidosis Regional Referral Center* during the analyzed timeframe. This value is in line with the majority of the cohorts of clinically evident neurosarcoidosis reported in the literature *(Dorman, 2019; Ramos-Casals 2021; Ungprasert, 2016a).*
A relevant metanalysis *(Fritz, 2016)* reviewed neurosarcoidosis cohorts published between 1965 and 2015 and identified 29 studies, with samples ranging from 5 to 305 patients (median: 27). Among them, nearly 50% (14 out of 29) included less than 22 patients. Moreover, most of these studies included both central and peripheral/neuromuscular neurosarcoidosis and clinical data regarding single patients were often poorly detailed. More recently, few larger cohorts have been published *(Leonhard, 2016; Dorman, 2019; Lord, 2020; Mani, 2020; Ramos-Casals, 2021),* mainly resulting from multicenter studies or big and nation-wide reference centers.
However, the detailed clinical data and the wide spectrum of clinical presentation of patients recruited in the present cohort allows us to express some new insights about some of the most debated issues regarding the disease.

First of all, a comment regarding the three currently proposed sets of diagnostic criteria seems appropriate.
Zajicek's 1999 criteria with subsequent modifications *(Zajicek, 1999; Marangoni, 2006; Tavee, 2014)* do not set limits to possible clinical presentations of neurosarcoidosis, taking in account also the great potential heterogeneity of the systemic disease. Indeed, these criteria do not describe or enumerate clinical syndromes which are included or

excluded by the criteria themselves, allowing the broadest possible applicability of the set. Moreover, the two conditions required for a *probable* neurosarcoidosis diagnosis focus the attention on the two main targets of the diagnostic process: confirming a neuro-inflammatory basis of the neurologic disease and investigating systemic sarcoidosis.

However, getting into details, some flaws can be detected. Indeed, concerning the demonstration of inflammation in CNS, it should be considered that CSF analysis is often not performed, especially in patients with milder presentations, including isolated seizures or single cranial nerve palsies, or in patients with isolated neuro-endocrine dysfunction (see, in our cohort, patients n. 01, 02, 03, 07, 12, 18, 21). On the other hand, some routinely performed biomarkers of systemic sarcoidosis are not considered in the criteria. For example, neither increased lymphocytes percentage in BAL nor serum CTO are included. Particularly, serum CTO has showed higher sensitivity and specificity in differentiating sarcoidosis from other diseases compared to other known biomarkers, and seemed to correlate with extrapulmonary involvement *(Bargagli, 2013; Bergantini, 2019)*. For instance, patient n. 20 was classified only as *possible* neurosarcoidosis according to Zajicek's criteria; instead, if those criteria included serum CTO among indirect indicators of systemic sarcoidosis, this patient would be classified as *probable* neurosarcoidosis.

Another issue which remains unclear in these criteria is PNS and skeletal muscle involvement. Zajicek's criteria, indeed, seem not to consider neuromuscular manifestations as "*neurosarcoidosis*", and however, the items required for at least a *probable* diagnosis are completely unsuited to evaluate peripheral nerves or skeletal muscle involvement.

By the way, the major limitation of Zajicek's criteria is probably relative to *definite* diagnosis, which necessarily requires a positive CNS pathology. This approach, which aims to reach the highest possible diagnostic accuracy and is certainly effective when applied to other organs, is less likely applicable in CNS, due to its extreme invasiveness. Indeed, in clinical practice the feasibility of a cerebral, spinal or meningeal biopsy is hardly ever evaluated and it may be taken into account in cases with very complex differential diagnosis or severe presentations, threatening patient's life or autonomies and however after careful evaluation of the procedure's risks. The present cohort is also an example of this criticism: the unique patient with a *definite* diagnosis (n. 11) underwent CNS biopsy only because his spinal lesion was suspected to be

lymphomatous or neoplastic, and not with the intention to rule out neurosarcoidosis.

An improvement regarding this criticism was achieved with 2014 WASOG criteria *(Judson, 2014)*. These criteria, although renaming the highest level of diagnostic accuracy from *definite* to *highly probable*, allowed the attribution of this definition also to patients who did not undergo CNS biopsy but fulfilled two other criteria: a clinical syndrome consistent with granulomatous inflammation of some part of the nervous system together with the demonstration of CNS inflammation, intended as CSF abnormalities or gadolinium enhancement on MRI.

By contrast, these criteria require positive pathological findings from at least one more organ as a precondition, not allowing any surrogate biomarker. This requirement limits their applicability, excluding all patients diagnosed with sarcoidosis due to a highly specific clinical presentation associated to other suggestive laboratory and radiologic findings, but who did not undergo any organ biopsy, as often happens in clinical practice and also as stated in the most recent consensus papers *(Crouser, 2020; Thillai, 2021)*. Indeed, in our cohort these criteria were applicable only in 17 of 22 patients.

Moreover, few clinical presentations included in WASOG criteria require a more critical evaluation. For example, isolated facial nerve palsy with negative MRI in a sarcoidosis patient may represent a simple casual association between systemic sarcoidosis and Bell's idiopathic palsy and not necessarily a *probable* neurosarcoidosis. Equally, isolated seizures or cognitive decline, both with normal or irrelevant MRI, should not be satisfactory to define a *possible* neurosarcoidosis, due to the high incidence of these common neurological manifestations also in general population.

NCCG's 2018 criteria *(Stern, 2018)* share some limitations with both previous sets. Mainly, they are strictly dependent on pathologic confirmation, both for a *definite* neurosarcoidosis diagnosis, which requires CNS biopsy as in Zajicek's criteria, and for systemic sarcoidosis confirmation, which requires pathologic confirmation of systemic granulomatous disease to define *probable* neurosarcoidosis and does not consider any surrogate biomarker, as WASOG's criteria do.

On the other hand, NCCG's criteria expand the spectrum of applicability, since they include EMG/NCS findings together with CSF and MRI findings to define neurological manifestations, thus allowing to consider also PNS and skeletal muscle involvement. Moreover, in Stern and colleagues paper, the authors underline two essential concepts to keep in mind when diagnosing neurosarcoidosis: first, the need to rigorously exclude other possible causes, which are extensively mentioned in supplementary material

together with proper diagnostic tests; second, the fact that even pathological evidence of granulomatous inflammation cannot be considered as a 100% certainty of the diagnosis, recommending that each patient included in any of the diagnostic categories should be constantly re-evaluated on the basis of clinical course and responsiveness to treatment.
However, some critical diagnostic issues remain unsolved by all proposed criteria. Above all, *isolated neurosarcoidosis* demonstration represents the main challenge, due to the absence of any extra-neurologic finding which makes the application of all criteria virtually impossible, unless a CNS biopsy is performed, with all the already mentioned issues that such procedure implies. The two patients with isolated neurosarcoidosis from our cohort are illustrative of this issue: one patient (n. 11) reaches the highest degree of diagnostic accuracy in all sets of criteria only because spinal cord biopsy was performed upon suspicion of malignancy, while the second patient (n. 20), who did not undergo CNS biopsy due to only mild symptoms, cannot be recognized with WASOG's criteria and reaches a *possible* diagnosis with the other two sets.
An indicator which could be useful for the diagnosis of neurosarcoidosis, although currently not considered by all proposed criteria, is response to treatment. Sarcoid lesions, both in CNS and other organs, present an excellent response to corticosteroid treatment, provided that the dosage is adequate. The features of this response are typical, with rapid and sustained therapeutic response which is both clinically and radiologically detectable. MRI findings, indeed, often show not only the disappearance of gadolinium enhancement, but also the reduction or disappearance of all radiologic abnormalities after appropriate corticosteroid treatment.

Some considerations should also be made regarding few possible differential diagnoses of neurosarcoidosis, which represent the most challenging questions for the clinicians.
CNS localizations of lymphomas are one of the most complex diseases to differentiate from neurosarcoidosis. These two conditions may share the same clinical, MRI and CSF findings (unless atypical cells are found in CSF, which however happens only in a minority of patients with CNS lymphoma). In this regard, it should also be considered that ACE, LSZ and sIL-2R have also been found in the CSF of cerebral lymphomas (*Oksanen, 1985; Oberg, 1987; Otto, 2020)* and that even whole-body imaging studies can be useless for differential diagnosis. In fact, CT images of multiple lymphadenopathies and/or pulmonary infiltrates, or hypermetabolic lesions on $^{18}$F-FDG PET/CT scan are both still consistent with either disease. Even therapeutic response to

corticosteroid treatment can be partly misleading because lymphomas often present an initial clinical and radiologic response to corticosteroids, although always showing early relapses after the improvement and a progressive loss of efficacy over time. Two patients from the present cohort were strongly suspected to have lymphomas: patient n. 9, who presented pulmonary infiltrates and multiple lymphadenopathies in the neck, chest and abdomen in addition to CNS lesions, and patient n. 11, who had an isolated spinal cord lesion which decreased in volume after corticosteroid treatment end relapsed after corticosteroid withdrawal. Both patients were finally diagnosed though lesion biopsy.

Multiple sclerosis is another complex differential diagnosis, mostly in isolated neurosarcoidosis involving brain and spinal cord parenchyma, which can mimic clinical presentation, neuroimaging and course of this demyelinating disease *(MacLean, 2015)*. However, as already mentioned, few clinical features and MRI findings can guide the diagnosis: the coexistence of parenchymal lesions with meningeal involvement strongly suggest neurosarcoidosis, while their association with the involvement of retrobulbar optic tract leads towards multiple sclerosis. CSF analysis can also be unremarkable, because typical multiple sclerosis abnormalities (i.e., increased IgG-index and presence of oligoclonal bands) can sometimes be seen also in neurosarcoidosis. On the other hand, CSF pleocytosis, absence of oligoclonal bands or their presence both in CSF and serum suggest neurosarcoidosis rather than multiple sclerosis. It should also be reminded that, although extremely rare, an association of the two diseases is possible, not only in patients with multiple sclerosis and extra-neurologic sarcoidosis, but also in exceptional cases presenting both neurosarcoidosis and multiple sclerosis, as seen in patient n. 14 from our cohort.

Meningeal presentations, especially when confined to pachymeninges, may be difficult to distinguish from chronic idiopathic pachymeninitis and mostly from *IgG4-RD*. This immune-mediated disease should always be considered in differential diagnosis, especially due to its possible multi-system presentation, which can involve different organs including lungs and lymph nodes and can consequently mimic multi-organ sarcoidosis *(Wallace, 2020; Bateman, 2015)*.

By contrast, the detection of small-sized T2-hyperintense NEWM lesions as an isolated finding in a sarcoidosis patient under evaluation for the suspect of neurosarcoidosis, especially when pauci-symptomatic, should not raise excessive concern. NEWM lesions are indeed described in sarcoidosis patients *(Bathla, 2020)*, but they are usually

asymptomatic, lacking signs of a clear inflammatory pathogenesis and not improving after therapy *(Bathla, 2016)*. On the other hand, signs of vascular involvement in brain MRI, such as perivascular enhancement, dilatation or tortuosity of lenticulostriate perforators and/or medullary veins, microhemorrhages or chronic infarcts should raise the suspect of neurosarcoidosis *(Bathla, 2021)*.

In conclusion, it is possible to say that CNS manifestations of sarcoidosis are well known and extensively detailed, allowing a relatively feasible diagnosis in patients with already known pulmonary or systemic sarcoidosis. The real challenge, however, remains the diagnosis of patients who present neurologic symptoms at the onset of the disease or as isolated localization. Our cohort demonstrates that these two conditions may together occur in more than half of neurosarcoidosis patients.
Neurosarcoidosis should be taken into account in each patient presenting acute-subacute and complex neurological symptoms, together with inflammatory signs on radiologic exams and CSF analysis, without specific findings leading to a determined neurological disease.
First line of investigations, when suspecting neurosarcoidosis, should include serum sarcoidosis biomarkers testing, chest HRCT scan, and analysis of lymphocytes subpopulation in serum and BAL. Systemic sarcoidosis should also be investigated with a whole-body $^{18}$F-FDG PET/CT scan, also for the purpose of revealing extra-neurologic lesions eligible for biopsy. Anyway, isolated presentations of neurosarcoidosis may still be missed by this approach. Hereby, besides the evaluation of the response to treatment, attention should be paid to the potential diagnostic role of putative CSF biomarker, which will be further addressed in the next chapter together with the first investigation in neurosarcoidosis patients of two more recent sarcoidosis biomarkers, namely CTO and KL-6.

Currently, there are no defined guidelines for neurosarcoidosis therapy, and although the treatment is based on the same groups of drugs used in systemic sarcoidosis, available evidence shows that a more aggressive approach, with higher dosages and longer treatments is required in these patients *(Ungprasert, 2017)*. In the present cohort, nearly 60% of patients have been treated at first with intravenous corticosteroids due to their severe presentation, and oral corticosteroid treatment was carried on for more than a year in the majority of them. Moreover, nearly 60% of patients required a second-line

immunosuppressive therapy, due to inadequate response to corticosteroids, relapses during corticosteroid reduction or unacceptable corticosteroids side effects. As reported in the literature *(Fritz, 2016)*, the most used second-line agent was MTX, which was effective in all but one treated patients. Patient n. 22, in fact, had a severe and refractory cerebrovascular presentation with recurrent ischemic episodes, which finally responded to RTX treatment. Similarly, patient n. 20, after complete clinical recovery and MRI findings improvement during oral corticosteroid treatment, experienced a clinical and radiological relapse during dose-reduction, and therefore we decided to use RTX as a second-line therapy. None of the patients from the current cohort presented relevant side effects due to MTX, confirming its positive safety profile *(Ungprasert, 2017)*. On the other hand, two out of three patients treated with AZA as second-line therapy reported gastrointestinal side effects, and therefore switched to MTX. Due to the complexity of the management of such patients, cooperation between neurologist and pneumologist is essential also during follow-up, in order to prevent both systemic and neurologic relapses.

Limitations of the present study include the retrospective nature of the sampling, the monocentric design of the study and the relatively small size of the population due to the rarity of the disease.

# CHAPTER 5: SERUM AND CSF BIOMARKERS IN CNS NEUROSARCOIDOSIS

## 5.1 Introduction

As already anticipated in previous chapters, the importance of serum and CSF biomarkers in the diagnosis of sarcoidosis and neurosarcoidosis is debated.

Established serum biomarkers of sarcoidosis, such as ACE, LSZ and sIL-2R, demonstrated a wide variability in sensitivity and specificity across different studies and, most of all, have been found increased in several other pathological conditions. On the other hand, more recently studied serum biomarkers, namely CTO and KL-6, seem promising, the former showing higher sensitivity and specificity in differentiating sarcoidosis from other diseases compared to other established biomarkers, the latter showing a close correlation with radiological and laboratory markers of pulmonary involvement (see chapter 1.4 for a detailed review of available literature).

Regarding CSF findings in neurosarcoidosis, apart from routine analysis demonstrating inflammatory alterations *(Pawate, 2009; Leonhard, 2016)*, specific sarcoidosis biomarkers have been investigated in several studies, with contradictory results.

CSF ACE was the most studied. Two large retrospective studies found low sensitivity (24-55%) but high specificity (94-95%), with remarkably elevated CSF ACE concentrations mostly in patients with widespread parenchymal and leptomeningeal involvement *(Dale, 1999; Tahmoush, 2002)*. Another study showed elevated CSF ACE concentrations in 20 out of 24 patients, with poor correlation between serum and CSF values, suggesting that CSF ACE may be intrathecally synthesized and not passively transferred from the serum *(Pawate, 2009)*. On the other hand, more recent results have been discouraging: Bridel et al. only recorded sensitivity and specificity around 65% for CSF ACE concentrations ≥ 3 U/l *(Bridel, 2015)*, while none of another cohort of 27 neurosarcoidosis patients tested positive for CSF ACE *(Arun, 2020)*. Moreover, increased concentrations of CSF ACE have also been reported in other conditions, including multiple sclerosis, Alzheimer disease, schizophrenia and CNS malignancies *(Kawajiri, 2009; Miners, 2009; Wahlbeck, 2000; Oksanen, 1985)*.

A few studies investigated sIL-2R in CSF, suggesting that elevated concentrations of this biomarker can effectively distinguish neurosaroidosis patients from other

neurologic inflammatory conditions such as multiple sclerosis, CNS vasculitis and Guillain-Barré syndrome *(Petereit, 2010; Otto, 2020)*. The authors themselves, however, underline that equally elevated levels were also found in other conditions, such as viral/bacterial meningitis, CNS lymphoma and neurotuberculosis, limiting the differential diagnostic potential of this biomarker. Another study showed elevated sIL2-R CSF concentrations in 3 out of 6 patients with clinically isolated neurosarcoidosis *(Wegener, 2015)*.

CSF lymphocyte subpopulations have been studied, with particular interest towards CD4/CD8 ratio. An increase of CSF CD4/CD8 ratio has been occasionally reported, in line with other organ-specific sarcoidosis involvement and also in comparison to multiple sclerosis and other neurological disorders *(Stern, 1987; Chazal, 2019; Nordstrom, 2020)*

A CD4/CD8 ratio >5 has even been proposed as an addition to Zajicek's criteria *(Marangoni, 2006)*. However, the low CSF cell count often found in neurosarcoidosis patients can significantly limit the reliability of this value.

A recent study investigated putative CSF biomarkers of neurosarcoidosis by proteomic analysis, finding an increase of low molecular weight kininogen, vitamin D-binding protein and fibrinogen-beta chain and a reduction of transthyretin compared to healthy subjects *(Taibi, 2017)*. In another study, serum and CSF neurofilament light chain levels were measured, comparing neurosarcoidosis patients with extra-neurologic patients and healthy controls. Significantly higher levels were found in neurosarcoidosis patients, which correlated with the extent of inflammation on MRI *(Byg, 2020)*.

Older studies also reported elevated CSF levels of lysozyme and β2-microglobulin, but available evidence are inadequate to evaluate their sensitivity and specificity *(Oksanen, 1986a)*.

To our knowledge, CTO and KL-6 were instead never systematically investigated in neurosarcoidosis patient.

Therefore, we decided to analyze several potential sarcoidosis biomarkers, namely ACE, CTO and KL-6, in serum and CSF samples from our cohort of CNS neurosarcoidosis patients.

## 5.2 Materials and methods

From the cohort of CNS neurosarcoidosis (NS) patients described in chapter 4, we retrospectively enrolled patients for whom samples of serum and CSF had been simultaneously collected and stored at -20°C and were available for further analysis. Moreover, we reviewed medical records of patients followed by *Neurology and Clinical Neurophysiology Unit* of Siena University Hospital, to enroll analogous CSF and serum samples from two control groups. We decided to select as control groups Multiple Sclerosis (MS) patients, as an example of an inflammatory CNS disease and a potential differential diagnosis of neurosarcoidosis (see chapter 2), and Amyotrophic Lateral Sclerosis (ALS) patients, as an example of a neurodegenerative disease. Unfortunately, serum from ALS patients was not available. Serum samples from healthy controls were also included as additional control group. The four groups were matched for sex and age. All CSF samples were collected during routine lumbar puncture performed for diagnostic purposes, before treatment with steroids, other immunosuppressants or immunomodulators. All patients were carefully evaluated to exclude comorbidities that could significantly affect biomarker detection. Demographic and clinical data, including comorbidities, family history, lung function parameters, radiological features and routine CSF analysis were obtained from medical records and entered into an electronic database for statistical analysis. All patients gave their written informed consent to participate in the study and for the use of their data and biological material, which was approved by our local ethics committee (CEAVSE 18712; Markerlung 17431).

- ACE activity was measured using a colorimetric method (FAR srl, Verona, Italy), for determination of ACE activity in serum and CSF. The normal range of serum ACE concentrations was 30-80 IU/l.
- CTO activity was determined by a fluorimetric method using 22 μM 4-methylumbelliferyl β-D-N,N′,N″-triacetylchitotriosidase (Sigma Chemical Co.) in citrate-phosphate buffer, pH 5.2; 100 μl substrate was incubated for 1 h at 37°C and the reaction was stopped with 1.4 ml 0.1 M glycine-NaOH buffer, pH 10.8. Fluorescence was read at 450 nm with a PerkinElmer Victor X4 fluorimeter (excitation wavelength 365 nm). Serum and CSF activity of CTO was expressed in nmol/ml/h.
- Serum and CSF samples from all patients were assayed for KL-6 concentrations by KL-6 reagent assay (Fujirebio Europe, Gand, Belgium). The principle of the assay is

agglutination of sialylated carbohydrate antigen in samples with KL-6 mAb by antigen–antibody reaction. The change in absorbance was measured to determine KL-6 levels. Serum concentrations of KL-6 were expressed in U/ml.

Data are presented as mean ± standard deviation and median and interquartile range (IQR). The Chi-squared test was used for categorical variables, as appropriate. Non-parametric one-way analysis of variance (Kruskal-Wallis test) and Dunn test were performed for multiple comparisons. Between-group comparisons were performed through non-parametric Mann-Whitney test. The Spearman test was used to look for correlations. A p-value less than 0.05 was considered statistically significant. Statistical analysis was performed by GraphPad Prism 9.3 software.

**Table 8. Demographic, clinical, functional and radiologic characteristics of NS patients**

| Pt | Sex/Age | Previous S | Chest HRCT (Scadding 0-IV) | DLCO (%) | Extra-thoracic localizations | CNS localization | NS diagnosis (NCCG, 2018) |
|---|---|---|---|---|---|---|---|
| **I (05 - MV)** | F/16 | No | III | **57** | – | Brain meninges | Probable |
| **II (06 – GM)** | F/46 | No | II | n.a. | LN, bones | Brain and spinal cord meninges and parenchyma, hypothalamus / pituitary gland | Probable |
| **III (11 - DA)** | M/61 | No | 0 | n.a. | – | Spinal cord parenchyma | Definite (type b) |
| **IV (13 - TG)** | M/53 | No | III | 95 | – | Brain and spinal cord parenchyma | Possible |
| **V (14 - CL)** | F/58 | No | II | **56,5** | LN | Brain and spinal meninges, brain parenchyma | Probable |
| **VI (17 - SM)** | F/51 | Yes | II | **65** | Orbit, kidney | Cauda equina roots | Probable |
| **VII (19 - SP)** | M/47 | Yes | II | n.a. | – | Spinal cord meninges | Probable |
| **VIII (20 - SD)** | M/31 | No | 0 | n.a. | – | Brain meninges and parenchyma | Possible |
| **IX (22 - MC)** | M/53 | Yes | IV | **52** | – | Cerebrovascular | Probable |

Abbreviations: Pt = patient; S = sarcoidosis; HRCT = high-resolution computed tomography; DLCO = diffusing lung capacity for carbon monoxide; CNS = central nervous system; NS = neurosarcoidosis; n.a. = not available; LN = lymph-nodes.
Reference values: DLCO: > 80%.
Abnormal values in bold.

