Title:

Distinguishing neurosarcoidosis from MS based on CSF analysis : A retrospective cohort study

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Abstract:

Objective: To characterise a cohort of neurosarcoidosis (NS) patients with particular focus on cerebrospinal fluid (CSF) analysis and investigate whether CSF values could help in distinguishing it from multiple sclerosis (MS).

Methods: This retrospective cohort study enrolled 85 patients with a diagnosis of neurosarcoidosis (possible, probable, or definite). CSF total protein (TP), white cell count (WCC) and angiotensin converting enzyme levels (ACE) were measured. CSF and serum oligoclonal IgG patterns were analysed using odds ratios and binary logistic regression.

Results: 80 patients had a probable (non-neural positive histology) or definite (neural positive histology) diagnosis of neurosarcoidosis. Most frequent findings on MRI were leptomeningeal enhancement (35%), white matter and spinal cord involvement (30% and 23%). PET scan showed avid areas in 74% of cases. CSF analysis frequently showed lymphocytosis (63%) and elevated protein (62%), but CSF selective oligoclonal bands were rare (3%). Serum ACE levels were elevated in 51% of patients, but in only 14% of those with isolated neurosarcoidosis. Elevated CSF-ACE was not found in any patient.

Conclusions: Large elevations in TP, WCC and serum ACE occur in neurosarcoidosis, but are rare in MS. The diagnostic use of these tests is, however, limited as minimal changes may occur in both. MS clinical mimics in neurosarcoidsosis are not common and intrathecal synthesis of oligoclonal IgG is a powerful discriminator as it is rare in neurosarcoidosis whilst occurring in 95-98% cases of MS. We suggest caution in making a diagnosis of neurosarcoidosis when intrathecal oligoclonal IgG synthesis is found.

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1. INTRODUCTION

Sarcoidosis is an inflammatory condition of unknown etiology featuring the formation of non-caseating granulomas in various tissues and is termed neurosarcoidosis (NS) when present in the central nervous system (CNS). Although neurosarcoidosis is rare, 5-15% of patients with sarcoidosis may develop the condition (1,2). Moreover, only around half of patients with positive histological evidence of neurosarcoidosis received a diagnosis prior to death, suggesting many patients either have sub-clinical disease or are misdiagnosed (3). This is a difficult challenge to overcome due to the current absence of definitive diagnostic criteria and the eloquence of the pathological location.

Under the current criteria, no test other than CNS biopsy and histology can be diagnostic of neurosarcoidosis (4), but it has been suggested that the combination of CSF analysis, imaging, and clinical features may be sufficient in future, thus avoiding the necessity of invasive biopsy (5).

We describe distinctive features of neurosarcoidosis by way of a well defined and large cohort of biopsy proven cases.

We seek to further the current understanding by analysis of total protein, white cell count, and angiotensin converting enzyme (ACE) in the CSF, however our specific focus is on oligoclonal bands (OCBs), as their high prevalence in MS may prove an invaluable differentiating diagnostic test (9,10,11).

2. METHODS

This retrospective study enrolled patients who had been referred to the large tertiary neurosarcoidosis clinic at the University Hospitals in Coventry and Warwickshire and had undergone further investigation following examination by the consultant neurologist. Patients were diagnosed over a 11 year period between January 2007 and April 2018. Only patients with 'definite (positive CNS biopsy) or 'probable' (non-CNS positive biopsy) neurosarcoidosis were included (1) and patients with 'possible' neurosarcoidosis (no histological support) were excluded. The diagnosis also requires the exclusion of other mimics.

Standard Protocol Approvals, Registrations, and Patient Consents.

The study was approved by the Biomedical and Scientific Research Ethics Committee: REGO-2018-2236.

We reviewed the readily collected and anonymised data in 85 consecutive patients with suspected and definite neurosarcoidosis. The data was analysed using the SPSS version 24 software, with tests of association including odds ratios, chi squares, and binary logistic regressions performed. Isoelectric focussing (IEF) was used to analyse oligoclonal bands, as this is the most sensitive method. Each paired sample of serum and CSF was analysed using the Hydragel CSF isofocussing (Sebia) gel, performed using Sebia Hydrasys system (Sebia; Norcross, GA). The procedure involves isoelectric focusing on agarsose followed by immunofixation with peroxidase labeled anti-IgG antiserum. IgG concentrations in both serum and CSF were adjusted to the same level. The presence of two or more discrete bands of IgG was interpreted as oligoclonal band positive. The laboratory that we use is a member of the external quality control program of UK NEQAS for CSF oligoclonal bands, and the internal quality control is also monitored regularly. All patients included in the study were tested using the same technique. All patients whose CSF results are reported in this paper were treatment naïve at the time of the CSF analysis.

CSF protein levels are reported with age-adjusted reference levels (12) , and the cut off for lymphocytic pleocytosis was 5/μL.

Data availability statement

Requests for access to the data reported in this article will be considered by the corresponding author.

3. RESULTS

3.1 DEMOGRAPHICS

The cohort included 45 (56%) female patients and 35 (44%) male. Age of diagnosis of neurosarcoidosis ranged from 19 to 76 years old, with an overall mean age of 47.8 years: 45.2 in males and 50.0 in females (Table 1).

3.2 CATEGORIES OF SARCOIDOSIS

Among the data from the 85 patients analysed, 80 patients were categorised as 'highly probable' (definite and probable as per the Zajicek criteria (4)). The category of highly probable patients included 11 definite (14%) and 69 probable (86%) patients. Five patients who were scored as possible under the Zajicek criteria were excluded from the analysis.

3.3 AREAS OF SARCOIDOSIS INVOLVEMENT

Of the patients included, 50/80 (63%) had an existing diagnosis of systemic sarcoidosis prior to onset of neurological symptoms, 22/80 (28%) presented with neurological symptoms and developed systemic involvement later in the course of their disease, and 8/80 (10%) had isolated biopsy proven neurosarcoidosis.

We found that by far the commonest area of systemic sarcoidosis involvement was pulmonary (74%), followed by lymph nodes (16%), ocular (14%), and cutaneous (13%) (Figure 1). Only eight (10%) patients had isolated neurosarcoidosis with no systemic involvement. We ran statistical tests

to investigate whether having isolated neurosarcoidosis had a marked effect on the probability of having abnormal CSF parameters, but no significant correlations were found. Patients with definite neurosarcoidosis were half as likely to have systemic involvement in general than patients with probable neurosarcoidosis, with definite patients having 18% isolated neurosarcoidosis, compared to 9% for probable patients (Figure 1). ***This can be may be attributed to the fact that patients with systemic involvement will have a non-neural tissue biopsy and thus be classified as probable, whereas in cases with only neurological involvement patients will have a CNS biopsy and thus be classified as definite.***

The commonest symptoms of neurological involvement were limb weakness and headaches with 41% and 35% prevalence respectively. Of those reporting limb weakness, 31% (25/80) had pyramidal symptoms, and 14% (11/80) of patients had peripheral nerve involvement alone. Other common symptoms included sensory disturbance (23%), ataxia (20%), facial weakness (16%), facial sensory disturbance (13%), optic neuritis (13%), and cognitive decline (11%). Less common symptoms were diplopia (9%), seizure (8%), pain (6%), hearing loss/tinnitus (6%), incontinence (6%), drowsiness (5%), dizziness/vertigo (4%), and vomiting (3%). One patient presented with transverse myelitis (1%), and one patient presented with stroke (1%).

3.4 CEREBROSPINAL FLUID

3.4.1 OCBs

CSF findings are shown in Table 2. Of patients who were identified as highly probable, 70 were analysed for the presence of OCBs. Only 2/70 (3%) were found to have unmatched OCBs (intrathecal synthesis alone). Among patients with definite neurosarcoidosis, no patient had CSF selective oligoclonal band synthesis. For patients with probable neurosarcoidosis, 8/60 (13%) tested positive for OCBs, and only 2/60 (3%) patients had OCBs isolated to the CSF.

3.4.2 CSF ELEVATED PROTEIN

Within our cohort, when we applied age adjusted reference levels (12), CSF protein levels were found to be elevated in 42/73 (57.5%) patients. The median protein elevation was 1.1 (range 0.5 -9.8) g/dl. Seven of ten patients with a diagnosis of definite neurological sarcoidosis had protein elevation greater than 1g/dl. Of patients that has isolated OCB synthesis within the CSF only, 2/3 (66%) had elevated protein compared to 33/60 (55%) of OCB negative patients. This shows the trend that OCB positive patients were more likely to have elevated CSF protein, but it was not statistically significant (p=0.11).

3.4.3 CSF WHITE CELL COUNT

Elevated white cell count was found to be present in 47/75 (63%), with a white cell count median of 40/μL (range 5-320). 9/11 (82%) of patients with definite neurosarcoidosis had white cell count over 5, and 7/11 (64%) had greatly elevated levels of over 50. Elevated white cell counts were invariably due to a lymphocytosis. There was significant association between elevated protein and lymphocytosis (OR 14.08, p=0.001), with 79% (58/73) of patients having the same result of either both raised or both negative.

3.4.4 CSF AND SERUM ACE

0/27 (0%) patients tested positive for CSF-ACE. Just over half of patients (28/75 (51%)) had elevated serum ACE, however of those patients with isolated neurosarcoidosis, only 14% (1/7) had elevated serum ACE.

We ran association analysis with systemic sarcoidosis involvement and CSF abnormalities, with one significant result found. Binary logistic regression revealed patients with ocular sarcoidosis involvement were significantly more likely to have OCBs present in the CSF (OR 6.90, p=0.035), with 3/7 (43%) patients with ocular symptoms being positive for OCBs. This is significantly higher than the 11% (7/63) prevalence found in patients without ocular sarcoidosis involvement. They were

all patients with a diagnosis of probable neurosarcoidosis. None of these patients were positive for CSF OCBs.

3.5 IMAGING

3.5.1 MRI

MRI of the brain and spine (entire neuraxis) was performed in 78 patients. 13 of 78 (17%) patients had a normal MRI. The proportion of patients with definite neurosarcoidosis who had a normal MRI was 10% (1/10), significantly lower than that of patients with probable neurosarcoidosis at 18% (12/68). Patients with normal MRI had a high prevalence of peripheral neuropathy at 54% (7/13). Other features included cranial neuropathies (4/13, 31%), audiovestibular involvement (1/13, 8%), and optic neuritis (1/13, 8%). None of the patients with either transverse myelitis or brain symptoms had a normal brain or spinal cord MRI.

Most patients had positive findings on MRI of the central nervous system (83%), with 59% having enhancing lesions (figure 3A and 3B) and 35% having specifically leptomeningeal enhancement. The commonest areas of the CNS involvement identified on MRI were the meninges (35%), the white matter (30%), the spinal cord (23%), and the brainstem (19%) (Figure 2). Cord lesions were frequently cervical 10/17 (59%) (Figure 3C and 3D) followed by the thoracic spinal cord 5/17 (29%) and conus involvement in 2/17 (12 %). All cord lesions were enhancing and 12/17 (70%) patients had long segment lesions (more than 3 vertebral segments), with involvement of the dorsal cord most frequently. Optic nerve lesions were anterior short segment lesions and showed high signal with contrast enhancement most commonly 6/10 (60%) (Figure 3E and 3F). The remainder of those with optic neuropathy was due to a compressive or infiltrative lesion at the orbital apex 4/10 (40%).