**Table 9. Serum and CSF laboratory findings in NS patient**

| Pt | Serum ACE (IU/l) | Serum CTO (nmol/ml/h) | Serum KL-6 (U/ml) | CSF P (mg/dl) | CSF Alb (mg/dl) | CSF IgG (mg/dl) | CSF cells/mm$^3$ | Alb index | IgG index | CSF OCB | CSF ACE (IU/l) | CSF CTO (nmol/ml/h) | CSF KL-6 (U/ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **I (05 - MV)** | **112,2** | **64** | 348 | 20 | 8,6 | 1 | 4 | 0,002 | 0,71 | - | 13,07 | n.m. | n.m. |
| **II (06 – GM)** | **141,4** | **98** | 324 | **251** | **149** | **52** | 3 | **0,349** | **0,85** | **+** | 27,84 | 2,65 | 7 |
| **III (11 - DA)** | **156,6** | **109** | 408 | 43 | 31,3 | 3,7 | **27** | **0,0084** | 0,52 | - | 14,3 | n.m. | 2 |
| **IV (13 - TG)** | **102,9** | **133** | 311 | **67,9** | **49,2** | **5,8** | **16** | **0,011** | 0,55 | - | 12 | 0,69 | 2 |
| **V (14 - CL)** | 79,5 | **43,3** | 242 | **103,3** | **65,4** | **20,4** | **19** | **0,015** | **1,35** | **+** | n.m. | 0,5 | 2 |
| **VI (17 - SM)** | 76,9 | **260** | 382 | **257,3** | **228** | **43,4** | **81** | **0,056** | **0,96** | - | 3,2 | n.m. | 10 |
| **VII (19 - SP)** | n.m. | **96,5** | 229 | 25 | 18,1 | 1,9 | 2 | 0,0039 | 0,57 | - | 6,97 | n.m. | n.m. |
| **VIII (20 - SD)** | 66,9 | **60** | 350 | **102,9** | **89,2** | **17,1** | **65** | **0,018** | **0,84** | - | n.m. | n.m. | 5 |
| **IX (22 - MC)** | **112,8** | **101** | 322 | **519,9** | **335** | **148** | **118** | **0,081** | **1,72** | **+** | 6,5 | n.m. | 15 |

Abbreviations: Pt = patient; ACE = angiotensin-converting enzyme; CTO = chitotriosidase; KL-6 = Krebs von den Lungen-6; CSF = cerebrospinal fluid; P = total proteins; Alb = albumin; OCB = oligoclonal bands; n.m. = not measurable.
Reference values: serum ACE: 30-80 IU/l; serum CTO: 20,4-51 nmol/ml/h; serum KL-6: < 465 U/ml; CSF proteins: 20-45 mg/dl; CSF albumin: 10-32 mg/dl; CSF IgG: 0-4 mg/dl; CSF cells: < 10 cells/mm$^3$; albumin index: < 0,0063; IgG index: < 0,71.
Abnormal values in bold.

**Table 10. Comparison of CSF findings in NS, MS and ALS patients**

| | | NS (n=9, M/F 5/4) | MS (n=9, M/F 5/4) | ALS (n=9, M/F 5/4) | P value |
|---|---|---|---|---|---|
| **Total proteins (mg/dl)** | Mean ± sd | 154,5 ± 163,4 | 46,67 ± 21,60 | 40,66 ±7,68 | 0,1832 |
| | Median (IQR) | 102,9 (34-254,2) | 46 (30,5-54,5) | 43,2 (31,75-45,85) | |
| **Albumin (mg/dl)** | Mean ± sd | 108,2 ± 110,2 | 33,57 ± 18,64 | 24,76 ± 8,44 | 0,0747 |
| | Median (IQR) | 65,4 (24,7-188,5) | 28,70 (21,45-38,90) | 24,6 (16,95-29,9) | |
| **IgG (mg/dl)** | Mean ± sd | 32,59 ± 47,03 | 5,42 ± 2,64 | 2,55 ± 0,90 | **0,0200*** |
| | Median (IQR) | 17,1 (2,8-47,7) | 5,1 (3,15-7,75) | 2,3 (1,88-3,15) | |
| **Cells/mm$^3$** | Mean ± sd | 37,22 ± 41,25 | 3,33 ± 2,29 | 3,29 ± 2,43 | **0,0166*** |
| | Median (IQR) | 19 (3,5-73) | 3 (1,5-5) | 2 (2-5) | |
| **Albumin index** | Mean ± sd | 0,0256 ± 0,0269 | 0,0078 ± 0,0049 | 0,0055 ± 0,0015 | 0,0610 |
| | Median (IQR) | 0,0150 (0,0061-0,0454) | 0,0066 (0,0049-0,0085) | 0,0056 (0,0041-0,0064) | |
| **IgG index** | Mean ± sd | 0,89 ± 0,4 | 0,80 ± 0,58 | 0,54 ± 0,07 | 0,0629 |
| | Median (IQR) | 0,84 (0,56-1,15) | 0,62 (0,46-0,79) | 0,56 (0,46-0,60) | |
| **OCB** | +/- | 3/6 | 9/0 | 0/9 | |
| **ACE (IU/l)** | Mean ± sd | 11,98 ± 8,07 | 12,79 ± 11,34 | 23,24 ± 12,28 | 0,0819 |
| | Median (IQR) | 12 (6,5-14,3) | 9,65 (2,42-24,33) | 24,28 (13,45-28,51) | |
| **KL-6 (U/ml)** | Mean ± sd | 6,14 ± 4,95 | n.m. | n.m. | |
| | Median (IQR) | 5 (2-10) | | | |

Abbreviations: CSF = cerebrospinal fluid; NS = neurosarcoidosis; MS = multiple sclerosis; ALS = amyotrophic lateral sclerosis; OCB = oligoclonal bands; ACE = angiotensin-converting enzyme; KL-6 = Krebs von den Lungen-6; sd = standard deviation; IQR = interquartile range; n.m. = not measured.

*** p<0,05**

## 5.3 Results

### *5.3.1 Study population*

Nine patients for each group were enrolled according to inclusion criteria. NS patients (M/F 5/4) had a mean age of 46,2 years (range: 16-61), MS patients (M/F 5/4) had a mean age of 46,3 years (range: 18-65) and ALS patients (M/F 5/4) had a mean age of 53,1 years (range: 37-65).

*Table 8* shows demographic, clinical, radiological and functional data of NS patients. Three patients had a previous diagnosis of sarcoidosis, while neurosarcoidosis was the first manifestation of the disease in 6 patients. Two patients had negative chest HRCT and were diagnosed with *isolated neurosarcoidosis*. Diffusing lung capacity for carbon monoxide was reduced in all but one tested patients. CNS localization of granulomatous lesions was heterogeneous, including brain and/or spinal meninges in 5 patients, brain and/or spinal cord parenchyma in 5 patients and hypothalamus/pineal gland, spinal roots and brain arteries in one patient, respectively; 4 out of 9 patients had more than one concomitant CNS localization. No patient had clinical manifestations suggesting concomitant neuromuscular involvement. Neurosarcoidosis diagnosis, according to NCCG's criteria *(Stern, 2018)* was *definite (type b)* in 1 patient, *probable* in 6 patients and *possible* in 2 patients.

Detailed laboratory findings from serum and CSF analysis of NS patients are reported in *Table 9*. Comparison of CSF findings between NS, MS and ALS patients is reported in *Table 10*.

### *5.3.2 Routine CSF analysis*

CSF total proteins were increased in 6 NS patients (66,6%). CSF albumin was increased in 6 NS patients (66,6%). CSF IgG were increased in 6 NS patients (66,6%). CSF pleocytosis was found in 6 NS patients (66,6%). Albumin index was increased in 7 NS patients (77,8%). IgG index was increased in 5 NS patients (55,5%).

Significant differences between the three study groups emerged regarding CSF cells (p=0,0166) and CSF IgG (p=0,0200). CSF cell count was significantly higher in NS patients compared to both MS patients (p=0,0399) and ALS patients (p=0,0455) *(Figure 11)*. CSF IgG were significantly higher in NS patients compared to ALS patients (p=0,0226), but not to MS patients *(Figure 11)*.

Regarding CSF total proteins, CSF albumin, Albumin index and IgG index, no

significant differences were found among the three study groups.

Multiple oligoclonal bands were found in 3 NS patients (33,3%), while they were present in all MS patients and in none of the ALS patients.

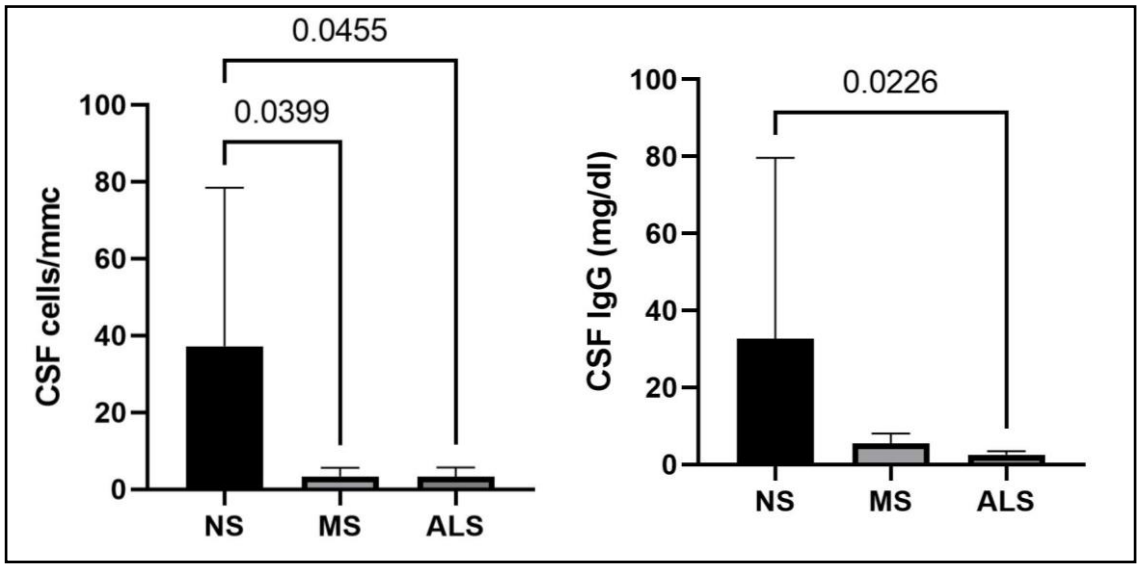



**Figure 11. Comparison of CSF cells and CSF IgG between NS, MS and ALS patients**

*5.3.3 Serum and CSF ACE*

Serum ACE was increased in 5 NS patients (55,5%, mean 106,2±31,59 IU/l, median (IQR) 107,6 (77,55-134,3)) and in 4 MS patients (44,4%, mean 66,70±29,25, median (IQR) 72,50 (40,65-84,30)). CSF ACE was measurable in 7 NS patients (77,8%). No correlation between serum and CSF ACE concentrations was found in NS patients (r=0,7714, p=0,1028).

Serum ACE concentrations were significantly higher in NS patients than in MS patients (p=0,0464) *(Figure 12)*. No significant differences in CSF ACE concentrations were found among the three study groups.

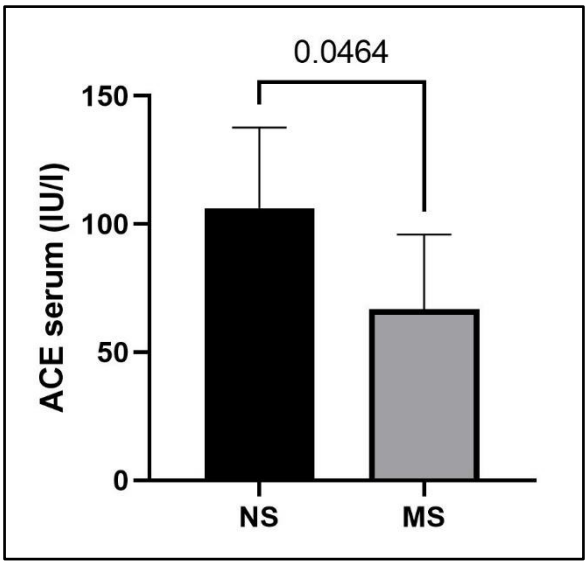



**Figure 12. Comparison of serum ACE between NS and MS patients**

*5.3.4 Serum and CSF CTO*

Serum CTO was increased in all NS patients (100%, mean 107,2±63,67 nmol/ml/h, median (IQR) 98 (62-121)) and in none of MS patients (mean 14,03±7,37, median (IQR) 13,5 (7-21,6)). Serum CTO activity was significantly higher in NS patients than in MS patients (p<0,0001) *(Figure 13)*.

CSF CTO was measurable only in 3 NS patients, with extremely low activity (respectively 0,5 nmol/ml/h, 0,7 nmol/ml/h and 2,7 nmol/ml/h). Moreover, it resulted measurable in one patient only from MS group (0.2 nmol/ml/h) and in no patients from ALS group.

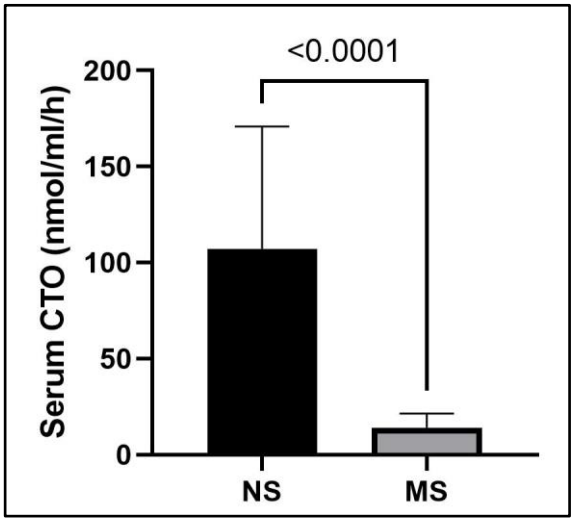



**Figure 13. Comparison of serum CTO between NS and MS patients**

*5.3.5 Serum and CSF KL-6*

Serum KL-6 concentrations did not exceed the proposed cut-off value of 465 U/ml in none of the NS patients (mean 324±58,73 U/ml, median (IQR) 324 (276,5-366)). Measurable CSF concentrations of KL-6 were detected in 7 NS patients (77,8%) but in none of MS or ALS patients. No correlation between serum and CSF KL-6 concentrations was found in NS patients (r=0,1853, p=0,7190). In NS patients, CSF KL-6 concentrations were directly correlated with CSF total proteins (r=0,8895, p=0,0143), CSF albumin (r=0,9636, p=0,0024), CSF IgG (r=0,8524, p=0,0286) and Albumin index (r=0,9636, p=0,0024) *(Figure 14)*. No significant differences in serum KL-6 concentrations were observed among NS patients, MS patients and healthy controls (p=0,7703).

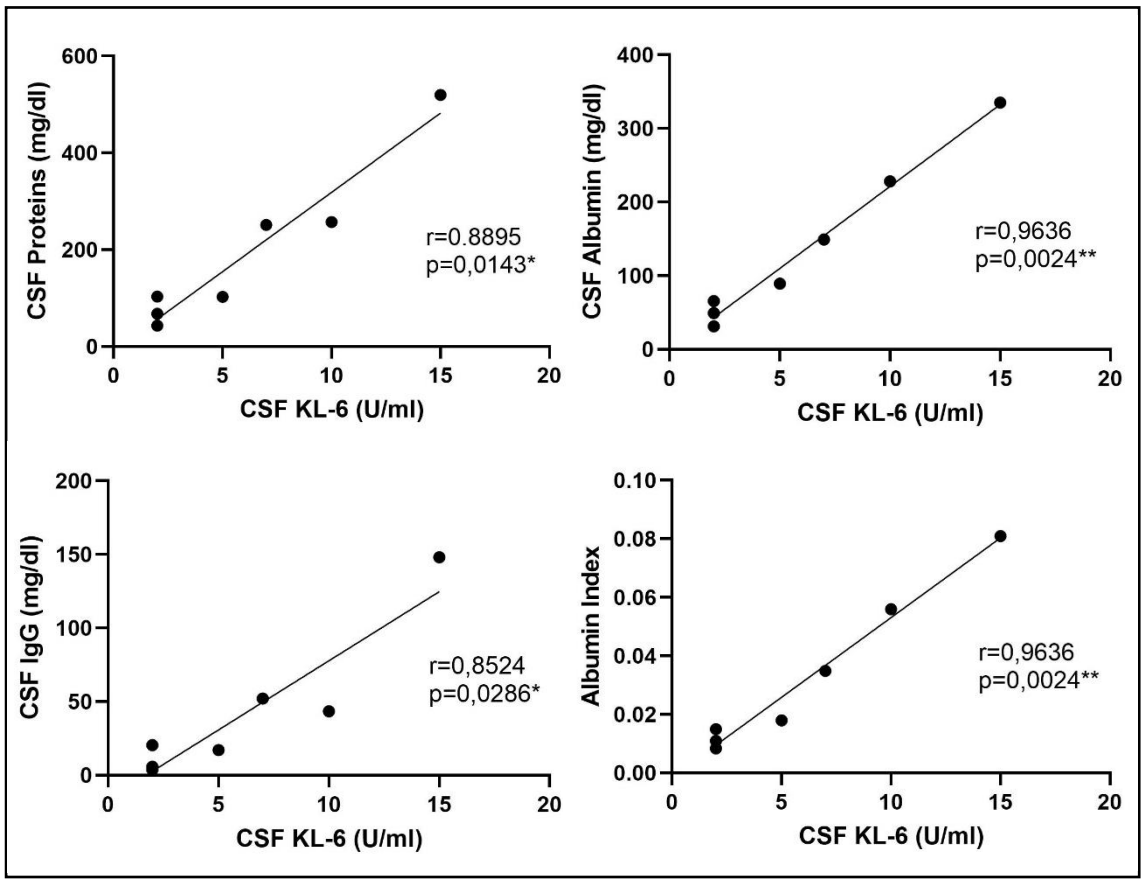



**Figure 14. Correlation between CSF KL-6 concentrations and CSF total proteins, CSF albumin, CSF IgG and Albumin index in NS patients**

* p<0,05

** p<0,01

## 5.4 Discussion

The present study compared serum and CSF findings among CNS neurosarcoidosis, multiple sclerosis and amyotrophic lateral sclerosis patients, also assessing sarcoidosis biomarkers such as ACE, CTO and KL-6. To our knowledge, both CTO and KL-6 were never systematically studied nor in serum or in CSF of neurosarcoidosis patients.

As already reported *(Pawate, 2009; Leonhard, 2016)*, routine CSF analysis showed inflammatory alterations in the majority of neurosarcoidosis patients, including elevated total proteins, albumin and IgG, CSF pleocytosis and increased Albumin index and IgG index. However, when compared to control groups, significative differences emerged only in CSF cell count and CSF IgG concentration. Moreover, while CSF pleocytosis was significantly higher in nerosarcoidosis patients when compared to both ALS and MS patients, the difference in CSF IgG concentrations was significant only between neurosarcoidosis and ALS patients and not when compared to MS patients. This

finding, alongside the demonstration of oligoclonal bands in one third of neurosarcoidosis patients, is in line with previous reports *(Arun, 2020)* and underlines the challenges in differentiating neurosarcoidosis from multiple sclerosis and the need to identify specific biomarkers to rely on for differential diagnosis.

Serum ACE, despite being increased in 55,5% of neurosarcoidosis patients and in 44,4% of multiple sclerosis patients, showed significantly higher concentrations in the first group. This finding confirms the need to consider as strongly suggestive of sarcoidosis only serum ACE concentrations twice greater than the upper normal limits, and to carefully interpret all results integrating them with other clinical and diagnostic elements *(Chopra, 2016)*.

In contrast to a recent study by Arun et al., which did not find measurable CSF ACE concentrations in none of 27 neurosarcoidosis patients *(Arun, 2020)*, in the current study ACE was measurable in CSF of 7 out of 9 neurosarcoidosis patients. However, CSF ACE was measured also in 8 out of 9 MS patients and in all 9 ALS patients and its concentrations did not differ among the three groups, confirming the poor specificity of this biomarker in CSF *(Bridel, 2015)*.

Serum CTO resulted increased in all neurosarcoidosis patients and in none of multiple sclerosis patients, showing a highly significant difference in its activity ($p<0,0001$). This finding is in line with previous studies on pulmonary and systemic sarcoidosis, which showed that CTO may have higher sensitivity and specificity in differentiating sarcoidosis from other diseases than other established biomarkers *(Bargagli, 2007)* and seems to correlate with disease severity and extrapulmonary involvement *(Bergantini, 2019; Bennet, 2020)*. Our finding of increased serum CTO activity also in patients with normal chest HRCT scan and isolated neurosarcoidosis (see patients n. III and n. VIII) and in patients with normal lung function tests (see patient n. IV) suggests that serum CTO could be a reliable biomarker also in neurologic presentations of sarcoidosis, regardless of the presence, extent and severity of pulmonary involvement.

Instead, CSF CTO was measurable only in 3 neurosarcoidosis patients, 1 MS patient and none of ALS patients; all measured activities were extremely low, ranging from 0,2 nmol/ml/h to 2,7 nmol/ml/h. Moreover, we observed an inadequate reproducibility of measures in test-retests, which led us to consider the possibility that the assay of enzymatic functional activity may be influenced by sample storage procedures. Therefore, we believe that further methodologic investigations are essential, and although these results seem to suggest a limited relevance of CSF CTO measurement,

the absence in the literature of similar studies demands caution in considering or excluding usefulness of this biomarker.

KL-6 is a mucin-like glycoprotein secreted by type II pneumocytes *(Kohno, 1989)*, which has been found increased in serum and BAL of patients with ILD and sarcoidosis *(D'Alessandro, 2020; Janssen, 2003)* and showed a close correlation with other radiological and laboratory markers of pulmonary involvement *(Bergantini, 2019; Miyoshi, 2010)*. In all our neurosarcoidosis patients, serum KL-6 concentrations resulted below the proposed cut-off of 465 U/ml *(Ohnishi, 2002)*. However, KL-6 was also found in CSF of 7 neurosarcoidosis patients and in none of MS and ALS patients. Notably, the highest concentration of CSF KL-6 was measured in the only patient who presented fibrotic pulmonary involvement and a Scadding *stage IV* at chest HRCT scan (patient n. IX). Measurable KL-6 concentrations were also detected in the two patients without any sign of pulmonary involvement and a Scadding *stage 0* at chest HRCT scan (patients n. III and n. VIII), suggesting the possibility of minimal lung parenchyma involvement even in patients with normal chest HRCT scan. On the other hand, the two patients without measurable CSF KL-6 concentrations (patients n. I and n. VII) were the only patients presenting with no other sign of blood-brain barrier damage in standard CSF analysis. Since KL-6 is a high molecular weight protein (200 kDa) produced by type II pneumocytes *(Kohno, 1989)*, under physiological conditions it is unlikely to cross the intact blood-brain barrier. However, when the barrier is damaged due to pathological conditions, as it occurs in neurosarcoidosis, KL-6 produced by even minimally affected lungs could cross the barrier together with other serum proteins as albumin (69 kDa) and IgG (108 kDa), as confirmed by the direct correlation found between CSF KL-6 concentrations and CSF total proteins, CSF albumin, CSF IgG and Albumin index. Our findings strongly suggest that this protein could be a specific CSF diagnostic biomarker of neurosarcoidosis, particularly useful in cases with little or no thoracic involvement and regardless its serum concentrations. Since no reliable biomarkers have yet been identified to support clinical and radiological diagnosis of neurosarcoidosis, our findings show that KL-6 assay in CSF is a simple, inexpensive and mini-invasive diagnostic tool in comparison to CSF biopsy, able to discriminate this specific phenotype of sarcoidosis with 81,2% sensitivity and 100% specificity.

Limitations of the present study include the retrospective nature of the sampling, the monocentric design of the study and the relatively small size of the study population due to the rarity of the disease. Moreover, a comparison with another control group

including patients with chronic inflammatory meningeal diseases (idiopathic or occurring in IgG4-RD or ANCA-related vasculitides) would provide more information regarding the specificity of our findings.