The great majority of patients who had abnormal CSF results also had abnormal findings on MRI of the neuroaxis: 94% of those with lymphocytosis, 96% of patients with elevated protein, and 100% of patients with OCBs. Association tests for any significant relationship between abnormal MRI and

CSF abnormalities showed that those with leptomeningeal enhancement on MRI were significantly less likely to have isolated intrathecal OCBs (OR 0.07, p=0.053), with only 1/25 (4%) patients with leptomeningeal enhancement testing positive for OCBs. Conversely, those with brainstem involvement on MRI were *more* likely to have OCBs (either intrathecal only or matched) (OR 9.01, p=0.021): 4/14 (29%) patients with brainstem involvement had OCBs (Table 3).

MRI scans were more likely to be normal in patients presenting with peripheral neuropathy, facial weakness, and trigeminal neuropathy, but this was not statistically significant.

3.5.2 PET

53 patients underwent a PET scan, 39 of which were positive (74%). Of these patients, 3/5 (60%) who had isolated neurosarcoidosis had a positive PET result, compared to 36/48 (75%) of patients with systemic involvement. Avid areas were seen in lymph nodes (68%), lungs (38%), bone (11%), liver (4%), skin (4%), and spinal cord (2%).

4. DISCUSSION

This is a large study histologically supported neurosarcoidosis and the largest group of definite neurosarcoid cases with CSF results. We show that CSF unmatched oligoclonal bands are rarely present in histologically supported neurosarcoidosis, and thus their presence is a red flag. Additionally, we have contributed to the evidence that elevated CSF protein and WBC counts are seen in the majority of patients.

We showed that both clinical characteristics and MRI findings in neurosarcoidosis are very distinct from multiple sclerosis. PET scans were helpful in many patients with isolated neurosarcoidosis.

Our results of our study were in agreement with a study by Joseph *et al.* (3) which found that three of eleven (27%) patients with probable neurosarcoidosis who underwent isoelectric focusing of their CSF were found to be unmatched oligoclonal band positive, but that four patients with definite

neurosarcodosis who were tested were all negative. In a study of 68 neurosarcodosis patients, Zajicek *et al.* (4) showed CSF oligoclonal bands were present in 34 patients, with 22 patients having CSF selective oligoclonal bands, however only one of the four patients with definite neurosarcoidosis tested positive for oligoclonal bands . Borucki *et al*. (15) and McLean *et al. (11)* also demonstrated that the occurrence of CSF selective oligoclonal bands was an uncommon occurrence. However, these studies had a relatively small number of patients. In contrast a recent larger study of patients with probable and definite neurosarcoidosis showed a greater prevelance of CSF selective oligoclonal bands in patients with isolated CNS involvement when compared to those with systemic disease (50% vs 23%), and importantly the presence of oligoclonal bands isolated CSF studies was seen only in patients with CNS isolated disease. This study included six patients with definite neurosarcoidosis (16) (Table 4).

Our findings have important implications in differentiating neurosarcoidosis from MS, one of the important differential diagnoses, where unmatched OCBs are present in 95-98% of cases (9-11). A review of previously proported multiple sclerosis cases without intrathecal synthesis revealed doubts about the diagnosis in 22 of the 34 patients, and in three of the six cases who underwent a further lumbar puncture, intrathecal synthesis later became apparent. Moreover, the few clinically definite but pathologically unconfirmed MS cases without intrathecal synthesis tended to have benign disease (10).

Our findings are supported by a postmortem study on two patients who had oligoclonal intrathecal IgG synthesis and a diagnosis of neurosarcoidosis during life, as it failed to reveal sarcoid granulomas in either. Indeed, multiple sclerosis was found in one case, and a necrotizing vasculitis was found in the other. Other immunological studies on the cerebrospinal fluid in neurosarcoidosis have demonstrated variable results (17-19) Table 4 , nevertheless we would strongly suggest caution in making a solitary diagnosis of neurosarcoidosis in the presence of intrathecal synthesis.

A literature review of other studies on CSF analysis in neurosarcoidosis is presented in Table 4. In two of the five studies data on CSF restricted oligoclonal bands were not available in patients with a diagnosis of definite neurosarcoidosis (4,18). In one study where identical oligoclonal bands were seen in the CSF and serum (10) the current Zajicek criteria for the diagnosis of sarcoidosis was not used and in the other study (15) where a small cohort of patients with definite neurosarcoidosis were reported, the reason for difference in results could not be identified. However given the important effect of inclusion criteria upon findings, the different incidence of intrathecal synthesis among published series may simply reflect different inclusion criteria. Furthermore, difficulties exist with the interpretation of iso-electric focusing, particularly when identical oligoclonal patterns are present in serum and CSF ('mirror pattern'). Mirror patterns are relatively common, often transient and occur most commonly in association with systemic infections. Importantly, a mirror pattern does not indicate intrathecal synthesis. In clinical practice identical bands in CSF and serum are not used for diagnostic purposes although some previous studies included this thus we have noted them in this paper for across study comparison only. Presence different oligoclonal band patterns in serum and CSF may be reflection of where the inflammatory response is being primarily driven. A peripheral drive may associate with identical oligoclonal bands patterns as in patients with neuromyelitis optica, sarcoidosis or systemic infection (37,38).

Other CSF parameters can also help distinguish neurosarcoidosis from its mimics. Large elevations in protein (>1g/dl) and white cell count in the CSF are rare in multiple sclerosis, but these are well-established changes found in around 60% of neurosarcoidosis patients (20), as supported by our results (63% had elevated protein and 62% had lymphocytosis). Although white count elevations may also be found in MS, the elevation is usually mild, with a cell count of greater than 50/μL rarely seen (21). Protein elevation found in MS is also usually mild, predominantly below the 0.5g/L cut off used in this study (6). However, the diagnostic use of these tests in an individual patient is limited since mild abnormalities may occur in both conditions.