# CHAPTER 6: CLINICO-PATHOLOGICAL VARIABILITY AND TNFα/TNFR1-2 EXPRESSION IN SARCOIDOSIS-ASSOCIATED MYOPATHY

## 6.1 Introduction

In sarcoidosis patients, asymptomatic involvement of skeletal muscles is reported in 25% to 75% of cases, while symptomatic involvement occurs in only 0.5% to 5% *(Kobak, 2015)*. Symptomatic sarcoid myopathy has historically been classified in three distinct clinical patterns: acute myopathy, chronic myopathy, and nodular myopathy *(Silverstein, 1969; Zisman, 2002)*. *Acute myopathy* tends to occur early during the course of sarcoidosis and in younger patients (<40 years of age). Clinical presentation is similar to other inflammatory myopathies, with rapid onset of proximal weakness, myalgia and often fever, with elevated creatinine kinase levels. EMG may show nonspecific myopathic findings and muscle biopsy shows non-caseating granulomas with pronounced lymphocytic infiltration *(Kobak, 2015; Bechman, 2018)*. *Chronic myopathy* is the most common form, mainly reported in females between 50 and 60 years of age. It usually presents with insidious onset of symmetrical proximal muscle weakness, sometimes involving trunk and neck muscles, and only occasionally with predominant distal weakness. Muscle enzyme levels are often normal. EMG demonstrates nonspecific myopathic changes, and muscle MRI may reveal muscle atrophy with fatty degeneration *(Moore, 2005; Bechman, 2018)*. Noncaseating granulomas infiltrating perimysial connective tissue, which cause muscle atrophy and fibrosis, are the main pathologic findings *(Kobak, 2015; Maeshima, 2015)*. *Nodular sarcoid myopathy* is the rarest presentation and is usually characterized by a symmetrical limb involvement and single or multiple nodules, palpable and often painful consisting of granuloma agglomerates. Serum levels of muscle enzymes and neurophysiological studies are usually normal. Pathological analysis demonstrates nodular lesions between muscle bundles without direct involvement of muscle fibers *(Bechman, 2018)*. MRI examinations can be useful for localizing this pattern of myopathy, showing round, ovoid or fusiform nodules extending alongside the muscle fibers. They usually present hypointensity in the center, due to persistent inflammation within the central portion of the granuloma, and a peripheral area which shows a mild

hyperintensity on T1-weighted images, due to its high cellular content, and significant hyperintensity on T2-weighted images due to peripheral oedema *(Otake, 1994; Prieto-González, 2014)*.

However, myalgia and fatigue are some of the most frequently reported symptoms by sarcoidosis patients *(Hinz, 2011)* and the wide clinical variability presented by patients with sarcoid myopathy is often difficult to classify inside the three historically proposed categories. A recent multicenter retrospective study, for instance, was unable to classify 18 of 48 patients according to the historical classification, and therefore proposed the identification of four alternative patterns: a *myopathic* pattern, in the presence of motor deficits; a *nodular* pattern, in the presence of nodular lesions without motor deficit; a *smoldering* pattern, in the absence of both nodular lesions and motor deficit; and a *combined myopathic and neurogenic* pattern, in the presence of neurogenic abnormalities on electrophysiological studies in addition to muscular involvement *(Cohen Aubart, 2018)*.

The presence of such different clinical and pathological findings suggests the existence of a more complex pathophysiology of muscular involvement in sarcoidosis patients, not limited to granuloma-mediated muscular damage. The presence of an inflammatory process without granuloma formation suggests that muscle damage may be caused by direct cytolytic effect of lymphocytes or indirectly through cytokines production *(Authier, 1997; Kobak, 2015)*.

In this context, a potential role of pro-inflammatory cytokines may be hypothesized. As previously described in chapters 1 and 2, local and circulating cytokines (mainly TNFα and IFNγ) play indeed a crucial role in the Th1 inflammatory response of sarcoidosis and the increase of local and circulating TNFα is also considered a possible cause of the loss of epidermic nerve fibers in sarcoidosis-associated SFN *(Kidd, 2020)*.

In addition, the role of pro-inflammatory cytokines including TNFα has been previously studied in other idiopathic inflammatory myopathies (IIM). In muscular biopsies from IIM patients, TNFα is expressed by infiltrating macrophages and lymphocytes, by injured/regenerating muscle fibers, by endothelial cells from muscular vessels and freely dispersed in endomysial and perimysial connective tissue *(Tateyama, 1997; De Bleecker, 1999; Kuru, 2003)*. Moreover, TNFα receptors TNF-R1 and TNF-R2 are also expressed by regenerating muscle fibers and by endothelial cells in the midst of inflammatory infiltrates in IIM muscle specimens *(De Bleecker, 1999)*. Increased endothelial expression of both TNFα and TNFR1-2 seems to be prominent in

dermatomyositis (DM) with respect to polymyositis (PM) and inclusion body myositis (IBM) *(De Bleecker, 1999)*. The soluble forms of receptors are increased also in the serum of IIM patients *(Shimizu, 2000; De Paepe, 2012)*.

Regarding possible effects of this increased expression, TNFα has shown to promote the accumulation of neutrophils and macrophages in skeletal muscle *(Peterson, 2006)*, enhance Fas-mediated apoptosis of muscle cells *(Kondo, 2009)*, induce macroautophagy and expression of MHC-II class molecules on muscle fibers *(Keller, 2011)* and seems to have even a direct role in causing muscle weakness and fatigue by blunting the response of muscle myofilaments to calcium activation *(Reid, 2002)*.

The aims of our study are to analyze pathological presentations of sarcoid myopathy beyond classic granulomatous myopathies and to investigate the potential role of pro-inflammatory cytokines in this subset of patients, examining tissue expression of TNFα and its receptors.

## 6.2 Materials and methods

The present study was performed at the University Hospital of Siena, Italy and originated from the cooperation between *Neurology and Clinical Neurophysiology Unit* and the *Sarcoidosis Regional Referral Center*, managed by *Respiratory Diseases and Lung Transplantation Unit*.

First, we performed a retrospective analysis of a cohort of sarcoidosis patients who underwent muscular biopsies for diagnostic purposes due to the suspect of an associated myopathy, in the timeframe from January 1$^{st}$ 2011 to December 31$^{st}$ 2017.

Inclusion criteria for the execution of muscle biopsy were:

- Complaint of myalgia or muscle cramps or muscular weakness (the last confirmed by MRC scale);

Plus

- Increased serum creatine kinase (CK) 1,5 times above the upper normal values or myopathic findings on EMG.

Sarcoidosis diagnosis, confirmed by pneumologist evaluation and investigations, was performed either previously or during the subsequent follow up.

Pathological specimens and medical records were retrospectively reviewed and information regarding demographics, medical history, clinical presentation, laboratory

and radiological data and pathologic findings were collected for each study patient and were entered into an electronic database.

Successively, we prospectively enrolled further sarcoidosis patients, presenting in the timeframe from January 1st 2018 to December 31st 2020, fulfilling the aforementioned inclusion criteria and subjected to muscular biopsies for diagnostic purposes.

Each patient gave its written informed consent to participate in the study and for the use of data and biological material, which was approved by our local ethics committee (CEAVSE 18712; Markerlung 17431).

Open biopsies were performed on muscles selected according to clinical and neurophysiological findings. Tissue blocks for histology and immunohistochemistry were frozen in liquid nitrogen precooled isopentane and stored at -80°C until use. In all cases, the search for granulomas was carried out by serial cutting of all frozen specimens. Routine histological and histochemical stains were carried out on 10 μm cryostat sections. Immunolocalization of markers of inflammatory cells (CD4, CD8, CD3, CD68) and immune tissue markers (Major Histocompatibility Complex-I, MHC-I, HLA-ABC; Major Histocompatibility Complex-II, MHC-II, HLA-DR; Membrane attack complex, terminal complement complex, MAC) was carried out on 6 μm-thick cryostat sections mounted on Starfrost slides. After air-drying, slides were fixed in cold acetone and washed in PBS. After blocking in PBS-1%BSA for 30 minutes, slides were incubated with the primary antibodies in PBS-0.5%BSA overnight at 4°C. A list of primary antibodies and working dilutions is given in *Table 11*. Immunoreactivity was evidenced by either peroxidase-antiperoxidase technique, with DAB or AEC as a cromogen, or indirect immunofluorescence. For transmission electron microscopy (TEM), tissue blocks were routinely processed for Epon embedding; thin sections were observed and photographed on a Philips CM 10 microscope.

Pathologic specimens of prospectively enrolled patients were also submitted to indirect immunofluorescence localization for TNFα and TNFR1-2. As TNFα tissue expression has already been reported IIM, we selected as control specimens samples of healthy skeletal muscles.

Continuous variables were presented as mean and data range, and non-parametric Mann-Whitney test was used for between-group comparisons. Categorical variables were presented as frequencies and proportions and Fisher's exact test was used for

between-group comparisons. A p-value of less than 0.05 was considered statistically significant. Statistical analysis was performed by GraphPad Prism 9.3 software.

**Table 11. List of primary antibodies and working dilutions**

| Antigen | Species | Specificity | Type | Dilution | Art.#/company |
|---|---|---|---|---|---|
| HLA-A,B,C (W6/32 clone) | Mouse Monoclonal | Anti Major Histocompatibility Complex -I | Anti human | 1:100 | M0736 Dako |
| MAC (ae11 clone) | Mouse monoclonal | Anti-C5b-9 complex | Anti human | 1:200 | M0777 Dako |
| CD8 Clone C8/144B | Mouse monoclonal | Anti human cytotoxic CD8 T cells | Anti human | 1:100 | M7103 Dako |
| CD4 Clone 4B12 | Mouse monoclonal | Anti Human CD4 | Anti human | 1:100 | M7310 Dako |
| CD68 Clone PG-M1 | Mouse monoclonal | Anti human monocytes and macrophages | Anti human | 1:100 | GA613 Dako |
| TNF alpha Clone 52B83 | Mouse monoclonal | Anti human TNF | Anti human | 1:100 | sc-52746 Santa Cruz Biotech |
| TNF-R1 Clone H5 | Mouse monoclonal | Anti human TNF receptor 1 | Anti human | 1:100 | sc-8436 Santa Cruz Biotech |
| TNF-R2 Clone D2 | Mouse monoclonal | Anti human TNF receptor 3 | Anti human | 1:100 | *sc-8041* Santa Cruz Biotech |

**Table 12. Demographic, clinical, laboratory and neurophysiological data**

| Pt | Sex | Age at biopsy | Time S-biopsy | Chest HRCT (Scadding 0-IV) | Extra-thoracic localizations | Muscular symptoms | Onset | CK (U/l) | EMG/NCS |
|---|---|---|---|---|---|---|---|---|---|
| **01 - NG** | F | 72 yrs | 11 yrs | I | – | LL weakness (distal>proximal) | Chronic | Normal | Diffuse myopathic changes |
| **02 - DD** | M | 57 yrs | 20 yrs | I | CNS, LN | Myalgia | Chronic | Normal | Proximal myopathic changes |
| **03 - LA** | M | 61 yrs | 8 yrs | I | – | Myalgia, fatigue | Chronic | n.a. | Diffuse myopathic changes, axonal pnp |
| **04 - PI** | F | 33 yrs | 2 yrs | II | Bones, lacrimal glands, LN | Myalgia, fatigue, LL weakness (distal>proximal) | Acute | Normal | Distal LL myopathic changes |
| **05 - CG** | F | 58 yrs | 1 yrs | I | Skin, kidney | Myalgia | Chronic | **240 U/l** | Proximal myopathic changes, axonal pnp |
| **06 - WR** | M | 28 yrs | 0 yrs | II | LN | Myalgia, fatigue, LL weakness (proximal>distal) | Acute | **2059 U/l** | Distal myopathic changes, axonal pnp |
| **07 - VL** | F | 56 yrs | 3 yrs | I | Skin | Myalgia | Chronic | Normal | Distal myopathic changes |
| **08 - CG** | F | 57 yrs | 5 yrs | III | Skin | Myalgia, fatigue, cramps | Chronic | 131 U/l | Proximal myopathic changes |
| **09 - NM** | F | 40 yrs | 9 yrs | I | Skin | Myalgia, fatigue | Chronic | **308 U/l** | Proximal myopathic changes |
| **10 - PM** | F | 73 yrs | 3 yrs | I | Heart | Myalgia, fatigue | Chronic | Normal | LL myopathic changes |
| **11 - DR** | M | 62 yrs | 12 yrs | II | Skin | Myalgia | Chronic | Normal | Diffuse myopathic changes |
| **12 - MG** | F | 51 yrs | 0 yrs | 0 | Skin | Myalgia, fatigue, LL and UL weakness (proximal>distal) | Acute | **957 U/L** | Proximal myopathic changes |
| **13 - SL** | F | 66 yrs | 20 yrs | II | – | Myalgia, LL and UL weakness (proximal>distal) | Chronic | **526 U/l** | Proximal myopathic changes |

| Pt | Sex | Age at biopsy | Time S-biopsy | Chest HRCT (Scadding 0-IV) | Extra-thoracic localizations | Muscular symptoms | Onset | CK (U/l) | EMG/NCS |
|---|---|---|---|---|---|---|---|---|---|
| **14 - DG** | F | 65 yrs | 11 yrs | II | Skin, parotid gland, larynx, bones | Myalgia, fatigue, cramps, LL and UL weakness (proximal>distal) | Acute | **225 U/l** | Proximal UL myopathic changes |
| **15 - BG** | F | 53 yrs | 3 yrs | III | Skin | Myalgia, fatigue, cramps | Chronic | **230 U/l** | Proximal LL myopathic changes |
| **16 - OF** | F | 27 yrs | 5 yrs | I | Skin | Myalgia, fatigue, cramps | Chronic | **325 U/l** | Normal |
| **17 - DJ** | M | 27 yrs | 3 yrs | II | Skin, LN | Myalgia, fatigue, cramps | Acute | **551 U/l** | Normal |
| **18 - AF** | F | 43 yrs | 0 yrs | II | Skin, eye, spleen, liver | Myalgia, fatigue + LL neuropathic pain | Chronic | **593 U/l** | LL myopathic changes |
| **19 - ML** | F | 71 yrs | 0 yrs | II | LN | Myalgia, fatigue, LL weakness (distal>proximal) | Chronic | 139 U/l | Distal LL myopathic changes |
| **20 - TE** | F | 50 yrs | 4 yrs | III | Skin | Myalgia, fatigue | Chronic | 70 U/l | Diffuse myopathic changes |
| **†21 - CI** | F | 38 yrs | 12 yrs | II | Spleen | Myalgia, fatigue | Chronic | 75 U/l | Proximal LL myopathic changes |
| **†22 - PF** | F | 44 yrs | 2 yrs | III | Skin | Myalgia | Chronic | 103 U/l | Proximal LL myopathic changes |
| **†23 - FE** | F | 78 yrs | 3 yrs | II | LN | Myalgia, fatigue, LL and UL weakness (proximal>distal) | Chronic | 172 U/l | Diffuse myopathic changes |
| **†24 - LM** | F | 59 yrs | 0 yrs | II | LN | Myalgia, fatigue + LL neuropathic pain | Chronic | **420 U/l** | Normal |
| **†25 - NG** | F | 72 yrs | 36 yrs | II | Skin, eye | Myalgia, fatigue, cramps | Chronic | **370 U/l** | Normal |
| **†26 - CC** | F | 36 yrs | 7 yrs | II | Skin | Myalgia, fatigue, cramps | Chronic | 56 U/l | Proximal LL myopathic changes |
| **†27 - TE** | F | 72 yrs | 43 yrs | III | Skin, bones | Myalgia, fatigue | Chronic | 108 U/l | Proximal UL myopathic changes |

| Pt | Sex | Age at biopsy | Time S-biopsy | Chest HRCT (Scadding 0-IV) | Extra-thoracic localizations | Muscular symptoms | Onset | CK (U/l) | EMG/NCS |
|---|---|---|---|---|---|---|---|---|---|
| **†28 - TG** | M | 59 yrs | 26 yrs | I | Skin | Myalgia, fatigue, LL and UL weakness (diffuse) | Chronic | **375 U/l** | Axonal pnp |
| **†29 - CL** | M | 30 yrs | 10 yrs | I | LN | Myalgia | Chronic | Normal | Proximal LL myopathic changes |

Abbreviations: Pt = patient; Time S-biopsy = time from sarcoidosis diagnosis to muscle biopsy; HRCT = high-resolution computed tomography; CK = creatine kinase; EMG/NCS = Electromyography / Nerve conduction studies; yrs = years; CNS = central nervous system; LN = lymph nodes; LL = lower limbs; UL = upper limbs; n.a. = not available; pnp = polyneuropathy.
† = prospectively enrolled patients.
Reference values: CK: 10-140 U/l.
Abnormal values in bold.

**Table 13. Histopathological findings**

| Pt | Site of biopsy | Granul. | Myopathic changes | Cellular inflammation | | | Necrosis | Regener. changes | MHC-I/II (HLA-ABC/DR) | MAC (C5b9) | Neurogenic changes | Associated changes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **01 - NG** | VL | + | +++ | +++ endomysial, perimysial, perivascular | | | +++ | +++ | n.p. | n.p | ++ | Fibrosis, mitochondrial |
| **02 - DD** | VL | - | + | + perivascular | | | - | - | ++ | ++ | - | - |
| | | | | CD4 + | CD8 - | CD68 + | | | | | | |
| **03 - LA** | VL | - | +/- | + endomysial, perivascular | | | - | + | +++ | ++ | + | - |
| | | | | CD4 + | CD8 + | CD68 + | | | | | | |
| **04 - PI** | TA | - | ++ | + endomysial, ++ perivascular | | | + | ++ | ++ | +++ | - | - |
| | | | | CD4 ++ | CD8 + | CD68 ++ | | | | | | |
| **05 - CG** | VM | - | +++ | - | | | - | - | n.p | n.p | - | Type II atrophy |
| **06 - WR** | VL | - | +++ | ++ endomysial, perivascular | | | + | + | +++ | +++ | + | - |
| | | | | CD4 ++ | CD8 + | CD68 ++ | | | | | | |
| **07 - VL** | TA | - | +/- | + perivascular | | | - | + | ++ | ++ | ++ | - |
| | | | | CD4 + | CD8 - | CD68 + | | | | | | |
| **08 - CG** | VL | - | +++ | +++ perimysial, perivascular | | | ++ | ++ | +++ | ++ | - | Fibrosis |
| | | | | CD4 + | CD8 - | CD68 + | | | | | | |
| **09 - NM** | D | - | + | +++ perivascular, ++ endomysial, perimysial | | | + | + | ++ | ++ | - | Mitochondrial |
| | | | | CD4 + | CD8 - | CD68 + | | | | | | |
| **10 - PM** | TA | - | ++ | ++ endomysial, perivascular | | | + | ++ | +++ | +++ | - | Fibrosis |
| | | | | CD4 ++ | CD8 + | CD68 ++ | | | | | | |
| **11 - DR** | TA | - | + | + endomysial, perivascular | | | - | + | +++ | ++ | - | - |
| | | | | CD4 + | CD8 - | CD68 + | | | | | | |
| **12 - MG** | VL | - | + | ++ perivascular | | | - | - | ++ | ++ | - | - |
| | | | | CD4 + | CD8 + | CD68 + | | | | | | |

| Pt | Site of biopsy | Granul. | Myopathic changes | Cellular inflammation | | | Necrosis | Regener. changes | MHC-I/II (HLA-ABC/DR) | MAC (C5b9) | Neurogenic changes | Associated changes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **13 - SL** | VL | - | +++ | +/- perivascular | | | + | + | + | - | - | Type II atrophy |
| | | | | CD3 +/- | | CD68 +/- | | | | | | |
| **14 - DG** | BB | - | ++ | +++ endomysial, perimysial, perivascular | | | - | + | + | - | - | Type II atrophy |
| | | | | CD4 +++ | CD8 + | CD68 +++ | | | | | | |
| **15 - BG** | VL | - | ++ | + perimysial, perivascular | | | - | - | + | - | - | |
| | | | | CD4 ++ | CD8 + | CD68 ++ | | | | | | |
| **16 - OF** | VL | - | - | - | | | - | +/- | - | - | - | - |
| **17 - DJ** | VL | + | ++ | +++ endomysial, perimysial, perivascular | | | ++ | ++ | ++ | - | - | Mitochondrial |
| | | | | CD4 +++ | CD8 + | CD68 +++ | | | | | | |
| **18 - AF** | RF | - | + | - | | | - | - | - | - | - | - |
| **19 - ML** | TA | + | +++ | +++ endomysial, perimysial, perivascular | | | +++ | +++ | n.p | n.p | - | Fibrosis |
| | | | | CD3 +++ | | CD68 +++ | | | | | | |
| **20 - TE** | VL | - | + | - | | | - | - | - | - | - | Mitochondrial |
| **†21 - CI** | VL | - | + | - | | | - | - | + | - | - | - |
| **†22 - PF** | RF | - | + | - | | | - | - | ++ | - | - | Mitochondrial |
| **†23 - FE** | D | + | +++ | +++ endomysial, perimysial, perivascular | | | ++ | + | +++ | + | - | Mitochondrial |
| | | | | CD4 +++ | CD8 + | CD68 +++ | | | | | | |

| Pt | Site of biopsy | Granul. | Myopathic changes | Cellular inflammation | | | Necrosis | Regener. changes | MHC-I/II (HLA-ABC/DR) | MAC (C5b9) | Neurogenic changes | Associated changes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **†24 - LM** | VL | - | + | - | | | - | - | + | - | - | - |
| **†25 - NG** | BB | - | +++ | + endomysial, perivascular | | | + | + | ++ | - | - | Type II atrophy, mitochondrial |
| | | | | CD4 n.p. | CD8 n.p. | CD68 + | | | | | | |
| **†26 - CC** | VL | - | +/- | - | | | - | + | - | - | - | - |
| **†27 - TE** | BB | - | +++ | - | | | - | - | - | - | + | - |
| **†28 - TG** | BB | - | +++ | ++ endomysial, perimysial | | | + | + | ++ | - | - | - |
| | | | | CD3 ++ | | CD68 ++ | | | | | | |
| **†29 - CL** | VL | - | - | - | | | - | + | - | - | - | - |

Abbreviations: Pt = patient; Granul. = granulomas; Regener. changes = regenerative changes; MHC-I/II (HLA-ABC/DR) = major histocompatibility complex I and II (human leukocyte antigen -A, -B, -C and -DR); MAC (C5b-9) = membrane attack complex (C5b9 complement fraction); VL = vastus lateralis muscle; TA = tibialis anterior muscle; VM = vastus medialis muscle; D = deltoid muscle; BB = brachial biceps muscle; RF = rectus femoris muscle; CD4 = CD4+ T-lymphocytes; CD8 = CD8+ T-lymphocytes; CD68 = CD68+ macrophages; CD3 = CD3+ T-lymphocytes; n.p. = not performed .

† = prospectively enrolled patients.

Semiquantitative evaluation, from – to +++.

**Table 14. Clinico-pathological patterns of sarcoidosis-associated myopathy in our study cohort**

| | | All (n=29) | Cellular inflammation (n=18) | Minor or no inflammatory changes (n=11) | P value |
|---|---|---|---|---|---|
| **Age at biopsy (yrs)** Mean (range) | | 53 (27-78) | 56,4 (27-78) | 47,5 (27-72) | 0,1556 |
| **Sex** M/F | | 7/22 | 6/12 | 1/10 | 0,2021 |
| **Muscular biopsy** | At S onset | 5 (17,2%) | 3 (16,7%) | 2 (18,2%) | 0,6027 |
| | At follow up | 24 (82,8%) | 15 (83,3%) | 9 (81,8%) | |
| **Time S-biopsy (yrs)** Mean (range) | | 10,8 (1-43) | 10,3 (2-36) | 11,6 (1-43) | 0,9886 |
| **Chest HRCT** (Scadding 0-IV) | 0 | 1 (3,4%) | 1 (5,6%) | 0 | >0,9999 |
| | I | 10 (34,5%) | 7 (38,9%) | 3 (27,3%) | 0,6942 |
| | II | 13 (44,8%) | 8 (44,4%) | 5 (45,4%) | >0,9999 |
| | III | 5 (17,2%) | 2 (11,1%) | 3 (27,3%) | 0,3386 |
| **Extra-thoracic localizations** | Skin | 17 (58,6%) | 10 (55,6%) | 7 (63,6%) | 0,7167 |
| | LN | 8 (27,6%) | 6 (33,3%) | 2 (18,2%) | 0,6706 |
| | Other | 9 (31%) | 5 (27,8%) | 4 (36,4%) | 0,6942 |
| **Muscular symptoms** | Myalgia | 28 (96,6%) | 17 (94,4%) | 11 (100%) | >0,9999 |
| | Fatigue | 21 (72,4%) | 14 (77,8%) | 7 (63,6%) | 0,4327 |
| | Cramps | 7 (24,1%) | 5 (27,8%) | 2 (18,2%) | 0,6765 |
| | Weakness | 9 (31%) | 8 (44,4%) | 1 (9,1%) | 0,0959 |
| **Onset** | Acute | 5 (17,2%) | 5 (27,8%) | 0 | 0,1261 |
| | Chronic | 24 (82,8%) | 13 (72,2%) | 11 (100%) | |
| **CK (U/l)** > 1,5 UNV | | 13 (44,8%) | 8 (44,4%) | 5 (45,4%) | >0,9999 |
| **ENG/EMG** | Myopathic changes | 24 (82,8%) | 15 (83,3%) | 9 (81,8%) | >0,9999 |
| | Axonal pnp | 4 (13,8%) | 3 (16,7%) | 1 (9,1%) | >0,9999 |
| **Muscle biopsy** | Granulomas | 4 (13,8%) | 4 (22,2%) | 0 | 0,2678 |
| | Neurogenic changes | 5 (17,2%) | 4 (22,2%) | 1 (9,1%) | 0,6221 |
| | Mitocondrial changes | 7 (24,1%) | 5 (27,8%) | 2 (18,2%) | 0,6765 |
| | Fibrosis | 4 (13,8%) | 4 (22,2%) | 0 | 0,2678 |
| | Type II atrophy | 4 (13,8%) | 2 (11,1%) | 2 (18,2%) | 0,6221 |

Abbreviations: Pt = patient; yrs = years; S = sarcoidosis; Time S-biopsy = time from sarcoidosis diagnosis to muscle biopsy; HRCT = high-resolution computed tomography; LN = lymph nodes; CK = creatine kinase; UNV = upper normal values; pnp = polyneuropathy.

**Table 15. TNFα and TNFR1-2 expression in prospectively enrolled patients vs healthy controls**

| Pt | TNFα | | | | TNFR1-2 | | | |
|---|---|---|---|---|---|---|---|---|
| | Endothelial | Sarcolemmal | Cytoplasmic | Inflammatory infiltrates | Endothelial | Sarcolemmal | Cytoplasmic | Inflammatory infiltrates |
| †21 - CI | +++ | - | - | Absent | ++ | + | + | Absent |
| †22 - PF | +++ | ++ | + | Absent | +++ | + | + | Absent |
| †23 - FE | +++ | +++ | +++ | +++ | +++ | ++ | +++ | +++ |
| †24 - LM | +++ | ++ | ++ | Absent | +++ | + | ++ | Absent |
| †25 - NG | +++ | ++ | ++ | +++ | +++ | ++ | + | +++ |
| †26 - CC | +++ | + | + | Absent | +++ | + | + | Absent |
| †27 - TE | ++ | + | ++ | Absent | ++ | - | + | Absent |
| †28 - TG | +++ | + | + | +++ | +++ | ++ | + | +++ |
| †29 - CL | +++ | ++ | ++ | Absent | +++ | ++ | ++ | Absent |
| HC (n=7) | + | - | - | Absent | + | - | - | Absent |

Abbreviations: Pt = patient; HC = healthy controls.
† = prospectively enrolled patients.
Semiquantitative evaluation, from – to +++.

**Figure 15. Representative histological and immunohistological muscle changes in sarcoidosis associated myopathies**

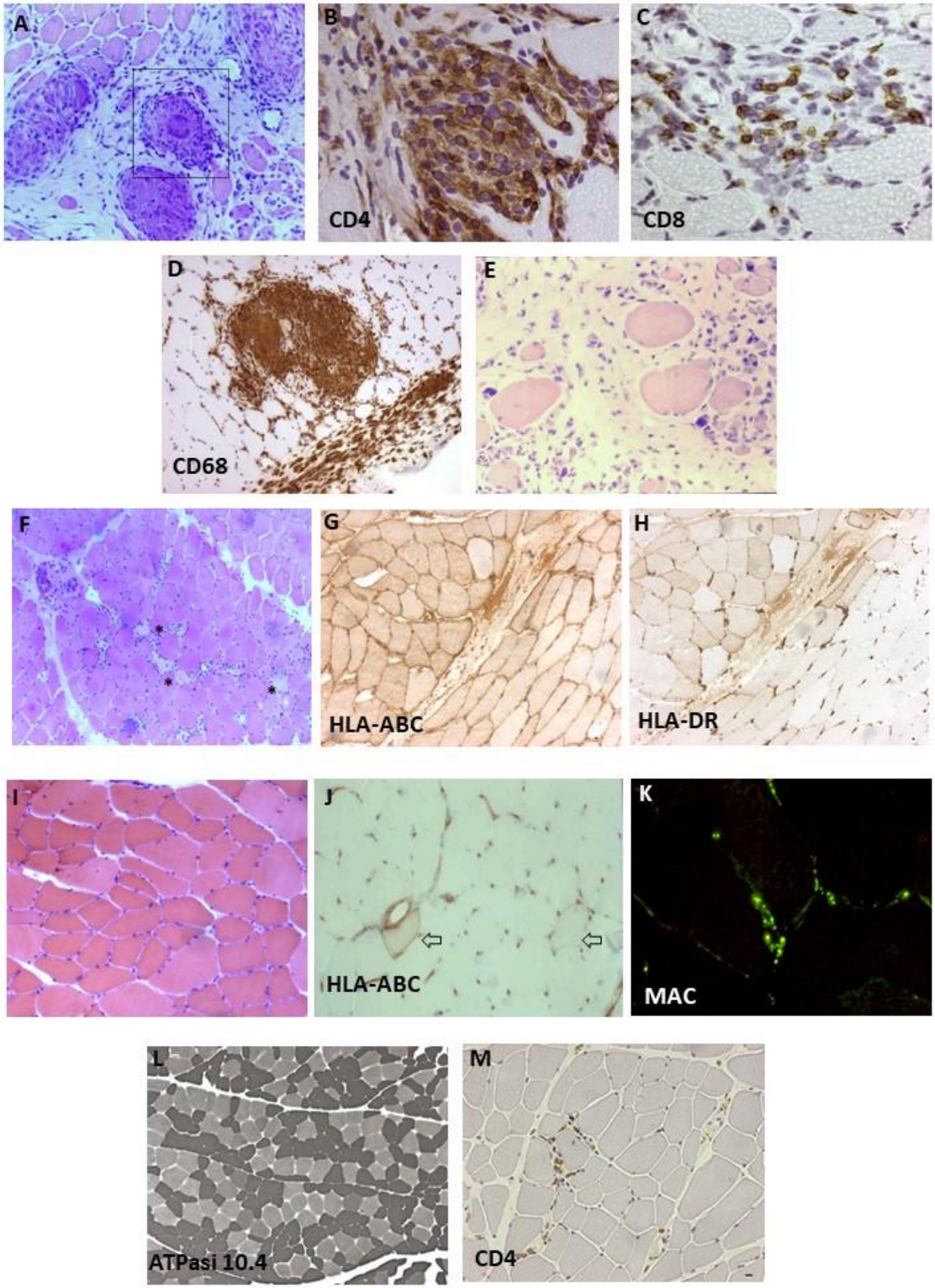


**Figure 15.** Scale bar: 10 μm. Bright field stains were carried out by immunoperoxidase technique, chromogen DAB. Indirect immunofluorescence for the terminal complement complex (MAC) was carried out by secondary FITC-conjugated antibody.
**A, B, C, D (pt. 17 - DJ):** granulomatous myopathy with subacute onset; granuloma (box in haematoxylin-eosin section) shows a predominance of CD4+ lymphocytes and CD68+ macrophages, with sparse CD8+ T cells.
**E (pt. 1 - NG):** granulomatous myopathy with very slow onset, wide fibroadipose replacement.
**F (pt. 06 - WR):** acute myositis with inflammatory infiltrates and prominent necrosis / regeneration (*). No granulomas were detected.
**G, H (pt. †28 - TG):** myositis with mild cellular inflammation; consecutive sections show a diffuse MHC-I (HLA-ABC) upregulation, where a smaller but consistent quote of fibers also displays MHC-II (HLA-DR) neolocalization.
**I, J, K (pt. †26 - CC):** muscle morphology within normal range; mild upregulation of MHC-I and localization of the terminal complement complex (MAC) on scattered muscle capillaries.
**L (pt. 14 - DG):** non granulomatous myositis with acute onset; reaction for myosin ATPase at pH 10.4 shows a reduction of the diameters of dark stained type 2 fibers.
**M (pt. 11 - DR):** non granulomatous myopathy with scarce cellular endomysial inflammation, composed of CD4+ T cells.

**Figure 16. Representative findings for TNFα, TNFR1-2 and in transmission electron microscopy**

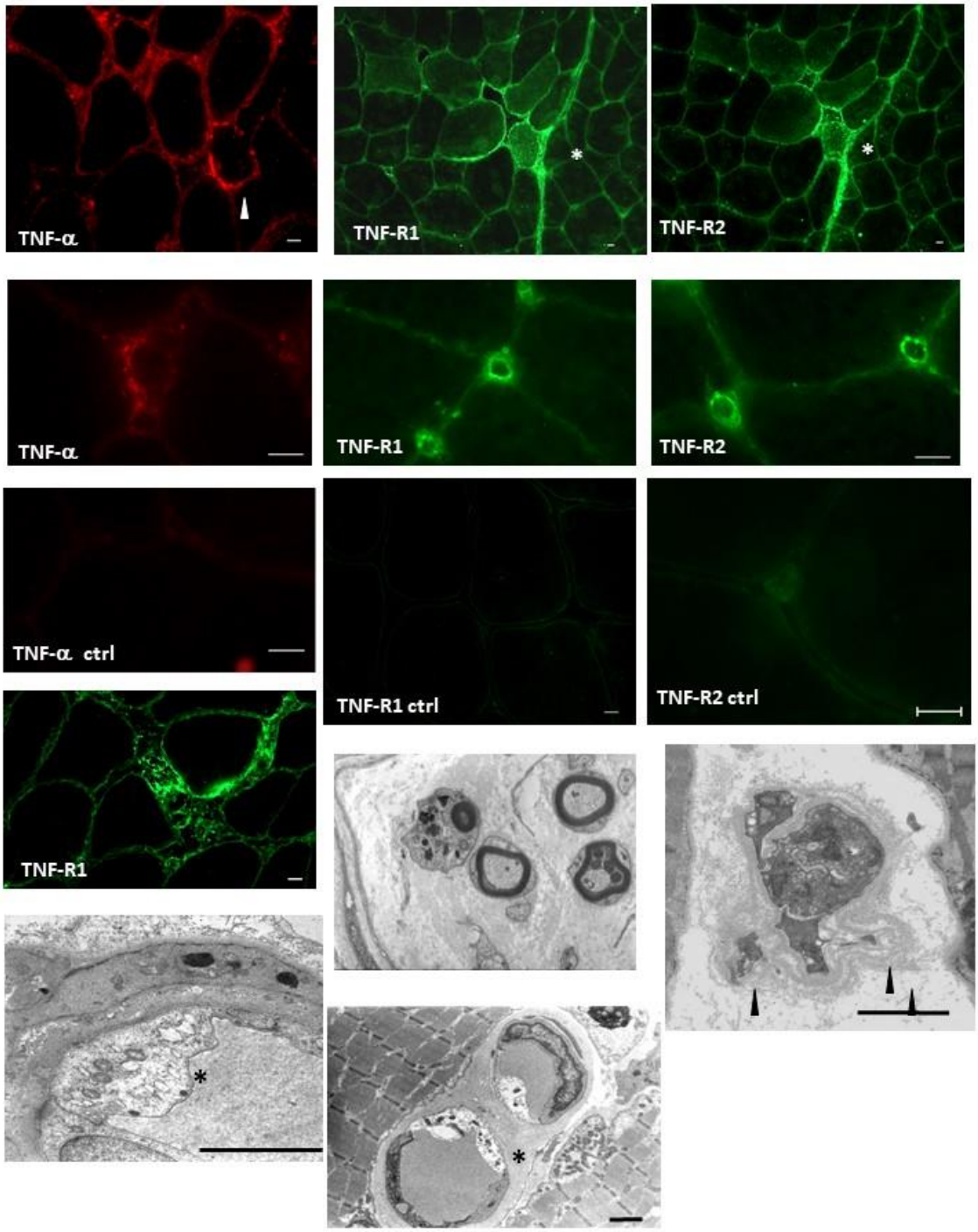


**Figure 16.** Scale bar: immunofluorescence 10 μm; TEM 2 μm.
**Indirect immunofluorescence** for TNF was performed by a secondary TRITC conjugated antibody; for the TNFR1-2 by a secondary FITC-conjugated antibody on consecutive sections.
In all cases inflammatory cells displayed a strong reactivity for TNFα and TNFR1-2, that were also detected diffusely on injured fibers, and resulted substantially coexpressed, as shown by consecutive sections (*). Tracts of sarcolemmal stain on apparently healthy fibers (arrowhead) were also observed. The walls of endomysial capillaries consistently expressed either TNFα and TNFR1-2 also in the cases with minor changes, as displayed in the table. In control cases, a very faint scattered reactivity was observed.
In **transmission electron microscopy**, injury of intramuscular nerve bundles was detected in a minority of cases, showing degeneration of small myelinated fibers. Moreover, TEM confirmed endothelial injury, with degeneration of the capillary wall and reduplications of the basal lamina (arrowhead), cell oedema, with lipid deposits and mitochondrial swelling (*).

## 6.3 Results

### *6.3.1 Study population*

Globally, twenty-nine patients met the inclusion criteria of the study. Twenty patients were retrospectively enrolled after reviewing medical records, and nine patients were prospectively enrolled during the study timeframe. Data regarding demographics and relevant clinical history of the study population are summarized in *Table 12*.

The study population included 7 males (24,1%) and 22 females (75,9%). Mean age at muscle biopsy was 53 years (range: 27-78). Muscular biopsy preceded sarcoidosis diagnosis in 5 patients while in the remaining 24 patients the mean interval between sarcoidosis diagnosis and muscle biopsy was 10,8 years (range: 1-43). Pulmonary disease staged from 0 to III according to Scadding staging system and 26 out of 29 patients (89,6%) presented at least one further extra-pulmonary localization of the disease. The most commonly involved organs were skin in 17 patients and extra-thoracic lymph nodes in 8 patients. One of the included patients (n. 02 - DD) had a concomitant CNS localization and was therefore included also in the CNS neurosarcoidosis cohort described in chapter 4 (patient n. 01 – DD). Myalgia was the most common muscular symptom, present in 28 out of 29 patients. Other reported symptoms were fatigue, reported by 21 patients, and cramps, reported by 7 patients. Muscular weakness was found in 9 patients and was predominantly proximal in 5 patients, diffuse in 1 patient and predominantly distal in 3 patients, of whom two (patients n. 01 and n. 19) with granulomatous myopathy, one (patient n. 01) with nodular pattern. Two patients (n. 18 and n. 24) reported concomitant neuropathic pain, predominant in lower limbs. Onset of symptoms was chronic in 24 patients and acute in 5 patients. EMG showed myopathic changes in 24 out of 29 patients. Nerve conduction abnormalities suggestive of large fiber axonal sensorimotor polyneuropathy were recorded in 4 patients, one without associated myopathic changes. CK values were 1,5 times above the upper normal value in 13 patients. All prospectively enrolled patients were tested for myositis-specific and myositis-associated antibodies (MSA and MAA: Anti-Mi2α, Anti-Mi2β, Anti-TIF1γ, Anti-MDA5, Anti-NXP2, Anti-SAE1, Anti-Ku, Anti-PM/Scl100, Anti-PM/Scl75, Anti-Jo1, Anti-SRP, Anti-PL7, Anti-PL12, Anti-EJ, Anti-OJ, Anti-HMGCR), which tested negative in all cases.

Muscular biopsy was performed in different muscles according to clinical and neurophysiological data; examined muscles were vastus lateralis (15 patients), tibialis

anterior (5 patients), brachial biceps (4 patients), rectus femoris (2 patients), deltoid (2 patients) and vastus medialis (1 patient). Data regarding histopathological findings in the study population are summarized in *Table 13*. Representative histological and immunohistological muscle changes detected are presented in *Figure 15*.

Despite extensive sectioning of standard-sized bioptic samples, granulomas were detected only in 4 out of 29 patients. General myopathic changes, such as increased variability of fiber diameter and nuclear internalization were detected in 27 out of 29 patients (93,1%). Associated neurogenic changes (namely, grouped atrophy and histotype grouping) were also detected in 5 patients. The most common associated changes were represented by mitochondrial proliferation, observed in 7 patients. Selective type II fibers atrophy and fibrous/adipose replacement were demonstrated in 4 patients each.

Different degrees of inflammation were detected, ranging from massive cellular infiltrates with diffuse myofibers necrosis/regeneration, to minimal perivascular lymphohistiocytic deposits. In cases with a significant amount of mononuclear cells, CD4+ T-lymphocytes were predominant, both in perivascular and perimysial infiltrates. Upregulation of tissue immunity markers was observed, namely MHC-I and MHC-II fibral neolocalization and recurrent MAC deposits on the wall of endomysial capillaries. Morphological examination in TEM (see *Figure 16*), which was performed in 15 patients, confirmed endothelial involvement in most of the cases. The most common finding was swelling of capillary endothelium, but mitochondrial swelling and necrosis of vessel walls were also present. Thickening or reduplications of basal lamina, indicative of degenerative and regenerative phenomena, were often associated with endothelial changes. Degenerative changes of intramuscular nerve bundles were also detected in patients with associated neurogenic changes.

### *6.3.2 Clinico-pathological patterns*

The overall study population can be divided in two main subgroups, according to the extent of pathological changes. The first subgroup comprises patients with pathologic evidence of *cellular inflammation* in skeletal muscles, with or without granuloma formation. The second subgroup of patients is characterized by the presence of *minor or absent inflammatory changes* in muscle specimens, such as different degrees of myopathic changes and sometimes increased expression of MHC-I and -II molecules,

without cellular infiltration.

An overview of the main clinicopathological findings of these two subgroups is reported in *Table 14*. Despite no significant differences between the two subgroups, all patients with the acute onset and/or clinically evident muscular weakness showed cellular inflammation in muscle specimens, while all patients with minor or absent inflammatory changes showed chronic onset of purely subjective symptoms. On the other hand, serum CK increase and myopathic changes on EMG were detected in very similar proportion of patients in both subgroups (about 45% for CK elevation and about 80% for myopathic changes). As expected, fibrous/adipose replacement was present only in patients with a relevant amount of cellular inflammation and was not demonstrated in any of the samples with minor or absent inflammatory changes.

#### *6.3.3 TNFα and TNFR1-2 expression*

Results of indirect immunofluorescence for TNFα and TNFR1-2 performed on specimens from prospectively enrolled patients are summarized in *Table 15*. Representative findings are presented in *Figure 16*.

As expected, granulomas and inflammatory infiltrates were diffusely reactive for both TNFα and TNFR1-2. Moreover, cytoplasmic and/or sarcolemmal deposition along myofibers was commonly detected in all patients, with or without granulomas, including samples with minor or absent inflammatory changes. Injured/necrotic fibers resulted consistently reactive for both TNFα and TNFR1-2. Nevertheless, TNFα and TNFR1-2 were also expressed by a minority of morphologically healthy myofibers, in cases with both active inflammation (either close to or remote from cellular infiltrates), and minor or no inflammatory changes.

The most consistent finding was however a strong endothelial staining for both TNFα and TNFR1-2, which was detected in each patient, whereas control cases displayed a far weaker endothelial immunolocalization of both markers.

### 6.4 Discussion

The present study assessed a cohort of 29 sarcoidosis patients who underwent muscular biopsy due to the presence of symptoms and/or signs of probable muscular involvement of the disease. Among all examined specimens, despite extensive sectioning of standard-sized bioptic samples, granulomas were demonstrated only in 13,8% of

samples, while the remaining 86,2% showed different grades of inflammatory and/or myopathic changes. Although the possibility of a sampling bias cannot be completely ruled out, albeit unlikely, this finding suggests the presence of different patterns of muscular involvement in sarcoidosis, not limited to granuloma-mediated muscular damage. This hypothesis expands the classical definition of symptomatic sarcoid myopathies described by the literature, which has always been limited to granulomatous forms with acute, chronic or nodular presentation *(Silverstein, 1969; Zisman, 2002)*. Although few recent reports begin to question such "historical" approach *(Cohen Aubart, 2018)*, to our knowledge no previous study has ever focused on non-granulomatous muscular manifestations of sarcoidosis.

Muscle samples without granulomas showed different degrees of myopathic and inflammatory changes, ranging from massive cellular infiltrates to minor inflammatory changes, namely increased expression of MHC-I and -II molecules (the former as non-specific hallmark of muscle inflammation, the latter as more specific marker of immune activation) or isolated myopathic alterations. A clear predominance of CD4+ T-lymphocytes was detected in samples with a relevant amount of cellular infiltrates, in line with the known CD4+ polarization of immune response in sarcoidosis localizations *(Sakthivel, 2017)*.

The overall histopathological alterations detected do not perfectly fit into anyone of the subgroups currently used to classify IIM (respectively PM, DM, IBM and necrotizing autoimmune myositis-NAM) *(Dalakas, 2015)*, as it often occurs in MSA and MAA seronegative inflammatory myopathies. However, all examined specimens, with or without cellular infiltrates, showed various signs of endothelial damage, such as recurrent MAC deposits on endomysial capillaries together with swelling of capillary endothelium, mitochondrial swelling and necrosis of vessel walls observed in TEM. Mitochondrial changes, found in a proportion of our patients, have also been described as an associated sign in various IIM, mainly in the pathologic subgroup of *myovasculopathies*, which includes most dermatomyositis syndromes *(Pestronk, 2011; Dalakas, 2015)*. These findings suggest a microvasculitic process supporting the generation of muscular damage in sarcoidosis patients.

Morevoer, the demonstration of associated type II fiber atrophy in a subset of our samples should remind to consider steroid-induced myopathy as a possible differential

diagnosis or, most likely, an association between the muscular involvement of the underlying disease and steroid-induced muscular damage.