Overall, we found that approximately a quarter of patients with probable or definite neurosarcoidosis had completely normal CSF results, in keeping with previous reporting of up to 30% (21,22). However, when looking at only patients with definite neurosarcoidosis, 0/10 (0%) had normal CSF results, supporting the view that normal CSF results are uncommon in neurosarcoidosis (24). We also found that 7/10 (70%) patients with a diagnosis of definite neurosarcoidosis had protein elevation over 1g/dl, and 7/11 (64%) had white cell counts over 50 in the CSF.

CSF angiotensin-converting enzyme (CSF-ACE) has been proposed as a valuable diagnostic tool with a high specificity for neurosarcoidosis (94-95%), however there is limited data to support its performance in routine clinical practice, with concerns over limited sensitivity (24-55%) (25,26). Our results support these concerns as no patient (0/27) patients tested positive for increased CSF-ACE activity. Just over half of patients (28/75 (51%)) had elevated serum ACE; this was higher than the 23% reportedly found in MS (27). However, of those patients with isolated neurosarcoidosis, only 14% (1/7) had elevated serum ACE. This supports the trend that prevalence of elevated serum ACE levels is lower for isolated neurosarcoidosis than for patients with systemic involvement (6), however the results were not statistically significant. In addition, elevated serum ACE is non-specific (28) and often affected by polymorphisms in the ACE gene in the healthy population (29) and is thus a poor diagnostic test for neurosarcoidosis.

It has been reported that it is very rare to have isolated neurosarcoidosis (range 1-17%) (7,30), with 90% of patients on average having coexistent systemic sarcoidosis (2). This is the exact prevalence our study also found: 8/80 (10%) had isolated neurosarcoidosis, with 63% having an existing diagnosis of systemic sarcoidosis at time of neurological onset, and ultimately 72/80 (90%) having coexistent systemic sarcoidosis by the point of data collection. In previous literature, cranial neuropathy is the commonest neurological symptom, present in 48% of patients. The facial nerve is reportedly the most frequently involved cranial nerve (2), as indeed it was in our study (16%).

Gadolinium-enhanced MRI is the preferred imaging modality for neurosarcoidosis (31), and 59% of patients in our study showed gadolinium enhancement on their brain and spinal cord imaging. In line with previous studies, MRI features were highly variable (2,4): Both enhancing and non-enhancing lesions were frequently found in various locations in the brain and spinal cord. 35% of our patients had leptomeningeal involvement, which supports the previously reported frequency of up to 40% of cases (7), a finding that is very rare in MS. Although 4/78 (5%) had MRI features indistinguishable for MS they did not meet the 2017 MS diagnostic criteria when the *no better explanation* clause was applied. We found that MRI appearances in general were different in the two conditions and played a important role in distinguishing them.

A few studies have suggested FDG-PET scan may be a useful alternative imaging test diagnosis (32-34), and it was found to have a sensitivity of 74% in our study, although this dropped to 60% for isolated neurosarcoidosis patients. We found that MRI abnormalities had an overall sensitivity of 83% in our study, and 100% for isolated neurosarcoidosis, hence we support the consensus that gadolinium-enhanced MRI should be the preferred imaging modality.

Neurosarcoidosis is typically a chronic condition that progresses over several years. It has a varied prognosis, with some patients recovering after corticosteroid therapy, and others suffering multiple relapses and developing serious functional sequelae (34).

Corticosteroids are first line treatment, with a range of options for second line treatment: azathioprine, methotrexate, mycophenolate, and hydroxychloroquine, among others. For refractory neurosarcoidosis, infliximab is third line (35). Treatment for MS is different to neurosarcoidosis and differs depending on the subtype, but for all types of MS corticosteroids are only used short-term for acute attacks (36), whereas many neurosarcoidosis sufferers need to be on long-term corticosteroid

therapy. Moreover, early symptomatic treatment is advised for neurosarcoidosis, thus there is a clear need for more prompt diagnosis to allow commencement of the appropriate therapy.

Limitations of this study include that it is retrospective, and we are thus dependent on the accuracy of existing medical records. We were also limited by the observational nature of the study, including analysis of patients at different times of their disease course, as well as missing data. These limitations are inherent to the study design, however we minimised the effect of missing data by presenting results as n of N (%). However, the strengths of our study include the separation of probable and definite neurosarcoidosis and the exclusion of possible neurosarcoidosis where the diagnosis may be incorrect and may thus lead to reports of CSF unmatched OCBs in neurosarcoidosis.

5. CONCLUSION

This study characterises a large number of histology supported neurosarcoidosis cases, covering clinical, imaging, and CSF findings. In contributing data on the usefulness of CSF parameters, we highlight that the absence of CSF OCBs is a useful distinguisher from MS and should be considered in future neurosarcoidosis criteria. Elevated WBC and protein levels may also be supporting criteria to distinguish from MS particularly when significantly elevated. Additionally, we found that measurement of CSF-ACE was not contributory and indeed was not seen in any patient, disputing the findings of previous studies.

REFERENCES

1. Hoitsma E, Faber C, Drent M, Sharma O. Neurosarcoidosis: a clinical dilemma. Lancet Neurol 2004;3(7):397-407.

2. Cação G, Branco A, Meireles M, Alves J, Mateus A, Silva A et al. Neurosarcoidosis according to Zajicek and Scolding criteria: 15 probable and definite cases, their treatment and outcomes. J Neurol Sci 2017;379(1):84-88.

3. M.L. Carlson, J.R. White, M. Espahbodi, D.S. Haynes, C.L. Driscoll, A.J. Aksamit, S. Pawat e, J.I.Lane, M.J. Link. Cranial base manifestations of neurosarcoidosis: a review of 305 patients. Otol Neurotol 2015;36(1):156-166.

4. J.P. Zajicek, N.J. Scolding, O. Foster, M. Rovaris, J. Evanson, I.F. Moseley, J.W. Scadding, E.J. Thompson, V. Chamoun, D.H. Miller, W.I. McDonald, D. Mitchell, Central nervous system sarcoidosis—diagnosis and management, *QJM: An International Journal of Medicine*, Volume 92, Issue 2, February 1999, Pages 103–117.

5. Reske D, Petereit H, Heiss W. Difficulties in the differentiation of chronic inflammatory diseases of the central nervous system - value of cerebrospinal fluid analysis and immunological abnormalities in the diagnosis. Acta Neurol Scand 2005;112(4): 207-213.

6. Gelfand J. Chapter 12 - Multiple sclerosis: diagnosis, differential diagnosis, and clinical presentation. In: Goodin D, ed. by. Handb Clin Neurol 2014;122:269-290.

7. Smith J, Matheus M, Castillo M. Imaging Manifestations of Neurosarcoidosis. AJR Am J Roentgenol 2004;182(2):289-295.

8. Dora Liu, Alexandra Ahmet, Leanne Ward, Preetha Krishnamoorthy, Efrem D Mandelcorn,Richard Leigh, Jacques P Brown, Albert Cohen, Harold Kim. A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. Allergy, Asthma & Clinical Immunology**volume 9**, Article number: 30 (2013)

9. Link H. The cerebrospinal fluid in multiple sclerosis. In: Swash M, Oxbury J, editors. Clin Neurol 1991;221(1):1128-1139.

10. Zeman AZ, Kidd D, McLean BN, Kelly MA, Francis DA, Miller DH, et al. A study of oligoclonal band negative multiple sclerosis. J Neurol Neurosurg Psychiatry Res 1996;60(1): 27-30.

11. McLean BN, Mitchell DN, Thompson EJ. Local synthesis of specific IgG in the cerebrospinal fluid of patients with neurosarcoidosis detected by antigen immunoblotting using Kveim material. J Neurol Sci 1990;99(2-3):165-75.

12. Mc Cudden et al, Clinical Chemistry 1856-1865 (2017)

13. Joseph FG, Scolding NJ. Neurosarcoidosis: a study of 30 new cases. J Neurol Neurosurg Psychiatry 2009;80(3):297–304.

14. Borucki SJ, Nguyen BV, Ladoulis CT, McKendall RR. Cerebrospinal Fluid Immunoglobulin Abnormalities in Neurosarcoidosis. *Arch Neurol* 1989;46(3):270-273

15. Kidd, D.P. J Neurol (2018) 265: 1906. Sarcoidosis of the central nervous system: clinical features, imaging, and CSF results.

16. Mitchell JD, Yap PL, Milne LA, Lachmann PJ, Pentland B (1985) Immunological studies on the cerebrospinal fluid in neurological sarcoidosis. J Neuroimmunol 7:249–253.

17. Stern BJ, Krumholz A, Johns C, Scott P, Nissim J. Sarcoidosis and its neurological manifestations. Arch Neurol. 1985;42(9):909-917.

18. Borucki SJ, Nguyen BV, Ladoulis CT, McKendall RR. Cerebrospinal fluid immunoglobulin abnormalities in neurosarcoidosis. Arch Neurol 1989;46:270–273.

19. Fritz D, van de Beek D, Brouwer M. Clinical features, treatment and outcome in neurosarcoidosis: systematic review and meta-analysis. BMC Neurol 2016;16(1):220.

20. Freedman M, Thompson E, Deisenhammer F, Giovannoni G, Grimsley G, Keir G et al. Recommended Standard of Cerebrospinal Fluid Analysis in the Diagnosis of Multiple Sclerosis. Arch Neurol 2005;62(6):865-870.

21. Oksanen V. Neurosarcoidosis: clinical presentations and course in 50 patients. Acta Neurol Scand 2009;73(3):283-290.

22. Terushkin V, Stern B, Judson M, Hagiwara M, Pramanik B, Sanchez M et al. Neurosarcoidosis. Neurologist. 2010;16(1):2-15.

23. Oliver Wengert, Eva Rothenfusser-Korber, Boris Vollrath, Georg Bohner, Franziska Scheibe, Carolin Otto, Jörg Hofmann, Klemens Angstwurm, Klemens Ruprecht, Neurosarcoidosis: Correlation of cerebrospinal fluid findings with diffuse leptomeningeal gadolinium enhancement on MRI and clinical disease activity, Journal of the Neurological Sciences, Volume 335, Issues 1–2, 2013, Pages 124-130.

24. Khoury J, Wellik K, Demaerschalk B, Wingerchuk D. Cerebrospinal Fluid Angiotensin-Converting Enzyme for Diagnosis of Central Nervous System Sarcoidosis. Neurologist 2009;15(2):108-111.

25. Bridel C, Courvoisier D, Vuilleumier N, Lalive P. Cerebrospinal fluid angiotensin-converting enzyme for diagnosis of neurosarcoidosis. J Neuroimmunol 2015;285(1):1-3.

26. Constantinescu CS, Goodman DB, Grossman RI, Mannon LJ, Cohen JA. Serum angiotensin-converting enzyme in multiple sclerosis. Arch Neurol 1997;54:1012–1015.

27. Bunting P, Szalai J, Katic M. Diagnostic aspects of angiotensin converting enzyme in pulmonary sarcoidosis. Clin Biochem 1987;20(3):213-219.