The two subgroups that we identified according to pathological findings did not demonstrate any significant difference in clinical presentation, probably due to the relatively small study population. However, few peculiarities seem to emerge. Indeed, acute presentations and clinically evident muscular weakness were found only in the cellular inflammation subgroup, while patients with minor or absent inflammatory changes all presented with a chronic and subtle onset of purely subjective symptoms. Moreover, fibrous/adipose replacement was only found in patients with relevant cellular inflammation (including patients with granulomas). On this basis, these two clinico-pathological patterns seem to represent a continuum, in which the increasing level of inflammatory changes (from their absence to their maximum extent represented by granuloma formation) seem to relate to an increasing severity of muscular signs and symptoms.

From this perspective, the demonstration of an increased expression of TNFα and TNFR1-2 also in muscles with minor or no inflammatory changes strengthens this hypothesis and suggests that a local increase of pro-inflammatory cytokines activity might be the first (and sometimes the only) sign of muscular involvement in sarcoidosis. Similarly, it is possible to hypothesize that muscular expression of TNFα may be first step in the progression of muscular inflammation and that, if untreated, it may afterwards lead to further inflammation in muscles, including lymphocytes infiltration and finally granuloma formation *(Peterson, 2006; Sakthivel, 2017*).

TNFα is a potent pleiomorphic proinflammatory cytokine, mainly secreted by activated macrophages and with multiple effects on immune system, including macrophage activation and differentiation, T-lymphocytes proliferation and expression of MHC antigens and cell adhesion molecules favoring leukocyte margination *(Vassalli, 1992)*. TNFα is also considered critical for the formation of sarcoid granulomas *(Sakthivel, 2017; Lepzien, 2021)*. The consistent and intense endothelial expression of both TNFα and TNFR1-2 that we detected in the muscle of sarcoidosis patients and the aforementioned signs of endothelial damage in immunohistochemistry and TEM may further support the hypothesis of a primary microvasculitic process underlying sarcoid myopathy. Indeed, evidence of endothelial lesions of bronchial capillaries in sarcoidosis has been obtained by morphological ultrastructural investigations *(Mochizuki, 2014)*.

The perivascular-limited inflammation that we detected in the skeletal muscle seems to indicate a shared pathogenetic mechanism, involving oxidative stress, mitochondrial swelling and disruption, with lipid endothelial deposits. More generally, a role of lipid dysmetabolism has been suggested in originating sarcoidosis *(Bargagli, 2017)*.

Moreover, cytoplasmic and sarcolemmal expression of TNFα and TNFR1-2 that we detected both along injured and morphologically healthy myofibers could play a direct role in developing fatigue and muscular weakness, as previously reported in experimental models *(Reid, 2002)*.

Circulating TNFα is considered as the possible cause of the loss of epidermic nerve fibers in sarcoidosis-associated SFN *(Kidd, 2020)*, as suggested by the clinical improvement of symptoms after treatment with IVIg and TNFα inhibitors *(Hoitsma, 2006; Parambil, 2011; Tavee, 2017)*. Our demonstration of increased expression of TNFα in the skeletal muscles of patients complaining of myalgia, irrespective of concomitant presence or extent of inflammatory changes, may lead to hypothesize a role of this cytokine also in the genesis of myopathic pain. In this perspective, the clinico-pathological picture of "minor" sarcoid inflammatory myopathy, presenting often with disabling myalgia but with no granulomas or cellular infiltration, could be considered, as well as SFN, inside the group of *non-organ manifestations* of sarcoidosis *(Drent, 2015)* (also called *parasarcoidosis* or *paraneurosarcoidosis (Judson, 2014; Tavee, 2014; Datema, 2015)*), together with other constitutional symptoms as fatigue, depression and cognitive changes.

Finally, all these issues concerning the role of TNFα in the development of sarcoid myopathy and in the genesis of muscular symptoms should point out the potential therapeutic role of TNFα inhibitors. These drugs are already widely used as third-line treatment in both systemic sarcoidosis and neurosarcoidosis *(Baughman, 2006; Jamilloux, 2017; Cohen-Aubart, 2017; Gelfand, 2017; Hutto, 2021)*. On the other hand, the use of TNFα inhibitors in IIM has been explored in several studies with conflicting outcomes *(De Paepe, 2015)* and some cases of drug-induced PM and DM have been reported after TNFα inhibitors treatment for other chronic inflammatory diseases *(Brunasso, 2014)*. However, therapeutic implications fall beyond the scope of this study, and would require further investigations with properly designed trials.

Limitations of the present study include the partially retrospective nature of the sampling, the monocentric design of the study and the relatively small size of the study population due to the rarity of the disease.

# CHAPTER 7: CONCLUSIONS

Our research study confirmed the complexity of neurologic involvement in sarcoidosis, for both CNS and muscular presentations, and underlined the challenges in diagnosis, which are still unsolved despite recent updates of available diagnostic criteria. In fact, patients presenting with apparently isolated neurologic disease elude such criteria, requiring a critical and extensive diagnostic workup, which is mandatory and should be performed in strict cooperation between neurologists and pneumologists.

In this perspective, CSF analysis for disease biomarkers may represent a powerful tool. The measurable concentrations of KL-6 that we detected in the CSF of CNS neurosarcoidosis patients reflect a new, promising, sensitive and specific biomarker of the disease.

Moreover, our demonstration of different patterns of muscular involvement in sarcoidosis beyond granulomatous myositis expands the conventional spectrum of the disease. The increased TNFα and TNFR1-2 expression in skeletal muscle of sarcoidosis patients represents a sensitive diagnostic marker, especially in milder presentations and underlines the relevance of capillary endothelium as the first site of the pathological process.

The relatively small size of both “central” and “muscular” study populations, due to the rarity of the assessed presentations, may have reduced the statistical power of our findings, which deserve further investigations to confirm and enforce our results.

# ACKNOWLEDGEMENTS

Firstly, I would like to thank Prof. Fabio Giannini for his constant help and support during the development of this work, and for his precious teachings all along many years of collaboration. A special thank to Prof. Paola Rottoli and Prof. Elena Bargagli, from *Respiratory Diseases and Lung Transplantation Unit* of *Siena University Hospital*, because without their precious collaboration this work would not have been possible. An important and sincere thank to Dr. Miriana D'Alessandro and Prof. Nila Volpi for the essential and priceless help they gave me in the realization of this work. Finally, I would like to thank my family, for their constant support and trust.

# REFERENCES


- Abehsera M, Valeyre D, Grenier P, Jaillet H, Battesti JP, Brauner MW. Sarcoidosis with pulmonary fibrosis: CT patterns and correlation with pulmonary function. *Am J Roentgenol*. 2000;174:1751–1757.
- Affan M, Mahajan A, Rehman T, Kananeh M, Schultz L, Cerghet M. The effect of race on clinical presentation and outcomes in neurosarcoidosis. *J Neurol Sci*. 2020;417:117073.
- Agnihotri SP, Singhal T, Stern BJ, Cho TA. *Neurosarcoidosis. Semin Neurol*. 2014;34(4):386-394.
- Akman-Demir FG, Eraksoy M, Gürvit IH, Saruhan-Direskeneli G, Aral O. Paroxysmal dysarthria and ataxia in a patient with Behçet's disease. *J Neurol*. 1995;242(5):344-347.
- Al-Kofahi K, Korsten P, Ascoli C, Virupannavar S, Mirsaeidi M, Chang I, Qaqish N, Saketkoo LA, Baughman RP, Sweiss NJ. Management of extrapulmonary sarcoidosis: challenges and solutions. *Ther Clin Risk Manag.* 2016;12:1623-1634.
- Androdias G, Maillet D, Marignier R, Pinède L, Confavreux C, Broussolle C, Vukusic S, Sève P. Mycophenolate mofetil may be effective in CNS sarcoidosis but not in sarcoid myopathy. *Neurology*. 2011;76(13):1168-1172.
- Anthony J, Esper GJ, Ioachimescu A. Hypothalamic-pituitary sarcoidosis with vision loss and hypopituitarism: case series and literature review. *Pituitary*. 2016;19(1):19-29.
- Antonovich DD, Callen JP. Development of sarcoidosis in cosmetic tattoos. *Arch Dermatol.* 2005;141(7):869-872.
- Ardehali H, Howard DL, Hariri A, Qasim A, Hare JM, Baughman KL, Kasper EK. A positive endomyocardial biopsy result for sarcoid is associated with poor prognosis in patients with initially unexplained cardiomyopathy. *Am Heart J*. 2005;150:459–463.
- Arkema EV, Grunewald J, Kullberg S, Eklund A, Askling J. Sarcoidosis incidence and prevalence: a nationwide register-based assessment in Sweden. *Eur Respir J*. 2016;48(6):1690-1699.

- Arkema EV, Cozier YC. Sarcoidosis epidemiology: recent estimates of incidence, prevalence and risk factors. *Curr Opin Pulm Med.* 2020;26(5):527-534.
- Arun T, Pattison L, Palace J. Distinguishing neurosarcoidosis from multiple sclerosis based on CSF analysis: A retrospective study. *Neurology*. 2020;94(24):e2545-e2554.
- Arun T, Palace J. Effects of immunotherapies and clinical outcomes in neurosarcoidosis: a retrospective cohort study. *J Neurol*. 2021;268(7):2466-2472.
- Aubart FC, Ouayoun M, Brauner M, Attali P, Kambouchner M, Valeyre D, Nunes H. Sinonasal involvement in sarcoidosis: a case-control study of 20 patients. *Medicine*. 2006;85:365–371.
- Authier F, Mhiri C, Chazaud B, Christov C, Cherin P, Barlovatz-Meimon G, Gherardi RK. Interleukin-1 expression in inflammatory myopathies: evidence of marked immunoreactivity in sarcoid granulomas and muscle fibres showing ischaemic and regenerative changes. *Neuropathol Appl Neurobiol*. 1997;23:132–140.
- Bagnato F, Stern BJ. Neurosarcoidosis: diagnosis, therapy and biomarkers. *Expert Rev Neurother*. 2015;15(5):533-548.
- Bargagli E, Margollicci M, Nikiforakis N, Luddi A, Perrone A, Grosso S, Rottoli P. Chitotriosidase activity in the serum of patients with sarcoidosis and pulmonary tuberculosis. *Respiration.* 2007;74(5):548-52.
- Bargagli E, Bianchi N, Margollicci M, Olivieri C, Luddi A, Coviello G, Grosso S, Rottoli P. Chitotriosidase and soluble IL-2 receptor: comparison of two markers of sarcoidosis severity. *Scand J Clin Lab Invest.* 2008;68(6):479-483.
- Bargagli E, Bennett D, Maggiorelli C, Di Sipio P, Margollicci M, Bianchi N, Rottoli P. Human Chitotriosidase: a Sensitive Biomarker of Sarcoidosis. *J Clin Immunol.* 2013;33:264-70.
- Bargagli E, Rosi E, Pistolesi M, Lavorini F, Voltolini L, Rottoli P. Increased Risk of Atherosclerosis in Patients with Sarcoidosis. *Pathobiology*. 2017;84(5):258-263.
- Bargagli E, Prasse A. Sarcoidosis: a review for the internist. *Intern Emerg Med.* 2018;13(3):325-331.

- Bateman AC, Ashton-Key MR, Jogai S. Lymph node granulomas in immunoglobulin G4-related disease. *Histopathology*. 2015;67(4):557-561.
- Bathla G, Singh AK, Policeni B, Agarwal A, Case B. Imaging of neurosarcoidosis: common, uncommon, and rare. *Clin Radiol*. 2016;71(1):96-106.
- Bathla G, Watal P, Gupta S, Nagpal P, Mohan S, Moritani T. Cerebrovascular manifestations of neurosarcoidosis: an underrecognized aspect of the imaging spectrum. *AJNR Am J Neuroradiol*. 2018;39(7):1194-1200.
- Bathla G, Freeman CW, Moritani T, Song JW, Srivastava S, Soni N, Derdeyn C, Mohan S. Retrospective, dual-centre review of imaging findings in neurosarcoidosis at presentation: prevalence and imaging sub-types. *Clin Radiol*. 2020;75(10):796.e1-796.e9.
- Bathla G, Abdel-Wahed L, Agarwal A, Cho TA, Gupta S, Jones KA, Priya S, Soni N, Wasserman BA. Vascular Involvement in Neurosarcoidosis: Early Experiences From Intracranial Vessel Wall Imaging. *Neurol Neuroimmunol Neuroinflamm.* 2021;8(6):e1063.
- Baughman RP, Teirstein AS, Judson MA, Rossman MD, Yeager H Jr, Bresnitz EA, DePalo L, Hunninghake G, Iannuzzi MC, Johns CJ, McLennan G, Moller DR, Newman LS, Rabin DL, Rose C, Rybicki B, Weinberger SE, Terrin ML, Knatterud GL, Cherniak R; Case Control Etiologic Study of Sarcoidosis (ACCESS) research group. Clinical characteristics of patients in a case control study of sarcoidosis. *Am J Respir Crit Care Med*. 2001;164(10 Pt.1):1885-1889.
- Baughman RP, Drent M, Kavuru M, Judson MA, Costabel U, du Bois R, Albera C, Brutsche M, Davis G, Donohue JF, Müller-Quernheim J, Schlenker-Herceg R, Flavin S, Lo KH, Oemar B, Barnathan ES, Sarcoidosis Investigators. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. *Am J Respir Crit Care Med*. 2006;174:795–802.
- Baughman RP, Sparkman BK, Lower EE. Six-minute walk test and health status assessment in sarcoidosis. *Chest*. 2007;132:207–213.
- Bechman K, Christidis D, Walsh S, Birring SS, Galloway J. A review of the musculoskeletal manifestations of sarcoidosis. *Rheumatology (Oxford)*. 2018;57(5):777-83.
- Bennett D, Cameli P, Lanzarone N, Carobene L, Bianchi N, Fui A, Rizzi L,

Bergantini L, Cillis G, d'Alessandro M, Mazzei MA, Refini RM, Sestini P, Bargagli E, Rottoli P. Chitotriosidase: a biomarker of activity and severity in patients with sarcoidosis. *Respir Res*. 2020;21(1):6.

- Benzagmout M, Boujraf S, Góngora-Rivera F, Bresson D, Van-Effenterre R. Neurosarcoidosis which manifested as acute hydrocephalus: diagnosis and treatment. *Intern Med*. 2007;46(18):1601-1604.
- Berek K, Kiechl S, Willeit J, Birbamer G, Vogl G, Schmutzhard E. Subarachnoid hemorrhage as presenting feature of isolated neurosarcoidosis. *Clin Investig*. 1993;71:54–56
- Bergantini L, Bianchi F, Cameli P, Mazzei MA, Fui A, Sestini P, Rottoli P, Bargagli E. Prognostic biomarkers of sarcoidosis: a comparative study of serum chitotriosidase, ACE, lysozyme, and KL-6. *Dis Markers*. 2019;2019:1–7.
- Bergner R, Hoffmann M, Waldherr R, Uppenkamp M. Frequency of kidney disease in chronic sarcoidosis. *Sarcoidosis Vasc Diffus Lung Dis*. 2003;20:126–132.
- Bergner R, Löffler C. Renal sarcoidosis: approach to diagnosis and management. *Curr Opin Pulm Med*. 2018;24:513–520.
- Berrios I, Jun-O'Connell A, Ghiran S, Ionete C. A case of neurosarcoidosis secondary to treatment of etanercept and review of the literature. *BMJ Case Rep*. 2015;2015.
- Besnier EH. Lupus pernio de la face: synovites fongueuses symétriques des extrémités supérieures. *Ann Derm Syph*. 1889;10:333-336.
- Bickett AN, Lower EE, Baughman RP. Sarcoidosis Diagnostic Score: A Systematic Evaluation to Enhance the Diagnosis of Sarcoidosis. *Chest*. 2018;154:1052–1060.
- Birnbaum AD, French DD, Mirsaeidi M, Wehrli S. Sarcoidosis in the national veteran population: association of ocular inflammation and mortality. *Ophthalmology.* 2015;122(5):934-938.
- Birnie D, Beanlands RSB, Nery P, Aaron SD, Culver DA, DeKemp RA, Gula L, Ha A, Healey JS, Inoue Y, Judson MA, Juneau D, Kusano K, Quinn R, Rivard L, Toma M, Varnava A, Wells G, Wickremasinghe M, Kron J. Cardiac sarcoidosis multi-center randomized controlled trial (CHASM CS- RCT). *Am Heart J*. 2020;220:246–252.

- Bitoun S, Bouvry D, Borie R, Mahevas M, Sacre K, Haroche J, Psimaras D, Pottier C, Mathian A, Hie M, Boutin DL, Papo T, Godeau B, Valeyre D, Nunes H, Amoura Z, Cohen Aubart F. Treatment of neurosarcoidosis - A comparative study of methotrexate and mycophenolate mofetil. *Neurology*. 2016;87(24):2517-2521.
- Bodaghi B, Touitou V, Fardeau C, Chapelon C, LeHoang P. Ocular Sarcoidosis. *La Presse Médicale.* 2012;41:e349–e354.
- Boeck C. Multiple benign sarcoid of the skin. *J Cutan & Genito-Urin Dis*. 1899;17:543.
- Bomprezzi R, Pati S, Chansakul C, Vollmer T. A case of neurosarcoidosis successfully treated with rituximab. *Neurology*. 2010;75(6):568-70.
- Boucly A, Weatherald J, Savale L, Jaïs X, Cottin V, Prevot G, Picard F, de Groote P, Jevnikar M, Bergot E, Chaouat A, Chabanne C, Bourdin A, Parent F, Montani D, Simonneau G, Humbert M, Sitbon O. Risk assessment, prognosis and guideline implementation in pulmonary arterial hypertension. *Eur Respir J*. 2017;50(2):1700889.
- Bradley DA, Lower EE, Baughman RP. Diagnosis and management of spinal cord sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis*. 2006;23(1):58-65.
- Bradshaw MJ, Pawate S, Koth LL, Cho TA, Gelfand JM. Neurosarcoidosis: Pathophysiology, Diagnosis, and Treatment. *Neurol Neuroimmunol Neuroinflamm*. 2021;8(6):e1084.
- Braksick S, Shah-Haque S, El-Haddad B, Moussa R. Neurosarcoidosis presenting as trigeminal nevralgia: a case report and review of the literature. *Sarcoidosis Vasc Diffuse Lung Dis*. 2013;30(2):153-156.
- Brennan NJ, Crean P, Long JP, Fitzgerald MX. High prevalence of familial sarcoidosis in an Irish population. *Thorax.* 1984;39:14–18.
- Bridel C, Courvoisier DS, Vuilleumier N, Lalive PH. Cerebrospinal fluid angiotensin-converting enzyme for diagnosis of neurosarcoidosis. *J Neuroimmunol*. 2015;285:1-3.
- Brown MM, Thompson AJ, Wedzicha JA, Swash M. Sarcoidosis presenting with stroke. *Stroke*. 1989;20(3):400-405.
- Brunasso AM, Aberer W, Massone C. New onset of dermatomyositis/polymyositis during anti-TNF-α therapies: a systematic

literature review. *ScientificWorldJournal*. 2014;2014:179180.

- Burns TM, Dyck PJB, Aksamit AJ, Dyck PJ. The natural history and long-term outcome of 57 limb sarcoidosis neuropathy cases. *J Neurol Sci*. 2006;244(1-2):77-87.
- Byg KE, Nielsen HH, Sejbaek T, Madsen JS, Olsen DA, Nguyen N, Kindt A, Grauslund J, Illes Z, Ellingsen T. Elevated Neurofilament Light Chain in Cerebrospinal Fluid and Plasma Reflect Inflammatory MRI Activity in Neurosarcoidosis. *Brain Sci*. 2021;11(2):238.
- Byrne JV, Lawton CA. Meningeal sarcoidosis causing intracranial hypertension secondary to dural sinus thrombosis. *Br J Radiol*. 1983;56:755-757.
- Caplan A, Rosenbach M, Imadojemu S. Cutaneous sarcoidosis. *Semin Respir Crit Care Med*. 2020;41:689–699.
- Carlson ML, White JR Jr, Espahbodi M, Haynes DS, Driscoll CL, Aksamit AJ, Pawate S, Lane JI, Link MJ. Cranial base manifestations of neurosarcoidosis: a review of 305 patients. *Otol Neurotol*. 2015;36(1):156-166.
- Chang B, Steimel J, Moller DR, Baughman RP, Judson MA, Yeager H Jr, Teirstein AS, Rossman MD, Rand CS. Depression in sarcoidosis. *Am J Respir Crit Care Med*. 2001;163(2):329-334.
- Chazal T, Costopoulos M, Maillart E, Fleury C, Psimaras D, Legendre P, Pineton de Chambrun M, Haroche J, Lubetzki C, Amoura Z, Legarff-Tavernier M, Cohen Aubart F. The cerebrospinal fluid CD4/CD8 ratio and interleukin-6 and -10 levels in neurosarcoidosis: a multicenter, pragmatic, comparative study. *Eur J Neurol*. 2019;26(10):1274-1280.
- Chen ES, Moller DR. Etiology of sarcoidosis. *Clin Chest Med*. 2008;29:365–377.
- Chokoeva AA, Tchernev G, Tana C, Ananiev J, Wollina U. Sarcoid-like pattern in a patient with tuberculosis. *J Biol Regul Homeost Agents*. 2014;28(4):783-788.
- Chopra A, Kalkanis A, Judson MA. Biomarkers in sarcoidosis. *Expert Rev Clin Immunol*. 2016;12(11):1191-1208.
- Cinetto F, Compagno N, Scarpa R, Malipiero G, Agostini C. Rituximab in refractory sarcoidosis: a single centre experience. *Clin Mol Allergy*. 2015;13(1):19.

- Cinetto F, Agostini C. Advances in understanding the immunopathology of sarcoidosis and implications on therapy. *Expert Rev Clin Immunol.* 2016;12(9):973-988.
- Coates D. The angiotensin converting enzyme (ACE). *Int J Biochem Cell Biol*. 2003;35(6):769-773.
- Cohen-Aubart F, Galanaud D, Grabli D, Haroche J, Amoura Z, Chapelon-Abric C, Lyon-Caen O, Valeyre D, Piette JC. Spinal cord sarcoidosis: clinical and laboratory profile and outcome of 31 patients in a case-control study. *Medicine (Baltimore)*. 2010;89(2):133-140.
- Cohen-Aubart F, Bouvry D, Galanaud D, Dehais C, Mathey G, Psimaras D, Haroche J, Pottier C, Hie M, Mathian A, Devilliers H, Nunes H, Valeyre D, Amoura Z. Long-term outcomes of refractory neurosarcoidosis treated with infliximab. *J Neurol*. 2017;264(5):891-897.
- Cohen Aubart F, Abbara S, Maisonobe T, Cottin V, Papo T, Haroche J, Mathian A, Pha M, Gilardin L, Hervier B, Soussan M, Morlat P, Nunes H, Benveniste O, Amoura Z, Valeyre D. Symptomatic muscular sarcoidosis: lessons from a nationwide multicenter study. *Neurol Neuroimmunol Neuroinflamm*. 2018;5(3):e452.
- Colover J. Sarcoidosis with involvement of the nervous system. *Brain*. 1948;71(Pt. 4):451-475.
- Coscia LA, Armenti DP, King RW, Sifontis NM, Constantinescu S, Moritz MJ. Update on the teratogenicity of maternal mycophenolate mofetil. *J Pediatr Genet*. 2015;4:42–55.
- Costabel U, Teschler H. Biochemical changes in sarcoidosis. Clin Chest Med. 1997;18:827-842.
- Costabel U, Bonella F, Ohshimo S, Guzman J. Diagnostic modalities in sarcoidosis: BAL, EBUS, and PET. *Semin Respir Crit Care Med*. 2010;31:404–408.
- Cozier YC, Berman JS, Palmer JR, Boggs DA, Serlin DM, Rosenberg L. Sarcoidosis in black women in the United States: Data from the black women's health study. *Chest.* 2011;139(1):144–150.
- Cozier YC, Coogan PF, Govender P, Berman JS, Palmer JR, Rosenberg L. Obesity and weight gain in relation to incidence of sarcoidosis in US black

women: data from the Black Women's Health Study. *Chest.* 2015;147(4):1086-1093.