28. Nesterovitch A, Hogarth K, Adarichev V, Vinokour E, Schwartz D, Solway J et al. Angiotensin I-converting enzyme mutation (Trp1197Stop) causes a dramatic increase in blood ACE. PLoS One 2009;4(12):8282.

29. Chapelon C, Ziza J, Piette J, Levy Y, Raguin G, Wechsler B et al. Neurosarcoidosis. Medicine. 1990;69(5):261-276.

30. Sherman J, Stern B. Sarcoidosis of the CNS: comparison of unenhanced and enhanced MR images. AJR Am J Roentgenol 1990;155(6):1293-1301.

31. Akaike G, Itani M, Shah H, Ahuja J, Yilmaz Gunes B, Assaker R et al. PET/CT in the Diagnosis and Workup of Sarcoidosis: Focus on Atypical Manifestations. RadioGraphics 2018;38(5):1536-1549.

32. Nishiyama Y, Yamamoto Y, Fukunaga K, Takinami H, Iwado Y, Satoh K, Ohkawa M. Comparative evaluation of 18F-FDG PET and 67Ga scintigraphy in patients with sarcoidosis. J Nucl Med 2006;47(10):1571-6.

33. Dubey N, Miletich R, Wasay M, Mechtler L, Bakshi R. Role of fluorodeoxyglucose positron emission tomography in the diagnosis of neurosarcoidosis. J Neurol Sci 2002;205(1):77-81.

34. Gascón-Bayarri J, Mañá J, Martínez-Yélamos S, Murillo O, Reñé R, Rubio F. Neurosarcoidosis: report of 30 cases and a literature survey. Eur J Intern Med 2011;22(6):125-132.

35. Hebel R, Dubaniewicz-Wybieralska M, Dubaniewicz A. Overview of neurosarcoidosis: recent advances. J Neurol 2014;262(2):258-67.

36. Compston A, Coles A. Multiple sclerosis. Lancet 2008;372(9648):1502–17.

37. Jarius S1, Paul F, Franciotta D, Ruprecht K, Ringelstein M, Bergamaschi R, Rommer P, Kleiter I, Stich O, Reuss R, Rauer S, Zettl UK, Wandinger KP, Melms A, Aktas O, Kristoferitsch W, Wildemann B. Cerebrospinal fluid findings in aquaporin-4 antibody positive neuromyelitis optica: Results from 211 lumbar punctures. J Neurol Sciences Volume 306, 2011

38. Zeman A, McLean B, Keir G, Luxton R, Sharief M, Thompson E. The significance of serum oligoclonal bands in neurological diseases. J Neurol Neurosurg Psychiatry. 1993 Jan;56(1):32-5

Table Headings & Figure Legends

Table 1: Patient and disease characteristics

Table 2. CSF results

Table 3. MRI findings and CSF values

Table 4. Review of Current Literature on CSF analysis in patients with neurosarcoidosis

Figure 1. Prevalence of areas of systemic sarcoidosis involvement

Data are the percentage prevalence of areas of systemic sarcoidosis involvement found in our patients. The left-hand bar is the prevalence for all 'highly probable' patients (n=80), the middle bar is the prevalence for all definite patients (n=11), and the right-hand bar is the prevalence for all probable patients (n=69). For clarity, actual percentage figures are included above the relevant bars.

Figure 2. Prevalence of areas of brain involvement on MRI

Data are the prevalence of areas of brain involvement on MRI for all 'highly probable' patients who underwent MRI (n=78).

Figure 3: **Imaging characteristics in our cohort of neurosarcoidosis patients**

A) T1 post contrast shows parenchymal lesions with avid enhancement (white arrows).

B) Multiple contrast enhancing small nodular lesions on the surface of the brain and on the infundibulum. There is sugar coating of the brain stem (white arrows). The findings are suggestive of a granulomatous disorder spreading in the subarachnoid space.

C) Sagittal imaging of cervical cord showing T2 high signal.

D) T1 post GAD imaging showing cervical spinal involvement with deposits on the surface of the cord and adjacent oedema (white arrows).

E) Avid anterior contrast enhancement on T1 post GAD imaging.

(F) Right optic nerve involvement with high signal on T2-weighted (white arrows).

Table 1: Patient and disease characteristics

Characteristics	n/N (%)	Characteristics	n/N (%)
Age at NS diagnosis	48 (19-74)	***Neurological Symptoms***	
Female Sex	45/80 (56%)		
Sarcoidosis known at onset of NS	30/80 (38%)	Diplopia	8/80 (10%)
		Facial sensory	10/80 (13%)
		Facial weakness	13/80 (16%)
		Optic neuritis	10/80 (13%)
		Hearing loss/tinnitus	5/80 (6%)
Systemic involvement		Limb weakness	33/80 (41%)
Pulmonary	59/80 (74%)	Sensory disturbance	23/80 (23%)
Cutaneous	10/80 (13%)	Headache	28/80 (35%)
Cardiac	2/80 (3%)	Drowsiness	4/80 (5%)
Lymph	13/80 (16%)	Vertigo	3/80 (4%)
Ocular	11/80 (14%)	Seizure	6/80 (8%)
Sialadens	4/80 (5%)	Cognitive decline	9/80 (11%)
Bone	1/80 (1%)	Urinary	5/80 (6%)
Joints	5/80 (6%)	Ataxia	16/80 (20%)
Spleen	2/80 (3%)	Pituitary	2/80 (3%)
Kidney	2/80 (3%)	Vomiting	2/80 (3%)
Liver	2/80 (3%)	Pyrexia	2/80 (3%)

Table 2. CSF results

Intrathecal Oligoclonal Band Synthesis	
Present in all 'Highly Probable' Patients	2/70 (3%)

Present in Definite NS Patients	0/10 (0%)
Present in Probable NS Patients	2/60 (3%)
Total Protein	
Total Protein ≥0.5g/L	45/73 (62%)
Protein Median* (Range*) g/dl	1.1 (0.5-9.8)
White Cell Count	
WCC ≥5/μL	47/75 (63%)
WCC Median* (Range*)	40 (5-320)
CSF ACE	
CSF ACE Elevation	0/27 (0%)

Medians and ranges are for abnormal values

Table 3. MRI findings and CSF values

	Prevalence	OCBs (CSF selective)	OCBs (matched in serum and CSF)	Elevated protein	Lymphocytosis
Abnormal MRI	65/78 (83%)	2/61 (3%)	8/61 (13%)	43/64 (67%)	43/64 (67%)
Enhancing lesions	43/73 (59%)	0/40 (0%)	4/40 (10%)	29/43 (67%)	29/43 (67%)
Leptomeningeal enhancement	28/78 (35%)	0/25 (0%)	1/25 (4%)	20/28 (71%)	20/28 (71%)
Area of Involvement					
Meninges	28/78 (35%)	0/25 (0%)	1/25 (4%)	20/28 (71%)	20/28 (71%)
White matter	23/78 (30%)	1/21 (5%)	3/21 (14%)	14/22 (64%)	14/22 (64%)
Cord	18/78 (23%)	0/17 (0%)	3/17 (18%)	16/18 (89%)	15/18 (83%)
Brainstem	15/78 (19%)	1/14 (7%)	3/14 (21%)	15/15 (100%)	13/15 (87%)
Optic nerve	5/78 (6%)	0/5 (0%)	1/5 (20%)	1/5 (20%)	1/5 (20%)
Hydrocephalus	5/78 (6%)	0/5 (0%)	1/5 (20%)	4/5 (80%)	5/5 (100%)
Cerebellum	4/78 (5%)	0/4 (0%)	0/4 (0%)	2/4 (50%)	3/4 (75%)
Cavernous sinus	3/78 (4%)	0/3 (0%)	1/3 (33%)	3/3 (100%)	2/3 (67%)
Pituitary	3/78 (4%)	0/3 (0%)	1/3 (33%)	2/3 (67%)	3/3 (100%)

Table 4. Review of Current Literature on CSF analysis in patients with neurosarcoidosis

* Different units for CSF cell count

Definite Neurosarcoidosis					Probable Neurosarcoidosis					Reference
OCB mat	**OCB unmat**	**Protein**	**WBC**	**CSF ACE**	**OCB mat**	**OCB unmat**	**Protein**	**WBC**	**CSF ACE**	
2/10	0/10	11/11	9/11	0/4	6/60	2/60	34/56	38/64	0/23	Current Study
DU	DU	6/8	4/8	2/3	DU	DU	39/54	DU	DU	Zajicek 1999 * (4)
DU	3/6	DU	DU	DU	37/160	0/160	DU	DU	DU	Kidd 2018 (15)
0/4	0/4	4/5	3/5	1/6	none	3/7	6/17	4/17	5/16	Joseph 2009 (13)
DU	12/19	DU	DU	DU	DU	DU	DU	DU	DU	Mc Lean 1990 ** (11)
DU	DU	DU	DU	DU	0/5	0/5	DU	4/5	DU	Borucki 1989 (14)

**Different definition of definite sarcoidosis
DU – Data Unavailable or no distinction between definite/probable/possible in data
OCB mat – OCB matched
OCB unmat – OCB unmatched