- Cremers JP, Drent M, Bast A, Shigemitsu H, Baughman RP, Valeyre D, Sweiss NJ, Jansen TL. Multinational evidence-based world association of sarcoidosis and other granulomatous disorders recommendations for the use of methotrexate in sarcoidosis: integrating systematic literature research and expert opinion of sarcoidologists worldwide. *Curr Opin Pulm Med*. 2013;19:545–561.
- Crommelin HA, van der Burg LM, Vorselaars AD, Drent M, van Moorsel CHM, Rijkers GT, Deneer VHM, Grutters JC. Efficacy of adalimumab in sarcoidosis patients who developed intolerance to infliximab. *Respir Med*. 2016;115:72–77.
- Crouser ED, Maier LA, Wilson KC, Bonham CA, Morgenthau AS, Patterson KC, Abston E, Bernstein RC, Blankstein R, Chen ES, Culver DA, Drake W, Drent M, Gerke AK, Ghobrial M, Govender P, Hamzeh N, James WE, Judson MA, Kellermeyer L, Knight S, Koth LL, Poletti V, Raman SV, Tukey MH, Westney GE, Baughman RP. Diagnosis and detection of sarcoidosis. An official American Thoracic Society clinical practice guideline. *Am J Respir Crit Care Med*. 2020;201:e26–e51.
- Dahan A, Dunne A, Swartjes M, Proto PL, Heij L, Vogels O, van Velzen M, Sarton E, Niesters M, Tannemaat MR, Cerami A, Brines A. ARA 290 improves symptoms in patients with sarcoidosis-associated small nerve fiber loss and increases corneal nerve fiber density. *Mol Med*. 2013;18:334-345.
- Dalakas MC. Inflammatory Muscle Diseases. *N Engl J Med*. 2015;373(4):393-394.
- Dale JC, O'Brien JF. Determination of angiotensin-converting enzyme levels in cerebrospinal fluid is not a useful test for the diagnosis of neurosarcoidosis. *Mayo Clin Proc*. 1999;74(5):535.
- D'Alessandro M, Carleo A, Cameli P, Bergantini L, Perrone A, Vietri L, Lanzarone N, Vagaggini C, Sestini P, Bargagli E. BAL biomarkers' panel for differential diagnosis of interstitial lung diseases. *Clin Exp Med*. 2020;20:207–216.
- Datema M, Tannemaat MR, Drent M, Hoitsma E. Neurosarcoidosis and paraneurosarcoidosis: new online registration of patients. *Ned Tijdschr Geneeskd*. 2015;159:A8383.

- De Bleecker JL, Meire VI, Declercq W, Van Aken EH. Immunolocalization of tumor necrosis factor-alpha and its receptors in inflammatory myopathies. *Neuromuscul Disord*. 1999;9(4):239-46.
- Delaney P. Neurologic manifestations in sarcoidosis: review of the literature, with a report of 23 cases. *Ann Intern Med*. 1977;87(3):336-345.
- Delaney P, Henkin RI, Manz H, Satterly RA, Bauer H. Olfactory sarcoidosis. Report of five cases and review of the literature. *Arch Otolaryngol*. 1977;103(12):717-724.
- De Paepe B, Creus KK, De Bleecker JL. The tumor necrosis factor superfamily of cytokines in the inflammatory myopathies: potential targets for therapy. *Clin Dev Immunol*. 2012;2012:369432.
- De Paepe B, Zschüntzsch J. Scanning for Therapeutic Targets within the Cytokine Network of Idiopathic Inflammatory Myopathies. *Int J Mol Sci*. 2015;16(8):18683-18713.
- Descamps V, Bouscarat F. Cutaneous manifestations of sarcoidosis. *Ann Dermatol Venereol.* 2016;143(1):39-50.
- Deutsch-Link S, Fortuna D, Weinberg EM. A comprehensive review of hepatic sarcoid. *Semin Liver Dis*. 2018;38:284–297.
- Dorman J, Warrior L, Pandya V, Sun Y, Ninan J, Trick W, Zhang H, Ouyang B. Neurosarcoidosis in a public safety net hospital: a study of 82 cases. *Sarcoidosis Vasc Diffuse Lung Dis*. 2019;36(1):25-32.
- Doty JD, Mazur JE, Judson MA. Treatment of corticosteroid-resistant neurosarcoidosis with a short-course cyclophosphamide regimen. *Chest*. 2003;124(5):2023-2026.
- Drent M, Mansour K, Linssen C. Bronchoalveolar lavage in sarcoidosis. *Semin Respir Crit Care Med*. 2007;28(5):486-495.
- Drent M, Lower EE, Vries JD. Sarcoidosis-associated fatigue. *Eur Respir J*. 2012;40:255–263.
- Drent M, Cremers JP, Jansen TL, Baughman RP. Practical eminence and experience-based recommendations for use of TNF-α inhibitors in sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis*. 2014;31(2):91-107.
- Drent M, Strookappe B, Hoitsma E, De Vries J. Consequences of sarcoidosis. *Clin Chest Med*. 2015;36:727–737.

- du Bois RM, Greenhalgh PM, Southcott AM, Johnson NM, Harris TA. Randomized trial of inhaled fluticasone propionate in chronic stable pulmonary sarcoidosis: a pilot study. *Eur Respir J*. 1999;13:1345–1350
- Duchemann B, Lavolé A, Naccache JM, Nunes H, Benzakin S, Lefevre M, Kambouchner M, Périé S, Valeyre D, Cadranel J. Laryngeal sarcoidosis: a case-control study. *Sarcoidosis Vasc Diffus Lung Dis.* 2014;31:227–234.
- Duhon BS, Shah L, Schmidt MH. Isolated intramedullary neurosarcoidosis of the thoracic spine: case report and review of the literature. *Eur Spine J*. 2012;21(Suppl.4):S390-5.
- Dumas O, Boggs KM, Cozier YC, Stampfer MJ, Camargo CA Jr. Prospective study of body mass index and risk of sarcoidosis in US women. *Eur Respir J*. 2017;50(4):1701397.
- Durel CA, Marignier R, Maucort-Boulch D, Iwaz J, Berthoux E, Ruivard M, André M, Le Guenno G, Pérard L, Dufour JF, Turcu A, Antoine JC, Camdessanche JP, Delboy T, Sève P. Clinical features and prognostic factors of spinal cord sarcoidosis: a multicenter observational study of 20 BIOPSY-PROVEN patients. *J Neurol*. 2016;263(5):981-990.
- Earle B, Wolf DS, Ramsay ES. Novel Use of Rituximab in Treatment of Refractory Neurosarcoidosis in an 11-Year-Old Girl. *J Clin Rheumatol*. 2019;25(6):e101-e103.
- Ehlers S. Tumor necrosis factor and its blockade in granulomatous infections: differential modes of action of infliximab and etanercept? *Clin Infect Dis*. 2005;41(Suppl.3):S199-203.
- Eishi Y, Suga M, Ishige I, Kobayashi D, Yamada T, Takemura T, Takizawa T, Koike M, Kudoh S, Costabel U, Guzman J, Rizzato G, Gambacorta M, du Bois R, Nicholson AG, Sharma OP, Ando M. Quantitative analysis of mycobacterial and propionibacterial DNA in lymphnodes of Japanese and European patients with sarcoidosis. *J Clin Microbiol*. 2002;40:198-204.
- Elfferich MD, Nelemans PJ, Ponds RW, Vries JD, Wijnen PA, Drent M. Everyday cognitive failure in sarcoidosis: the prevalence and the effect of anti-TNF-alpha treatment. *Respiration*. 2010;80:212–219.
- Fazzi P, Solfanelli S, Di Pede F, Begliomini E, Diviggiano E, Giuntini C. Sarcoidosis in Tuscany. A preliminary report. *Sarcoidosis*. 1992;9(2):123–126.

- Fidler LM, Balter M, Fisher JH, To T, Stanbrook MB, Gershon A. Epidemiology and health outcomes of sarcoidosis in a universal healthcare population: a cohort study. *Eur Respir J.* 2019;54(4):1900444.
- Flanagan EP, Kaufmann TJ, Krecke KN, Aksamit AJ, Pittock SJ, Keegan BM, Giannini C, Weinshenker BG. Discriminating long myelitis of neuromyelitis optica from sarcoidosis. *Ann Neurol*. 2016;79(3):437-447.
- Floe A, Hoffmann HJ, Nissen PH, Møller HJ, Hilberg O. Genotyping increases the yield of angiotensin-converting enzyme in sarcoidosis--a systematic review. Dan Med J. 2014;61(5):A4815.
- Folz SJ, Johnson CD, Swensen SJ. Abdominal manifestations of sarcoidosis in CT studies. *J Comput Assist Tomogr*. 1995;19:573–579.
- Fritz D, van de Beek D, Brouwer MC. Clinical features, treatment and outcome in neurosarcoidosis: systematic review and meta-analysis. *BMC Neurol*. 2016;16(1):220.
- Fritz D, Voortman M, van de Beek D, Drent M, Brouwer MC. Many faces of neurosarcoidosis: from chronic meningitis to myelopathy. *Curr Opin Pulm Med*. 2017;23(5):439-446.
- Fritz D, van de Beek D, Brouwer MC, Booij J. Whole-Body 18F-FDG PET-CT in the Diagnosis of Neurosarcoidosis. *Mayo Clin Proc*. 2020;95(5):1082-1084.
- Fritz D, Timmermans WMC, van Laar JAM, van Hagen PM, Siepman TAM, van de Beek D, Brouwer MC. Infliximab treatment in pathology-confirmed neurosarcoidosis. *Neurol Neuroimmunol Neuroinflamm*. 2020;7(5):e847.
- Gelfand JM, Bradshaw MJ, Stern BJ, Clifford DB, Wang Y, Cho TA, Koth LL, Hauser SL, Dierkhising J, Vu NH, Sriram S, Moses H, Bagnato F, Kaufmann JA, Ammah DJ, Yohannes TH, Hamblin MJ, Venna N, Green AJ, Pawate S. Infliximab for the treatment of CNS sarcoidosis: A multi-institutional series. *Neurology*. 2017;89(20):2092–2100.
- Ghrenassia E, Mekinian A, Chapelon-Albric C, Levy P, Cosnes J, Sève P, Lefèvre G, Dhôte R, Launay D, Prendki V, Morell-Dubois S, Sadoun D, Mehdaoui A, Soussan M, Bourrier A, Ricard L, Benamouzig R, Valeyre D, Fain O, and the Groupe Sarcoïdose Francophone. Digestive-tract sarcoidosis: French nationwide case-control study of 25 cases. *Medicine*. 2016;95:e4279.
- Gibson GJ, Prescott RJ, Muers MF, Middleton WG, Mitchell DN, Connolly CK,

Harrison BD. British Thoracic Society Sarcoidosis study: effects of long term corticosteroid treatment. *Thorax*. 1996;51:238–247

- Götestam Skorpen C, Hoeltzenbein M, Tincani A, Fischer-Betz R, Elefant E, Chambers C, da Silva J, Nelson-Piercy C, Cetin I, Costedoat-Chalumeau N, Dolhain R, Förger F, Khamashta M, Ruiz-Irastorza G, Zink A, Vencovsky J, Cutolo M, Caeyers N, Zumbühl C, Østensen M. The EULAR points to consider for use of antirheumatic drugs before pregnancy, and during pregnancy and lactation. *Ann Rheum Dis*. 2016;75(5):795–810.
- Gott PS, Kumar V, Kadakia J, Sharma OP. Significance of multimodality evoked potential abnormalities in sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis*. 1997;14(2):159-164.
- Gribbin J, Hubbard RB, Jeune IL, Smith CJP, West J, Tata LJ. Incidence and mortality of idiopathic pulmonary fibrosis and sarcoidosis in the UK. *Thorax*. 2006;61(11):980–985.
- Grutters JC, Fellrath JM, Mulder L, Janssen R, van den Bosch JM, van Velzen-Blad H. Serum soluble interleukin-2 receptor measurement in patients with sarcoidosis: a clinical evaluation. *Chest.* 2003;124(1):186-195.
- Gullapalli D, Phillips LH 2nd. Neurosarcoidosis. *Curr Neurol Neurosci Rep*. 2004;4(6):441-447.
- Gupta D, Agarwal R, Agarwal AN, Jindal SK. Molecular evidence for the role of mycobacteria in sarcoidosis: A meta-analysis. *Eur Respir J*. 2007;30:508-516.
- Haimovic A, Sanchez M, Judson MA, Prystowsky S. Sarcoidosis: a comprehensive review and update for the dermatologist. *J Am Acad Dermatol*. 2012;66(5):699.e1–18.
- Hajj-Ali RA, Calabrese LH. Primary angiitis of the central nervous system. *Autoimmun Rev*. 2013;12(4):463-466.
- Hamzeh N, Voelker A, Forssén A, Gottschall EB, Rose C, Mroz P, Maier LA. Efficacy of mycophenolate mofetil in sarcoidosis. *Respir Med*. 2014;108:1663–1669.
- Harper LJ, Gerke AK, Wang XF, Ribeiro Neto ML, Baughman RP, Beyer K, Drent M, Judson MA, Maier LA, Serchuck L, Singh N, Culver DA. Income and Other Contributors to Poor Outcomes in U.S. Patients with Sarcoidosis. *Am J Respir Crit Care Med.* 2020;201(8):955-964.

- Heerfordt CF. On febris uveo-parotidea subchronica localized in the parotid gland and uvea of the eye, frequently complicated by paralysis of the cerebrospinal nerves. *Grefes Arch Ophtal*. 1909;70:254-258.
- Heij L, Niesters M, Swartjes M, Hoitsma E, Drent M, Dunne A, Grutters JC, Vogels O, Brines M, Cerami A, Dahan A. Safety and efficacy of ARA 290 in sarcoidosis patients with symptoms of small fiber neuropathy: a randomized, double-blind pilot study. *Mol Med*. 2012;18:1430-1436.
- Heij L, Dahan A, Hoitsma E. Sarcoidosis and Pain Caused by Small-Fiber Neuropathy. *Pain Res Treat*. 2012;2012:256024.
- Henke CE, Henke G, Elveback LR, Beard CM, Ballard DJ, Kurland LT. The epidemiology of sarcoidosis in Rochester Minnesota: A population-based study of incidence and survival. *Am J Epidemiol.* 1986;123(5):840–845.
- Herring AB, Urich H. Sarcoidosis of the central nervous system. *J Neurol Sci*. 1969;9:405–422.
- Hillerdal G, Nou E, Osterman K, Schmekel B. Sarcoidosis: epidemiology and prognosis. A 15-year European study. *Ann Rev Respir Dis*. 1984;130(1):29–32.
- Hinz A, Fleischer M, Brähler E, Wirtz H, Bosse-Henck A. Fatigue in patients with sarcoidosis, compared with the general population. *Gen Hosp Psychiatry*. 2011;33:462-468.
- Hiramatsu J, Kataoka M, Nakata Y, Okazaki K, Tada S, Tanimoto M, Eishi Y. Propionibacterium acnes DNA detected in bronchoalveolar lavage cells from patients with sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis*. 2003;20:197-203.
- Hofmann S, Franke A, Fischer A, Jacobs G, Nothnagel M, Gaede KI, Schürmann M, Müller-Quernheim J, Krawczak M, Rosenstiel P, Schreiber S. Genome-wide associationstudy identifies ANXA11 as a new susceptibility locus for sarcoidosis. *Nat Genet*. 2008;40:1103–1106.
- Hoitsma E, Marziniak M, Faber CG, Reulen JP, Sommer C, De Baets M, Drent M. Small fibre neuropathy in sarcoidosis. *Lancet*. 2002;359(9323):2085-2086.
- Hoitsma E, Faber CG, van Santen-Hoeufft M, De Vries J, Reulen JP, Drent M. Improvement of small fiber neuropathy in a sarcoidosis patient after treatment with infliximab. *Sarcoidosis Vasc Diffuse Lung Dis*. 2006;23(1):73-77.
- Honda K, Okada F, Ando Y, Mori H, Umeki K, Ishii H, Kadota J, Ando M, Miyazaki E, Kumamoto T. Comparison of pulmonary thin section CT findings

and serum KL-6 levels in patients with sarcoidosis. *Br J Radiol*. 2011;84:229–235.

- Hostettler KE, Bratu VA, Fischmann A, Tamm M, Studler U. Whole-body magnetic resonance imaging in extrathoracic sarcoidosis. *Eur Respir J*. 2014;43(6):1812-5.
- Hunninghake GW, Bedell GN, Zavala DC, Monick M, Brady M. Role of interleukin-2 release by lung T-cells in active pulmonary sarcoidosis. *Am Rev Respir Dis*. 1983;218:634–638.
- Hunter JB, Rivas A. Multiple cranial neuropathies following etanercept administration. *Am J Otolaryngol*. 2016;37(3):259-262.
- Hutto SK, Kyle K, Cavanagh JJ, Reda H, Venna N. Adalimumab for CNS sarcoidosis: single-center experience and literature review. *J Neurol*. 2021:Epub ahead of print.
- Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. *N Engl J Med*. 2007;357(21):2153-2165.
- Ibitoye RT, Wilkins A, Scolding NJ. Neurosarcoidosis: a clinical approach to diagnosis and management. *J Neurol*. 2017;264:1023-1028.
- Ishige I, Usui Y, Takemura T, Eishi Y. Quantitative PCR of mycobacterial and propionibacterial DNA in lymph nodes of Japanese patients with sarcoidosis. *Lancet*. 1999:354:120–123.
- Ishige I, Eishi Y, Takemura T, Kobayashi I, Nakata K, Tanaka I, Nagaoka S, Iwai K, Watanabe K, Takizawa T, Koike M. Propionibacterium acnes is the most common bacterium commensal in peripheral lung tissue and mediastinal lymph nodes from subjects without sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis*. 2005;22:33-42.
- Iwai K, Tachibana T, Takemura T, Matsui Y, Kitaichi M, Kawabata Y. Pathological studies on sarcoidosis autopsy. I. Epidemiological features of 320 cases in Japan. *Acta Pathol Jpn*. 1993;43(7-8):372-376.
- James DG, Carstairs LS, Trowell J, Sharma OP. Treatment of sarcoidosis. Report of a controlled therapeutic trial. *Lancet*. 1967;2:526.
- Jachiet V, Lhote R, Rufat P, Pha M, Haroche J, Crozier S, Dupel-Potier C, Psimaras D, Amoura Z, Cohen Aubart F. Clinical, imaging, and histological presentations and outcomes of stroke related to sarcoidosis. *J Neurol*.

2018;265(10):2333-2341.

- Jamilloux Y, Cohen-Aubart F, Chapelon-Abric C, Maucort-Boulch D, Marquet A, Pérard L, Bouillet L, Deroux A, Abad S, Bielefeld P, Bouvry D, André M, Noel N, Bienvenu B, Proux A, Vukusic S, Bodaghi B, Sarrot-Reynauld F, Iwaz J, Amoura Z, Broussolle C, Cacoub P, Saadoun D, Valeyre D, Sève P, Groupe Sarcoïdose Francophone. Efficacy and safety of tumor necrosis factor antagonists in refractory sarcoidosis: A multicenter study of 132 patients. *Semin Arthritis Rheum*. 2017;47(2):288–294.
- Janssen R, Sato H, Grutters JC, Bernard A, Van Velzen-Blad H, Du Bois RM, van den Bosch JMM. Study of Clara cell 16, KL-6, and surfactant protein-D in serum as disease markers in pulmonary sarcoidosis. *Chest*. 2003;124:2119–2125.
- Jefferson M. The nervous system in sarcoidosis. *Postgrad Med J*. 1958;34(391):259-261.
- Jeny F, Bernaudin JF, Cohen Aubart F, Brillet PY, Bouvry D, Nunes H, Valeyre D. Diagnosis Issues in Sarcoidosis. *Respir Med Res*. 2020;77:37–45.
- Joseph FG, Scolding NJ. Neurosarcoidosis: a study of 30 new cases. *J Neurol Neurosurg Psychiatry*. 2009;80(3):297-304.
- Joubert B, Chapelon-Abric C, Biard L, Saadoun D, Demeret S, Dormont D, Resche-Rigon M, Cacoub P. Association of prognostic factors and immunosuppressive treatment with long-term outcomes in neurosarcoidosis. *JAMA Neurol*. 2017;74(11):1336-1344.
- Judson MA, Baughman RP, Teirstein AS, Terrin ML, Yeager H Jr. Defining organ involvement in sarcoidosis: the ACCESS proposed instrument. ACCESS Research Group. A Case Control Etiologic Study of Sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis*. 1999;16(1):75-86.
- Judson MA, Thompson BW, Rabin DL, Steimel J, Knattereud GL, Lackland DT, Rose C, Rand CS, Baughman RP, Teirstein AS. The diagnostic pathway to sarcoidosis. *Chest*. 2003;123:406–412.
- Judson MA, Hirst K, Iyengar SK, Rybicki BA, El-Ghormli L, Baughman RP, Donohue JF, Elston RC, Kavuru MS, Moller DR, Newman LS, Rabin DL, Rossman MD, Teirstein AS, Iannuzzi MC, SAGA Study Consortium. Comparison of sarcoidosis phenotypes among affected African-American

siblings. *Chest*. 2006;130(3):855-862.

- Judson MA. The diagnosis of sarcoidosis. *Clin Chest Med*. 2008;29:415–427.
- Judson MA, Boan AD, Lackland DT. The clinical course of sarcoidosis: presentation, diagnosis, and treatment in a large white and black cohort in the United States. 2012;29(2):119-127.
- Judson MA, Costabel U, Drent M, Wells A, Maier L, Koth L, Shigemitsu H, Culver DA, Gelfand J, Valeyre D, Sweiss N, Crouser E, Morgenthau AS, Lower EE, Azuma A, Ishihara M, Morimoto S, Tetsuo Yamaguchi T, Shijubo N, Grutters JC, Rosenbach M, Li HP, Rottoli P, Inoue Y, Prasse A, Baughman RP, Organ Assessment Instrument Investigators TW. The WASOG Sarcoidosis Organ Assessment Instrument: An update of a previous clinical tool. *Sarcoidosis Vasc Diffuse Lung Dis*. 2014;31(1):19-27.
- Junger SS, Stern BJ, Levine SR, Sipos E, Marti-Masso JF. Intramedullary spinal sarcoidosis: clinical and magnetic resonance imaging characteristics. *Neurology*. 1993;43(2):333-337.
- Kandolin R, Lehtonen J, Airaksinen J, Vihinen T, Miettinen H, Ylitalo K, Kaikkonen K, Tuohinen S, Haataja P, Kerola T, Kokkonen J, Pelkonen M, Pietilä-Effati P, Utrianen S, Kupari M. Cardiac sarcoidosis: epidemiology, characteristics, and outcome over 25 years in a nationwide study. *Circulation*. 2015;131:624–632.
- Kane K. Deafness in sarcoidosis. *J Laryngol Otol*. 1976;90(6):531-537.
- Karakaya B, Kaiser Y, van Moorsel C, Grunewald J. Löfgren's Syndrome: Diagnosis, Management, and Disease Pathogenesis. *Semin Respir Crit Care Med*. 2017;38:463–476.
- Kawajiri M, Mogi M, Higaki N, Matsuoka T, Ohyagi Y, Tsukuda K, Kohara K, Horiuchi M, Miki T, Kira JI. Angiotensin-converting enzyme (ACE) and ACE2 levels in the cerebrospinal fluid of patients with multiple sclerosis. *Mult Scler*. 2009;15(2):262-265.
- Keicho N, Kitamura K, Takaku F, Yotsumoto H. Serum concentration of soluble interleukin-2 receptor as a sensitive parameter of disease activity in sarcoidosis. *Chest*. 1990;98(5):1125-1129.
- Keijsers RGM, Grutters JC. In which patients with sarcoidosis is FDG PET/CT indicated? *J Clin Med*. 2020;9:890.