Figure 1. Prevalence of areas of systemic sarcoidosis involvement

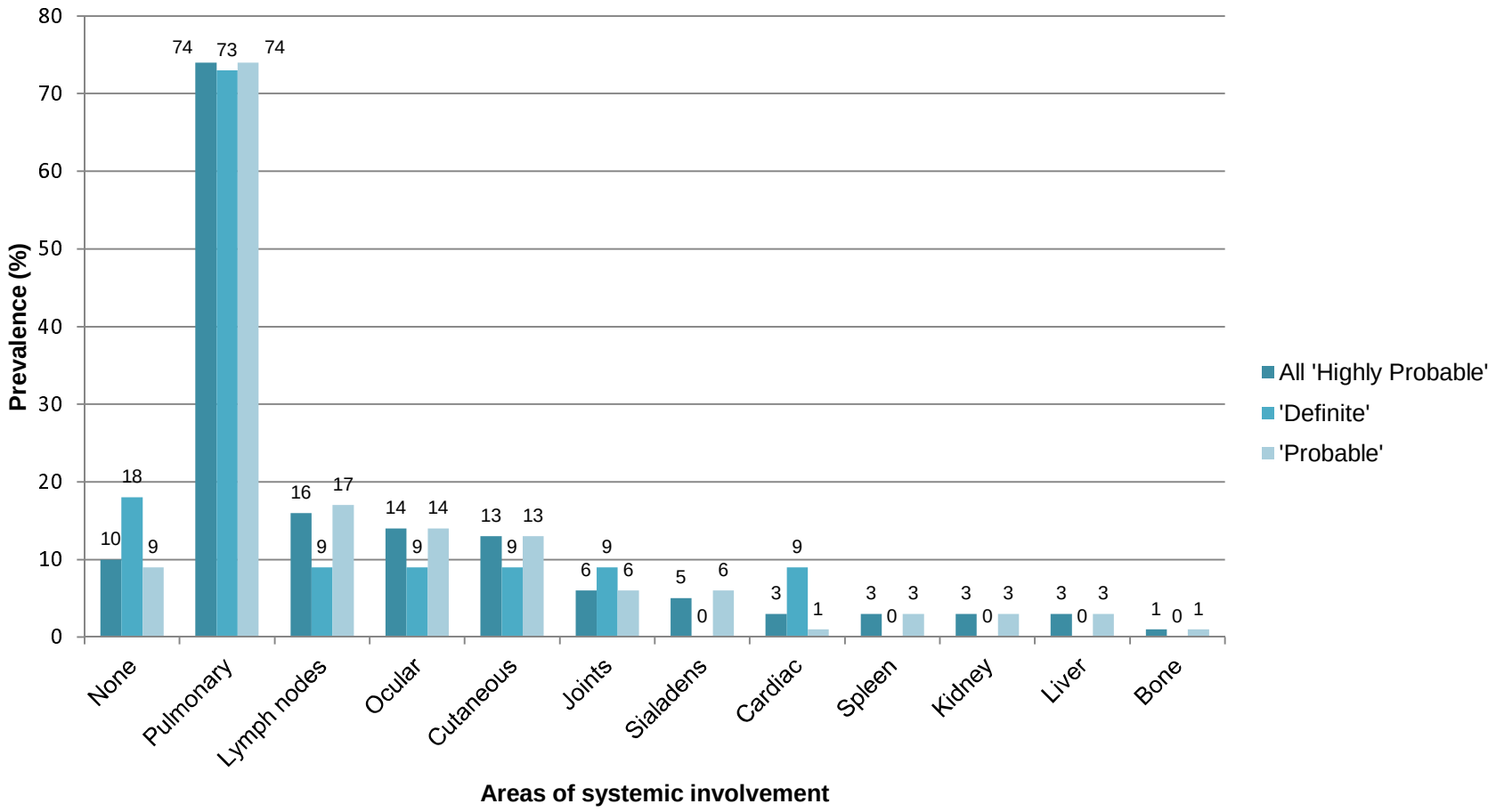

Figure 2. Prevalence of areas of brain involvement on MRI

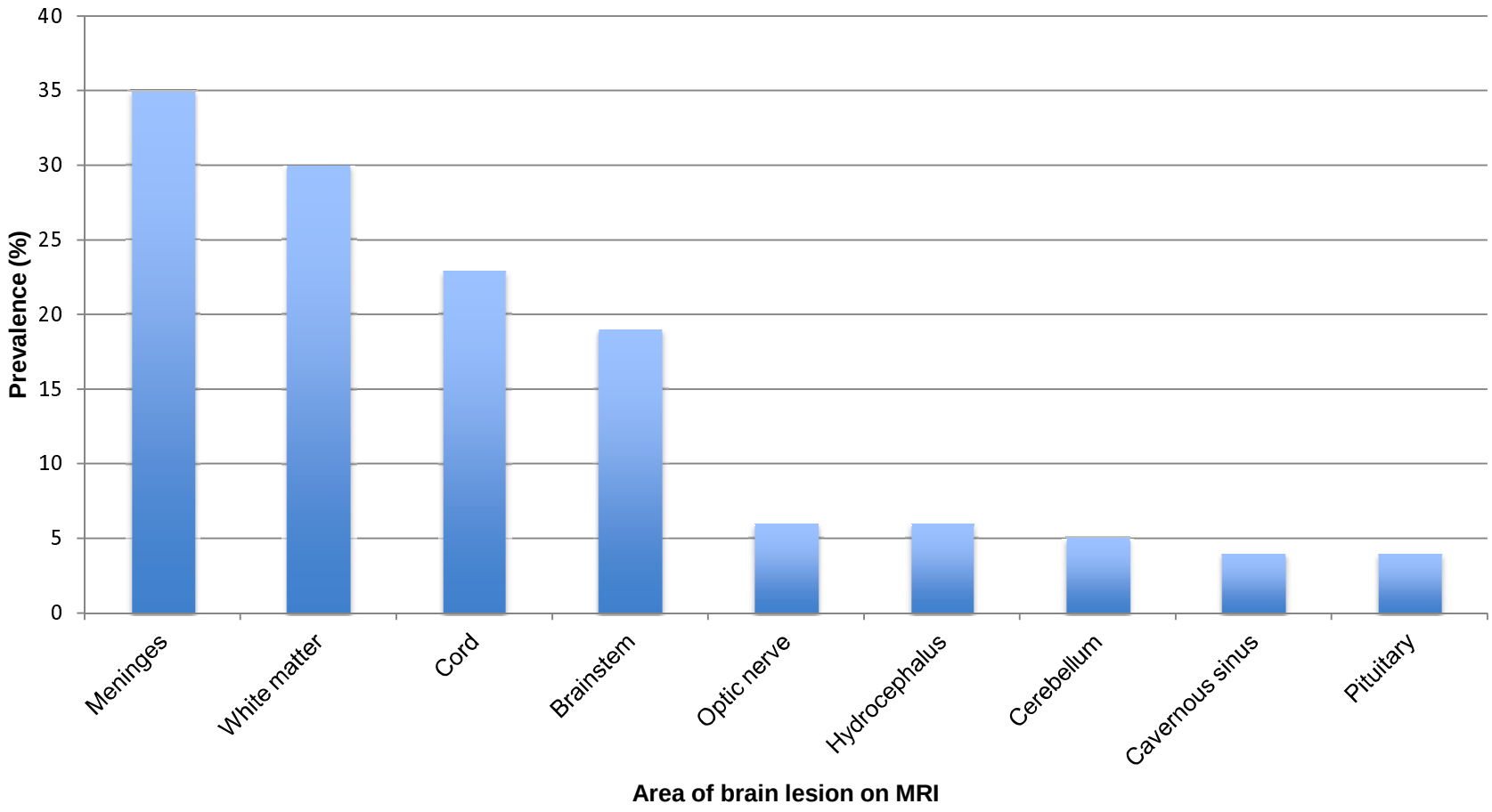

Figure 3: **Imaging characteristics in our cohort of neurosarcoidosis patients**