- Keller CW, Fokken C, Turville SG, Lünemann A, Schmidt J, Münz C, Lünemann JD. TNF-alpha induces macroautophagy and regulates MHC class II expression in human skeletal muscle cells. *J Biol Chem*. 2011;286(5):3970-80.
- Khan NA, Donatelli CV, Tonelli AR, Wiesen J, Ribeiro Neto ML, Sahoo D, Culver DA. Toxicity risk from glucocorticoids in sarcoidosis patients. *Respir Med*. 2017;132:9–14.
- Kidd DP. Sarcoidosis of the central nervous system: clinical features, imaging and CSF results. *J Neurol*. 2018;265(8):1906-1915.
- Kidd DP. Neurosarcoidosis: clinical manifestations, investigation and treatment. *Pract Neurol*. 2020;20(3):199-212.
- Kirkland JL, Pearson DJ, Goddard C, Davies I. Polyuria and inappropriate secretion of arginine vasopressin in hypothalamic sarcoidosis. *J Clin Endocrinol Metab*. 1983;56(2):269-272.
- Kitaichi N, Kotake S, Shibuya H, Yamada Y, Chiba H, Namba K, Ohno S. Increase of KL-6 in sera of uveitis patients with sarcoidosis. *Graefes Arch Clin Exp Ophthalmol*. 2003;241:879–883.
- Ko JK, Lee SW, Choi CH. Moyamoya-like vasculopathy in neurosarcoidosis. *J Korean Neurosurg Soc*. 2009;45(1):50-52.
- Kobak S. Sarcoidosis: a rheumatologist's perspective. *Ther Adv Musculoskelet Dis*. 2015;7(5):196-205.
- Kobayashi J, Kitamura S. Serum KL-6 for the evaluation of active pneumonitis in pulmonary sarcoidosis. *Chest*. 1996;109:1276–1282.
- Koczman JJ, Rouleau J, Gaunt M, Kardon RH, Wall M, Lee AG. Neuro-ophthalmic sarcoidosis: the University of Iowa experience. *Semin Ophthalmol*. 2008;23(3):157-168.
- Koffmann B, Junck L, Elias SB, Feit HW, Levine SR. Polyradiculopathy in sarcoidosis. *Muscle Nerve*. 1999;22(5):608-613.
- Kohno N, Kyoizumi S, Awaya Y, Fukuhara H, Yamakido M, Akiyama M. New serum indicator of interstitial pneumonitisactivity. Sialylated carbohydrate antigen KL-6. *Chest*. 1989;96:68–73.
- Kondo M, Murakawa Y, Harashima N, Kobayashi S, Yamaguchi S, Harada M. Roles of proinflammatory cytokines and the Fas/Fas ligand interaction in the pathogenesis of inflammatory myopathies. *Immunology*.

2009;128(1Suppl):e589-99.

- Korsten P, Strohmayer K, Baughman RP, Sweiss NJ. Refractory pulmonary sarcoidosis - proposal of a definition and recommendations for the diagnostic and therapeutic approach. *Clin Pulm Med*. 2016;23(2):67-75.
- Kosjerina Z, Zaric B, Vuckovic D, Lalosevic D, Djenadic G, Murer B. The sarcoid granuloma: 'epithelioid' or 'lymphocytic-epithelioid' granuloma? *Multidiscip Respir Med*. 2012;7(1):11.
- Kouranos V, Tzelepis GE, Rapti A, Mavrogeni S, Aggeli K, Douskou M, Prasad S, Koulouris N, Sfikakis P, Wells A, Gialafos E. Complementary role of CMR to conventional screening in the diagnosis and prognosis of cardiac sarcoidosis. *JACC Cardiovasc Imaging*. 2017;10:1437–1447.
- Krasowski MD, Savage J, Ehlers A, Maakestad J, Schmidt GA, La'ulu S, Rasmussen NN, Strathmann FG, Genzen JR. Ordering of the serum angiotensin-converting enzyme test in patients receiving angiotensin-converting enzyme inhibitor therapy: an avoidable but common error. Chest. 2015;148(6):1447-1453.
- Kruit A, Grutters JC, Gerritsen WBM, Kos S, Wodzig WKWH, van den Bosch JMM, Ruven HJT. ACE I/D-corrected Z-scores to identify normal and elevated ACE activity in sarcoidosis. *Respir Med*. 2007;101:510–515.
- Krumholz A, Stern BJ, Stern EG. Clinical implications of seizures in neurosarcoidosis. *Arch Neurol*. 1991;48:842–844.
- Krumholz A, Stern BJ. Neurologic manifestations of sarcoidosis. *Handb Clin Neurol*. 2014;119:305-333.
- Kumar V, Abbas AK, Fausto N, Aster JC. Infiammazione acuta e cronica. In: Kumar V, Abbas AK, Fausto N, Aster JC. Robbins e Cotran. *Le basi patologiche delle malattie.* 8ª ed. it. Milano:Elsevier;2010.
- Kurmann R. Echocardiography in sarcoidosis. *Curr Cardiol Rep*. 2018;20:118.
- Kuru S, Inukai A, Kato T, Liang Y, Kimura S, Sobue G. Expression of tumor necrosis factor-alpha in regenerating muscle fibers in inflammatory and non-inflammatory myopathies. *Acta Neuropathol*. 2003;105(3):217-24.
- Laney AS, Cragin LA, Blevins LZ, Sumner AD, Cox-Ganser JM, Kreiss K, Moffatt SG, Lohff CJ. Sarcoidosis, asthma, and asthma-like symptoms among occupants of a historically water-damaged office building. *Indoor Air*.

2009;19(1):83-90.

- Langrand C, Bihan H, Raverot G, Varron L, Androdias G, Borson-Chazot F, Brue T, Cathebras P, Pinede L, Muller G, Broussolle C, Cotton F, Valeyre D, Seve P. Hypothalamo-pituitary sarcoidosis: a multicenter study of 24 patients. *QJM*. 2012;105(10):981-995.
- Lavergne F, Clerici C, Sadoun D, Brauner M, Battesti JP, Valeyre D. Airway obstruction in bronchial sarcoidosis. *Chest*. 1999;116:1194–1199.
- Lazarus A. Sarcoidosis: epidemiology, etiology, pathogenesis, and genetics. *Dis Mon.* 2009;55(11):649-660.
- Ledingham J, Gullick N, Irving K, Gorodkin R, Aris M, Burke J, Gordon P, Christidis D, Galloway S, Hayes E, Jeffries A, Mercer S, Mooney J, van Leuven S, Galloway J, BSR and BHPR Standards, Guidelines and Audit Working Group. BSR and BHPR guideline for the prescription and monitoring of non-biologic disease-modifying anti-rheumatic drugs. *Rheumatology*. 2017;56:865–868.
- Leonhard SE, Fritz D, Eftimov F, van der Kooi AJ, van de Beek D, Brouwer MC. Neurosarcoidosis in a Tertiary Referral Center: A Cross-Sectional Cohort Study. *Medicine (Baltimore)*. 2016;95(14):e3277.
- Lepzien R, Liu S, Czarnewski P, Nie M, Österberg B, Baharom F, Pourazar J, Rankin G, Eklund A, Bottai M, Kullberg S, Blomberg A, Grunewald J, Smed-Sörensen A. Monocytes in sarcoidosis are potent tumour necrosis factor producers and predict disease outcome. *Eur Respir J*. 2021;58(1):2003468.
- Li X, Sundquist J, Hamano T, Sundquist K. Neighborhood Deprivation and Risks of Autoimmune Disorders: A National Cohort Study in Sweden. *Int J Environ Res Public Health.* 2019;16(20):3798.
- Lieberman J. Elevation of serum angiotensin-converting-enzyme (ACE) level in sarcoidosis. *Am J Med*. 1975;59(3):365-372.
- Lord J, Paz Soldan MM, Galli J, Salzman KL, Kresser J, Bacharach R, DeWitt LD, Klein J, Rose J, Greenlee J, Clardy SL. Neurosarcoidosis: Longitudinal experience in a single-center, academic healthcare system. *Neurol Neuroimmunol Neuroinflamm*. 2020;7(4):e743.
- Lower EE, Broderick JP, Brott TG, Baughman RP. Diagnosis and management of neurological sarcoidosis. *Arch Intern Med*. 1997;157(16):1864-1868.

- Lower EE, Sturdivant M, Grate L, Baughman RP. Use of third-line therapies in advanced sarcoidosis. *Clin Exp Rheumatol*. 2020;38(5):834–840.
- Lucantoni C, De Bonis P, Doglietto F, Esposito G, Larocca LM, Mangiola A, Martini M, Papacci F, Teofili L, Pompucci A. Primary cerebral lymphomatoid granulomatosis: report of four cases and literature review. *J Neurooncol*. 2009;94(2):235-242.
- Lury KM, Smith JK, Matheus MG, Castillo M. Neurosarcoidosis--review of imaging findings. *Semin Roentgenol*. 2004;39(4):495-504.
- MacLean HJ, Abdoli M. Neurosarcoidosis as an MS Mimic: The trials and tribulations of making a diagnosis. *Mult Scler Relat Disord*. 2015;4(5):414-429.
- Maeshima S, Koike H, Noda S, Noda T, Nakanishi H, Iijima M, Ito M, Kimura S, Sobue G. Clinicopathological features of sarcoidosis manifesting as generalized chronic myopathy. *J Neurol*. 2015;262(4):1035-1045.
- Mahévas M, Lescure FX, Boffa JJ, Delastour V, Belenfant X, Chapelon C, Cordonnier C, Makdassi R, Piette JC, Naccache JM, Cadranel J, Duhaut P, Choukroun G, Ducroix JP, Valeyre D. Renal sarcoidosis: clinical, laboratory, and histologic presentation and outcome in 47 patients. *Medicine*. 2009;88:98–106.
- Majcherczyk PA, Langen H, Heumann D, Fountoulakis M, Glauser MP, Moreillon P. Digestion of Streptococcus pneumoniae cell walls with its major peptidoglycan hydrolase releases branched stem peptides carrying proinflammatory activity. *J Biol Chem*. 1999;274:12537–12543.
- Mañá J, Rubio-Rivas M, Villalba N, Marcoval J, Iriarte A, Molina-Molina M, Llatjos R, García O, Martínez-Yélamos S, Vicens-Zygmunt V, Gámez C, Pujol R, Corbella X. Multidisciplinary Approach and Long-Term Follow-up in a Series of 640 Consecutive Patients with Sarcoidosis: Cohort Study of a 40-Year Clinical Experience at a Tertiary Referral Center in Barcelona, Spain. *Medicine*. 2017;96(29):e7595.
- Mani AM, Prabhakar AT, Mannam P, Benjamin RN, Ahmed Shaikh AI, Mathew D, Singh P, Nair A, Alexander PT, Vijayaraghavan A, Sivadasan A, Mani S, Mathew V, Aaron S, Alexander M. Clinical Spectrum and Outcome of Neurosarcoidosis: A Retrospective Cohort Study from a Teaching Hospital in India. *Ann Indian Acad Neurol*. 2020;23(4):528-535.

- Manz HJ. Pathobiology of neurosarcoidosis and clinicopathologic correlation. *Can J Neurol Sci*. 1983;10(1):50-55.
- Marangoni S, Argentiero V, Tavolato B. Neurosarcoidosis. Clinical description of 7 cases with a proposal for a new diagnostic strategy. *J Neurol*. 2006;253(4):4884-95.
- Marcoval J, Mañá J, Rubio M. Specific cutaneous lesions in patients with systemic sarcoidosis: relationship to severity and chronicity of disease. *Clin Exp Dermatol*. 2011;36:739–744.
- Matsui M, Tomimoto H, Sano K, Hashikawa K, Fukuyama H, Shibasaki H. Paroxysmal dysarthria and ataxia after midbrain infarction. *Neurology*. 2004;63(2):345-347.
- McCaskill JG, Chason KD, Hua X, Neuringer IP, Ghio AJ, Funkhouser WK, Tilley SL. Pulmonary immune responses to Propionibacterium acnes in C57BL/6 and BALB/c mice. *Am J Respir Cell Mol Biol*. 2006;35:347–356.
- Melani AS, Bigliazzi C, Cimmino FA, Bergantini L, Bargagli E. A comprehensive review of sarcoidosis treatment for pulmonologists. *Pulm Ther*. 2021;7(2):325-344.
- Meyer JS, Foley JM, Campagna-Pinto D. Granulomatous angiitis of the meninges in sarcoidosis. *AMA Arch Neurol Psychiatry*. 1953:69:587–600.
- Meyer KC, Raghu G, Baughman RP, Brown KK, Costabel U, du Bois RM, Drent M, Haslam PL, Kim DS, Nagai S, Rottoli P, Saltini C, Selman M, Strange C, Wood B; American Thoracic Society Committee on BAL in Interstitial Lung Disease. An official American Thoracic Society clinical practice guideline: the clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease. *Am J Respir Crit Care Med.* 2012;185(9):1004-1014.
- Michelakakis H, Dimitriou E, Labadaridis I. The expanding spectrum of disorders with elevated plasma chitotriosidase activity: an update. *J Inherit Metab Dis*. 2004;27:705–706.
- Milman N, Graudal N, Grode G, Munch E. No effect of high-dose inhaled steroids in pulmonary sarcoidosis: a double-blind, placebo-controlled study. *J Intern Med*. 1994;236:285–290.
- Miners S, Ashby E, Baig S, Harrison R, Tayler H, Speedy E, Prince JA, Love S, Kehoe PG. Angiotensin-converting enzyme levels and activity in Alzheimer's

disease: differences in brain and CSF ACE and association with ACE1 genotypes. *Am J Transl Res*. 2009;1(2):163-177.

- Mirfakhraee M, Crofford MJ, Guinto FC Jr, Nauta HJ, Weedn VW. Virchow-Robin space: a path of spread in neurosarcoidosis. *Radiology*. 1986;158(3):715-720.
- Miyoshi S, Hamada H, Kadowaki T, Hamaguchi N, Ito R, Irifune K, Higaki J. Comparative evaluation of serum markers in pulmonary sarcoidosis. Chest. 2010;137:1391–1397.
- Mochizuki I, Kubo K, Hond T. Widespread heavy damage of capillary endothelial cells in the pathogenesis of sarcoidosis--Evidence by monoclonal von Willebrand factor immunohistochemistry in the bronchus and lung of patients with sarcoidosis. Sarcoidosis *Vasc Diffuse Lung Dis*. 2014;31(3):182-190.
- Moller DR, Rybicki BA, Hamzeh NY, Montgomery CG, Chen ES, Drake W, Fontenot AP. Genetic, immunologic, and environmental basis of sarcoidosis. *Ann Am Thorac Soc*. 2017;14(suppl 6):S429–S436.
- Moore SL, Teirstein A, Golimbu C. MRI of sarcoidosis patients with musculoskeletal symptoms. *Am J Roentgenol*. 2005;185(1):154-159.
- Morgenthau AS, Teirstein AS. Sarcoidosis of the upper and lower airways. *Expert Rev Respir Med.* 2011;5(6):823-833.
- Morimoto T, Azuma A, Abe S, et al. Epidemiology of sarcoidosis in Japan. *Eur Resp J*. 2008;31(2):372-379.
- Mostard RL, Prompers L, Weijers RE, van Kroonenburgh MJ, Wijnen PA, Geusens PP, Drent M. F-18 FDG PET/CT for detecting bone and bone marrow involvement in sarcoidosis patients. *Clin Nucl Med*. 2012;37:21–25.
- Mrówka-Kata K, Kata D, Lange D, Namysłowski G, Czecior E, Banert K. Sarcoidosis and its otolaryngological implications. *Eur Arch Otorhinolaryngol*. 2010; 267(10):1507–1514.
- Murphy OC, Salazar-Camelo A, Jimenez JA, Barreras P, Reyes MI, Garcia MA, Moller DR, Chen ES, Pardo CA. Clinical and MRI phenotypes of sarcoidosis-associated myelopathy. *Neurol Neuroimmunol Neuroinflamm*. 2020;7(4):e722.
- Naccache JM, Lavolé A, Nunes H, Lamberto C, Letoumelin P, Brauner M, Valeyre D, Brillet PY. High-resolution computed tomographic imaging of

airways in sarcoidosis patients with airflow obstruction. *J Comput Assist Tomogr*. 2008;32:905–912.

- Newman KL, Newman LS. Occupational causes of sarcoidosis. *Curr Opin Allergy Clin Immunol.* 2012;12(2):145-50.
- Newman LS, Rose CS, Bresnitz EA, Rossman MD, Barnard J, Frederick M, Terrin ML, Weinberger SE, Moller DR, McLennan G, Hunninghake G, DePalo L, Baughman RP, Iannuzzi MC, Judson MA, Knatterud GL, Thompson BW, Teirstein AS, Yeager H Jr, Johns CJ, Rabin DL, Rybicki BA, Cherniack R; ACCESS Research Group. A case control etiologic study of sarcoidosis: environmental and occupational risk factors. *Am J Respir Crit Care Med.* 2004;170(12):1324-1330.
- Nordgaard-Lassen I, Dahlerup JF, Belard E, Gerstoft J, Kjeldsen J, Kragballe K, Ravn P, Sørensen IJ, Theede K, Tjellesen L, Danish Society for Gastroenterology. Guidelines for screening, prophylaxis and critical information prior to initiating anti-TNF-alpha treatment. *Dan Med J*. 2012;59:C4480.
- Nordström S, Andersson B, Malmeström C. Cerebrospinal fluid CD4+/CD8+ ratio in diagnosing neurosarcoidosis. *Acta Neurol Scand*. 2020;142(5):480-485.
- Nozaki K, Judson MA. Neurosarcoidosis: Clinical manifestations, diagnosis and treatment. *Presse Med*. 2012;41(6 Pt.2):e331-48.
- Nunes H, Freynet O, Naggara N, Soussan M, Weinman P, Diebold B, Brillet PY, Valeyre D. Cardiac sarcoidosis. *Semin Respir Crit Care Med*. 2010;31:428–441.
- Oberg G, Hällgren R, Venge P. Beta 2-microglobulin, lysozyme and lactoferrin in cerebrospinal fluid in patients with lymphoma or leukaemia: relationship to CNS involvement and the effect of prophylactic intrathecal treatment with methotrexate. *Br J Haematol*. 1987;66(3):315-322.
- O'Dwyer JP, Al-Moyeed BA, Farrell MA, Pidgeon CN, Collins DR, Fahy A, Gibney J, Swan N, Dempsey OJ, Kidd DP, Reid JM, Smyth S, McCabe DJ. Neurosarcoidosis-related intracranial haemorrhage: three new cases and a systematic review of the literature. *Eur J Neurol*. 2013;20(1):71-78.
- Ohnishi H, Yokoyama A, Kondo K, Hamada H, Abe M, Nishimura K, Hiwada K, Kohno N. Comparative study of KL-6, surfactant protein-A, surfactant protein-D, and monocyte chemoattractant protein-1 as serum markers for

interstitial lung diseases. *Am J Respir Crit Care Med*. 2002;165(3):378-381.

- Okamoto H, Mizuno K, Horio T. Monocyte-derived multinucleated giant cells and sarcoidosis. *J Dermatol Sci*. 2003;31:119–128.
- Oksanen V, Fyhrquist F, Somer H, Grönhagen-Riska C. Angiotensin converting enzyme in cerebrospinal fluid: a new assay. *Neurology*. 1985;35(8):1220-1223.
- Oksanen V, Grönhagen-Riska C, Tikanoja S, Somer H, Fyhrquist F. Cerebrospinal fluid lysozyme and beta 2-microglobulin in neurosarcoidosis. *J Neurol Sci*. 1986;73(1):79-87.
- Oksanen V, Salmi T. Visual and auditory evoked potentials in the early diagnosis and follow-up of neurosarcoidosis. *Acta Neurol Scand*. 1986;74(1):38-42.
- Ostermann PO, Westerberg CE. Paroxysmal attacks in multiple sclerosis. *Brain*. 1975 Jun;98(2):189-202.
- Otake S. Sarcoidosis involving skeletal muscle: imaging findings and relative value of imaging procedures. *AJR Am J Roentgenol*. 1994;162(2):369-375.
- Otto C, Wengert O, Unterwalder N, Meisel C, Ruprecht K. Analysis of soluble interleukin-2 receptor as CSF biomarker for neurosarcoidosis. *Neurol Neuroimmunol Neuroinflamm*. 2020;7(4):e725
- Pande A, Culver DA. Knowing when to use steroids, immunosuppressants or biologics for the treatment of sarcoidosis. *Expert Rev Respir Med*. 2020;14(3):285–298.
- Panselinas E, Rodgers JK, Judson MA. Clinical outcomes in sarcoidosis after cessation of infliximab treatment. *Respirology*. 2009;14(4):522-528.
- Parambil JG, Tavee JO, Zhou L, Pearson KS, Culver DA. Efficacy of intravenous immunoglobulin for small fiber neuropathy associated with sarcoidosis. *Respir Med*. 2011;105(1):101-105.
- Park JE, Kim YS, Kang MJ, Kim CJ, Han CH, Lee SM, Park SC. Prevalence, incidence, and mortality of sarcoidosis in Korea, 2003-2015: A nationwide population-based study. *Respir Med.* 2018;144S:S28-S34.
- Parker, HL. Periodic ataxia. *Collect Papers Mayo Clinic Mayo Found*. 1946-1947;38:642-645.
- Pawate S, Moses H, Sriram S. Presentations and outcomes of neurosarcoidosis: a study of 54 cases. *QJM*. 2009;102(7):449-460.

- Pestronk A. Acquired immune and inflammatory myopathies: pathologic classification. *Curr Opin Rheumatol*. 2011;23(6):595-604.
- Petereit HF, Reske D, Tumani H, Jarius S, Markus Leweke F, Woitalla D, Pfister HW, Rubbert A. Soluble CSF interleukin 2 receptor as indicator of neurosarcoidosis. *J Neurol*. 2010;257(11):1855-1863.
- Peterson JM, Feeback KD, Baas JH, Pizza FX. Tumor necrosis factor-alpha promotes the accumulation of neutrophils and macrophages in skeletal muscle. *J Appl Physiol (1985)*. 2006;101(5):1394-9.
- Pietinalho A, Hiraga Y, Hosoda Y, Löfroos AB, Yamaguchi M, Selroos O. The frequency of sarcoidosis in Finland and Hokkaido, Japan. A comparative epidemiological study. *Sarcoidosis.* 1995;12(1):61-67.
- Pietinalho A, Ohmichi M, Hirasawa M, Hiraga Y, Lofroos AB, Selroos O. Familial sarcoidosis in Finland and Hokkaido, Japan - a comparative study. *Respir Med*. 1999;93:408–412.
- Piffer S, Turri G, Acler M, Richelli S, Cerini R, Fiaschi A, Monaco S, Bonetti B. Paroxysmal dysarthria-ataxia in remitting-relapsing Bickerstaff's-like encephalitis. *J Neurol Sci*. 2014;341(1-2):85-87.
- Plotkin GR, Patel BR. Neurosarcoidosis presenting as chronic lymphocytic meningitis. *Pa Med*. 1986;89(7):36-37.
- Popević S, Šumarac Z, Jovanović D, Babić D, Stjepanović M, Jovičić S, Šobić-Šaranović D, Filipović S, Gvozdenović B, Omčikus M, Milovanović A, Videnović-Ivanov J, Radović A, Žugić V, Mihailović-Vučinić V. Verifying Sarcoidosis Activity: Chitotriosidase versus ACE in Sarcoidosis - a Case-control Study. *J Med Biochem*. 2016;35(4):390-400.
- Prasse A. The diagnosis, differential diagnosis and treatment of sarcoidosis. *Dtsch Arztebl Int.* 2016;113(33-34):565-574.
- Prieto-González S, Grau JM. Diagnosis and classification of granulomatous myositis. *Autoimmun Rev*. 2014;13(4-5):372-374.
- Rahaghi FF, Baughman RP, Saketkoo LA, Sweiss NJ, Barney JB, Birring SS, Costabel U, Crouser ED, Drent M, Gerke AK, Grutters JC, Hamzeh NY, Huizar I, Ennis James W 4th, Kalra S, Kullberg S, Li H, Lower EE, Maier LA, Mirsaeidi M, Müller-Quernheim J, Carmona Porquera EM, Samavati L, Valeyre D, Scholand MB. Delphi consensus recommendations for a treatment algorithm

in pulmonary sarcoidosis. *Eur Respir Rev*. 2020;29(155):190146.

- Ramos-Casals M, Retamozo S, Sisó-Almirall A, Pérez-Alvarez R, Pallarés L, Brito-Zerón P. Clinically-useful serum biomarkers for diagnosis and prognosis of sarcoidosis. *Expert Rev Clin Immunol*. 2019;15:391–405.
- Ramos-Casals M, Pérez-Alvarez R, Kostov B, Gómez-de-la-Torre R, Feijoo-Massó C, Chara-Cervantes J, Pinilla B, González-García A, Garcia-Morillo JS, López-Dupla M, De-Escalante B, Rascón J, Perez-Guerrero P, Bonet M, Cruz-Caparrós G, Alguacil A, Callejas JL, Calvo E, Soler C, Robles A, de Miguel-Campo B, Oliva-Nacarino P, Estela-Herrero J, Pallarés L, Brito-Zerón P, Blanco Y; SarcoGEAS-SEMI Registry. Clinical characterization and outcomes of 85 patients with neurosarcoidosis. *Sci Rep*. 2021;11(1):13735.
- Rao R, Dimitriades VR, Weimer M, Sandlin C. Neurosarcoidosis in Pediatric Patients: A Case Report and Review of Isolated and Systemic Neurosarcoidosis. *Pediatr Neurol*. 2016;63:45-52.
- Reich JM. A critical analysis of sarcoidosis incidence assessment. *Multidiscip Respir Med.* 2013;8(1):57.
- Reid MB, Lännergren J, Westerblad H. Respiratory and limb muscle weakness induced by tumor necrosis factor-alpha: involvement of muscle myofilaments. *Am J Respir Crit Care Med*. 2002;166(4):479-484.
- Rivera A, Siracusa MC, Yap GS, Gause WC. Innate cell communication kickstarts pathogen-specific immunity. *Nat Immunol*. 2016;17:356–363.
- Rochepeau C, Jamilloux Y, Kerever S, Febvay C, Perard L, Broussolle C, Burillon C, Kodjikian L, Sève P. Long-term visual and systemic prognoses of 83 cases of biopsy-proven sarcoid uveitis. *Br J Ophthalmol*. 2017;101:856–861.
- Rosini F, Bennett D, Cerase A, Volterrani L, Federico A, Rottoli P, Rufa A. Cavernous sinus syndrome due to neurosarcoidosis in adolescence: a diagnosis not to be missed. *Neurol Sci*. 2017;38(3):517-519.
- Rossides M, Grunewald J, Eklund A, Kullberg S, Di Giuseppe D, Askling J, Arkema EV. Familial aggregation and heritability of sarcoidosis: a Swedish nested case-control study. *Eur Respir J.* 2018;52(2):1800385.
- Rottoli P, Bargagli E, Chidichimo C, Nuti D, Cintorino M, Ginanneschi C, Caruso G. Sarcoidosis with upper respiratory tract involvement. *Respir Med*. 2006;100(2):253-257.

- Rubin LA, Nelson DL. The soluble interleukin-2 receptor: biology, function, and clinical application. *Ann Intern Med*. 1990;113:619.
- Rubio-Rivas M, Franco J, Corbella X. Sarcoidosis Presenting with and without Löfgren's Syndrome: Clinical, Radiological and Behavioral Differences Observed in a Group of 691 Patients. *Jt Bone Spine*. 2020;87:141–147.
- Rybicki BA, Major M, Popovich J Jr, Maliarik MJ, Iannuzzi MC. Racial differences in sarcoidosis incidence: A 5-year study in a health maintenance organization. *Am J Epidemiol.* 1997;145(3):234–241.
- Sahoo DH, Bandyopadhyay D, Xu M, Pearson K, Parambil JG, Lazar CA, Chapman JT, Culver DA. Effectiveness and safety of leflunomide for pulmonary and extrapulmonary sarcoidosis. *Eur Respir J*. 2011;38:1145–1150.
- Said G, Lacroix C, Plante-Bordeneuve V, Le Page L, Pico F, Presles O, Senant J, Remy P, Rondepierre P, Mallecourt J. Nerve granulomas and vasculitis in sarcoid peripheral neuropathy: a clinicopathological study of 11 patients. *Brain*. 2002;125:264–275.
- Said G. Sarcoidosis of the peripheral nervous system. *Handb Clin Neurol*. 2013;115:485-495.
- Sakthivel P, Bruder D. Mechanism of granuloma formation in sarcoidosis. *Curr Opin Hematol*. 2017;24:59-65.
- Saygin D, Jones S, Sundaram P, Calabrese LH, Messner W, Tavee JO, Hajj-Ali RA. Differentiation between neurosarcoidosis and primary central nervous system vasculitis based on demographic, cerebrospinal and imaging features. *Clin Exp Rheumatol*. 2020;38Suppl.124(2):135-138
- Scadding JG. Prognosis of intrathoracic sarcoidosis in England. A review of 136 cases after five years' observation. *Br Med J.* 1961;2(5261):1165-1172.
- Schaeffeler E, Jaeger SU, Klumpp V, Yang JJ, Igel S, Hinze L, Stanulla M, Schwab M. Impact of NUDT15 genetics on severe thiopurine-related hematotoxicity in patients with European ancestry. *Genet Med*. 2019;21(9):2145–2150.
- Schrezenmeier E, Dörner T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. *Nat Rev Rheumatol*. 2020;16(3):155–166.
- Schutt AC, Bullington WM, Judson MA. Pharmacotherapy for pulmonary

sarcoidosis: a Delphi consensus study. *Respir Med.* 2010;104(5):717-723.

- Seror R, Mahr A, Ramanoelina J, Pagnoux C, Cohen P, Guillevin L. Central nervous system involvement in Wegener granulomatosis. *Medicine (Baltimore)*. 2006;85:54–65.
- Sève P, Pacheco Y, Durupt F, Jamilloux Y, Gerfaud-Valentin M, Isaac S, Boussel L, Calender A, Androdias G, Valeyre D, El Jammal. Sarcoidosis: a clinical overview from symptoms to diagnosis. *Cells*. 2021;10(4):766.
- Shah R, Roberson GH, Curé JK. Correlation of MR imaging findings and clinical manifestations in neurosarcoidosis. *AJNR Am J Neuroradiol*. 2009;30(5):953-961.
- Sheffield EA. Pathology of sarcoidosis. Clin Chest Med. 1997;18(4):741-754.
- Shimizu T, Tomita Y, Son K, Nishinarita S, Sawada S, Horie T. Elevation of serum soluble tumour necrosis factor receptors in patients with polymyositis and dermatomyositis. *Clin Rheumatol*. 2000;19(5):352-359.
- Silva M, Nunes H, Valeyre D, Sverzellati N. Imaging of sarcoidosis. *Clin Rev Allergy Immunol*. 2015;49(1):45-53.
- Silverstein A, Siltzbach LE. Muscle involvement in sarcoidosis. Asymptomatic, myositis, and myopathy. *Arch Neurol*. 1969;21(3):235-241.
- Silverstein E, Friedland J, Ackerman T. Elevation of granulomatous lymph-node and serum lysozyme in sarcoidosis and correlation with angiotensin-converting enzyme. *Am J Clin Pathol.* 1977;68(2):219-224.
- Skoie IM, Wildhagen K, Omdal R. Development of sarcoidosis following etanercept treatment: a report of three cases. *Rheumatol Int*. 2012;32:1049–1053.
- Smith JK, Matheus MG, Castillo M. Imaging manifestations of neurosarcoidosis. *Am J Roentgenol*. 2004;182:289-295.
- Sones M, Israel HL, Dratman MB, Frank JH. Effect of cortisone in sarcoidosis. *N Engl J Med*. 1951;244:209–213.
- Spector WG. Immunologic components of granuloma formation. Epithelioid cells, giant cells, and sarcoidosis. *Ann N Y Acad Sci*. 1976;278:3–6.
- Stern BJ, Krumholz A, Johns C, Scott P, Nissim J. Sarcoidosis and its neurological manifestations. *Arch Neurol*. 1985;42(9):909-917.
- Stern BJ, Griffin DE, Luke RA, Krumholz A, Johns CJ. Neurosarcoidosis:

cerebrospinal fluid lymphocyte subpopulations. *Neurology*. 1987;37(5):878-881.

- Stern BJ, Aksamit A, Clifford D, Scott TF; Neurosarcoidosis Study Group. Neurologic presentations of sarcoidosis. *Neurol Clin*. 2010;28(1):185-198.
- Stern BJ, Royal W III, Gelfand JM, Clifford DB, Tavee J, Pawate S, Berger JR, Aksamit AJ, Krumholz A, Pardo CA, Moller DR, Judson MA, Drent M, Baughman RP. Definition and consensus diagnostic criteria for neurosarcoidosis: from the Neurosarcoidosis Consortium Consensus Group. *JAMA Neurol*. 2018;75(12):1546-1553.
- Stjepanović MI, Vucinić VM, Jovanović D, Mijajlović M, Trifunović VS, Ivanov JV. Radiological presentation of neurosarcoidosis. *Med Pregl*. 2014;67(1-2):24-27.
- Stjernberg N, Cajander S, Truedsson H, Uddenfeldt P. Muscle involvement in sarcoidosis. *Acta Med Scand*. 1981;209(3):213-216.
- Stokes GS, Monaghan JC, Schrader AP, Glenn CL, Ryan M, Morris BJ. Influence of angiotensin converting enzyme (ACE) genotype on interpretation of diagnostic tests for serum ACE activity. *Aust N Z J Med*. 1999;29(3):315-318.
- Stone JH, Zen Y, Deshpande V. IgG4-related disease. *N Engl J Med*. 2012;366(6):539-551.
- Sverrild A, Backer V, Kyvik KO, Kaprio J, Milman N, Svendsen CB, Thomsen SF. Heredity in sarcoidosis: a registry-based twin study. *Thorax.* 2008;63:894–896.
- Swaisgood CM, Oswald-Richter K, Moeller SD, Klemenc JM, Ruple LM, Farver CF, Drake JM, Culver DA, Drake WP. Development of a sarcoidosis murine lung granuloma model using. Mycobacterium superoxide dismutase A peptide. *Am J Respir Cell Mol Biol*. 2011;44:166–174.
- Sweiss NJ, Lower EE, Mirsaeidi M, Dudek S, Garcia JG, Perkins D, Finn PW, Baughman RP. Rituximab in the treatment of refractory pulmonary sarcoidosis. *Eur Respir J*. 2014;43(5):1525-1528.
- Tabuchi S, Uno T. Hydrocephalus with panventricular enlargement as the primary manifestation of neurosarcoidosis: a case report. *J Med Case Rep*. 2013;7:240.
- Tadros M, Forouhar F, Wu GY. Hepatic sarcoidosis. *J Clin Transl Hepatol*. 2013;1:87–93.

- Tahmoush AJ, Amir MS, Connor WW, Farry JK, Didato S, Ulhoa-Cintra A, Vasas JM, Schwartzman RJ, Israel HL, Patrick H. CSF-ACE activity in probable CNS neurosarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis*. 2002;19(3):191-197.
- Taibi L, Boursier C, Clodic G, Bolbach G, Bénéteau-Burnat B, Vaubourdolle M, Baudin B. Search for biomarkers of neurosarcoidosis by proteomic analysis of cerebrospinal fluid. *Ann Biol Clin (Paris)*. 2017;75(4):393-402.
- Takemori N, Nakamura M, Kojima M, Eishi Y. Successful treatment in a case of Propionibacterium acnes-associated sarcoidosis with clarithromycin administration: a case report. *J Med Case Rep.* 2014;8:15.
- Tana C, Wegener S, Borys E, Pambuccian S, Tchernev G, Tana M, Giamberardino MA, Silingardi M. Challenges in the diagnosis and treatment of neurosarcoidosis. *Ann Med*. 2015;47(7):576-591.
- Tateyama M, Nagano I, Yoshioka M, Chida K, Nakamura S, Itoyama Y. Expression of tumor necrosis factor-alpha in muscles of polymyositis. *J Neurol Sci*. 1997;146(1):45-51.
- Tavee JO, Culver D. Sarcoidosis and small-fiber neuropathy. *Curr Pain Headache Rep*. 2011;15(3):201-206.
- Tavee JO, Stern BJ. Neurosarcoidosis. *Continuum (Minneap Minn)*. 2014;20(3):545-559.
- Tavee JO, Stern BJ. Neurosarcoidosis. *Clin Chest Med*. 2015;36(4):643-656.
- Tavee JO, Karwa K, Ahmed Z, Thompson N, Parambil J, Culver DA. Sarcoidosis-associated small fiber neuropathy in a large cohort: Clinical aspects and response to IVIG and anti-TNF alpha treatment. *Respir Med*. 2017;126:135-138.
- Thillai M, Atkins CP, Crawshaw A, Hart SP, Ho LP, Kouranos V, Patterson KC, Screaton NJ, Whight J, Wells AU. BTS Clinical Statement on pulmonary sarcoidosis. *Thorax*. 2021;76:4–20.
- Tinelli C, De Silvestri A, Richeldi L, Oggionni T. The Italian register for diffuse infiltrative lung disorders (RIPID): a four-year report. *Sarcoidosis Vasc Diffuse Lung Dis.* 2005;22:S4-S8.
- Tobias S, Prayson RA, Lee JH. Necrotizing neurosarcoidosis of the cranial base resembling an en plaque sphenoid wing meningioma: case report. *Neurosurgery*. 2002;51(5):1290-4;discussion 1294.

- Tomita H, Sato S, Matsuda R, Sugiura Y, Kawaguchi H, Niimi T, Yoshida S, Morishita . Serum lysozyme levels and clinical features of sarcoidosis. *Lung*. 1999;177:161–7.
- Turton CW, Grundy E, Firth G, Mitchell D, Rigden BG, Turner-Warwick M. Value of measuring serum angiotensin I converting enzyme and serum lysozyme in the management of sarcoidosis. *Thorax.* 1979;34(1):57-62.
- Ungprasert P, Carmona EM, Utz JP, Ryu JH, Crowson CS, Matteson EL. Epidemiology of sarcoidosis 1946-2013: A population based study. *Mayo Clin Proc.* 2016;91:183-188.
- Ungprasert P, Crowson CS, Matteson EL. Clinical Characteristics of Parotid Gland Sarcoidosis: A Population-Based Study. *JAMA Otolaryngol Head Neck Surg*. 2016;142(5):503-504.
- Ungprasert P, Crowson CS, Matteson EL. Smoking, obesity and risk of sarcoidosis: A population-based nested case-control study. *Respir Med.* 2016;120:87-90.
- Ungprasert P, Crowson CS, Matteson EL. Risk of cardiovascular disease among patients with sarcoidosis: a population-based retrospective cohort study, 1976-2013. *Eur Respir J*. 2017;49(2):1601290.
- Ungprasert P, Matteson EL. Neurosarcoidosis. *Rheum Dis Clin North Am*. 2017;43(4):593-606.
- Valentonyte R, Hampe J, Huse K, Rosenstiel P, Albrecht M, Stenzel A, Nagy M, Gaede KI, Franke A, Haesler R, Koch A, Lengauer T, Seegert D, Reiling N, Ehlers S, Schwinger E, Platzer M, Krawczak M, Müller-Quernheim J, Schürmann M, Schreiber S. Sarcoidosis is associated with a truncating splice site mutation in BTNL2. *Nat Genet*. 2005;37:357–364.
- Valeyre D, Bernaudin JF, Uzunhan Y, Kambouchner M, Brillet PY, Soussan M, Nunes H. Clinical presentation of sarcoidosis and diagnostic work-up. *Semin Respir Crit Care Med*. 2014;35:336–351.
- van Dellen JR. "Equo ne credite, Teucri. Quidquid id est, timeo Danaos et dona ferentes". *World Neurosurg*. 2013;80(6):e215-217.
- van Eijk M, van Roomen CP, Renkema GH, Bussink AP, Andrews L, Blommaart EF, Sugar A, Verhoeven AJ, Boot RG, Aerts JM. Characterization of human phagocyte-derived chitotriosidase, a component of innate immunity.

*Int Immunol.* 2005;17(11):1505-1512.

- Vanhoof J, Wilms G, Bouillon R. Hypothalamic hypopituitarism with hyperphagia and subacute dementia due to neurosarcoidosis: case report and literature review. *Acta Clin Belg*. 1992;47(5):319-328.
- Vassalli P. The pathophysiology of tumor necrosis factors. *Annu Rev Immunol*. 1992;10:411-452.
- Vellodi A, Foo Y, Cole TJ. Evaluation of three biochemical markers in the monitoring of Gaucher disease. *J Inherit Metab Dis*. 2005;28:585–592.
- Vigne C, Tebib JG, Pacheco Y, Coury F. Sarcoidosis: an underestimated and potentially severe side effect of anti-TNF-alpha therapy. *Joint Bone Spine*. 2013;80(1):104-107.
- Visser H. Sarcoid arthritis: clinical characteristics, diagnostic aspects, and risk factors. *Ann Rheum Dis*. 2002;61:499–504.
- Vital A, Lagueny A, Ferrer X, Louiset P, Canron MH, Vital C. Sarcoid neuropathy: clinico-pathological study of 4 new cases and review of the literature. *Clin Neuropathol*. 2008;27(2):96-105.
- Voortman M, Hendriks CM, Elfferich MDP, Bonella F, Møller J, De Vries J, Costabel U, Drent M. The burden of sarcoidosis symptoms from a patient perspective. *Lung*. 2019;197:155–161.
- Voortman M, Drent M, Baughman RP. Management of neurosarcoidosis: a clinical challenge. *Curr Opin Neurol*. 2019;32(3):475-483.
- Vorselaars AD, Wuyts WA, Vorselaars VMM, Zanen P, Deneer VHM, Veltkamp M, Thomeer M, van Moorsel CHM, Grutters JC. Methotrexate vs azathioprine in second-line therapy of sarcoidosis. *Chest.* 2013;144(3):805-812.
- Vorselaars ADM, Verwoerd A, Van Moorsel CHM, Keijsers RGM, Rijkers GT, Grutters JC. Prediction of relapse after discontinuation of infliximab therapy in severe sarcoidosis. *Eur Respir J.* 2014;43:602–609.
- Wahlbeck K, Ahokas A, Nikkilä H, Miettinen K, Rimón R. Cerebrospinal fluid angiotensin-converting enzyme (ACE) correlates with length of illness in schizophrenia. *Schizophr Res*. 2000;41(2):335-340.
- Wallace ZS, Naden RP, Chari S, Choi HK, Della-Torre E, Dicaire JF, Hart PA, Inoue D, Kawano M, Khosroshahi A, Lanzillotta M, Okazaki K, Perugino CA, Sharma A, Saeki T, Schleinitz N, Takahashi N, Umehara H, Zen Y, Stone JH;

Members of the ACR/EULAR IgG4-RD Classification Criteria Working Group. The 2019 American College of Rheumatology/European League Against Rheumatism classification criteria for IgG4-related disease. *Ann Rheum Dis*. 2020;79(1):77-87.

- Wegener S, Linnebank M, Martin R, Valavanis A, Weller M. Clinically isolated neurosarcoidosis: a recommended diagnostic path. *Eur Neurol*. 2015;73(1-2):71-77.
- Welker L, Jorres RA, Costabel U, Magnussen H. Predictive value of BAL cell differentials in the diagnosis of interstitial lung diseases. *Eur Respir J*. 2004;24:1000–1006.
- Wu CH, Chung PI, Wu CY, Chen YT, Chiu YW, Chang YT, Liu HN. Comorbid autoimmune diseases in patients with sarcoidosis: A nationwide case-control study in Taiwan. *J Dermatol.* 2017;44(4):423-430.
- Yanardağ H, Pamuk ON, Karayel T. Cutaneous involvement in sarcoidosis: analysis of the features in 170 patients. *Respir Med.* 2003;97(8):978-982.
- Yanardağ H, Tetikkurt C, Bilir M. Clinical and prognostic significance of muscle biopsy in sarcoidosis. *Monaldi Arch Chest Dis*. 2018;88(1):910.
- Yoshitomi M, Uchikado H, Hattori G, Sugita Y, Morioka M. Endoscopic biopsy for the diagnosis of neurosarcoidosis at the fourth ventricle outlet with hydrocephalus. *Surg Neurol Int*. 2015;6(Suppl 25):S633-6.
- Zajicek JP, Scolding NJ, Foster O, Rovaris M, Evanson J, Moseley IF, Scadding JW, Thompson EJ, Chamoun V, Miller DH, McDonald WI, Mitchell D. Central nervous system sarcoidosis--diagnosis and management. *QJM*. 1999;92(2):103-117.
- Zamora C, Hung SC, Tomingas C, Atkinson C, Castillo M. Engorgement of deep medullary veins in neurosarcoidosis: a common-yet underrecognized cerebrovascular finding on SWI. *AJNR Am J Neuroradiol*. 2018;39:2045e50.
- Zella S, Kneiphof J, Haghikia A, Gold R, Woitalla D, Thöne J. Successful therapy with rituximab in three patients with probable neurosarcoidosis. *Ther Adv Neurol Disord*. 2018;11:1756286418805732.
- Zisman DA, Shorr AF, Lynch JP 3rd. Sarcoidosis involving the musculoskeletal system. *Semin Respir Crit Care Med*. 2002;23(6):555-570.
- Zissel G, Prasse A, Müller-Quernheim J. Immunologic response of sarcoidosis.

*Semin Respir Crit Care Med*. 2010;31: 390–403.