SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2023; 40 (2): e2023019 DOI: 10.36141/svdld.v40i2.12900

Krebs von den Lungen-6 (KL-6) in cerebrospinal fluid from neurosarcoidosis patients

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To the Editor,

Krebs von den Lungen-6 (KL-6) is a high molecular weight (200 kDa) glycoprotein mainly secreted by type II pneumocytes as a result of lung damage or during regeneration (1). It has been widely studied in idiopathic pulmonary fibrosis, fibrotic hypersensitivity pneumonitis and recently in sarcoidosis (1).

Sarcoidosis is a systemic granulomatous disease of unknown aetiology, characterised by multiple non-caseating, non-necrotizing granulomas. The lung is most frequently involved, but granulomatous inflammation can affect any organ or system.

Neurosarcoidosis, where sarcoid granulomas involve the nervous system, is uncommon, occurring in 3-16% of patients with sarcoidosis. Clinical presentation varies, including cranial neuropathies, chronic meningitis, brain parenchyma or spinal cord inflammation, vascular involvement, peripheral neuropathies and granulomatous myopathies (2). Radiological features are also diverse and often non-specific. They are often unable to reliably distinguish neurosarcoidosis from other chronic inflammatory, infectious or neoplastic CNS diseases (3). Serum analysis is equally inadequate: classic biomarkers, such as angiotensin-converting enzyme (ACE), show poor sensitivity and specificity for neurosarcoidosis and systemic sarcoidosis.

Currently proposed diagnostic criteria for neurosarcoidosis (4) therefore restrict "definite" diagnosis to patients with histologically demonstrated CNS or neuromuscular granulomas, and outline "probable" and "possible" cases with a combination of clinical, imaging and cerebrospinal fluid (CSF) features, plus a confirmed diagnosis of systemic sarcoidosis. Such criteria are unable to identify patients with isolated neurosarcoidosis (up to 10% of cases and 1% of sarcoidosis patients) or patients whose neurosarcoidosis manifestations precede systemic disease (almost 50% of cases).

Several studies have investigated potential CSF biomarkers of neurosarcoidosis, with contradictory results. Most focused on ACE in CSF. Two large retrospective studies found low sensitivity (24-55%) but high specificity (94-95%). Remarkably elevated CSF concentrations of ACE were found mostly in patients with widespread parenchymal and leptomeningeal involvement (Dale). On the other hand, more recent results have been discouraging: Bridel et al. only recorded sensitivity and specificity around 65% for CSF concentrations of ACE ³3 U/l, while none of another cohort of 27 neurosarcoidosis patients tested positive for ACE in CSF. Elevated CSF concentrations of ACE have also been reported in other pathologies, including multiple sclerosis, Alzheimer disease and schizophrenia.

Received: 13 February 2022 - Accepted: 16 May 2023
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The few studies of other possible CSF biomarkers, such as soluble interleukin-2 receptor (sIL-2R) and CD4/CD8 lymphocyte ratio, always showed poor reliability due to low specificity in the first case and often low CSF cell count in the second.

There is some evidence suggesting serum and bronchoalveolar lavage (BAL) concentrations of KL-6 as differential and prognostic markers of pulmonary sarcoidosis. No data is currently available on KL-6 in serum or CSF of neurosarcoidosis patients. The aim of the present study was to compare KL-6 concentrations in serum and CSF of neurosarcoidosis patients with CNS involvement versus others with neurodegenerative (ND) or chronic inflammatory demyelinating (DM) diseases.

We studied medical records of patients monitored at Siena Regional Referral Center for Sarcoidosis, identifying 22 patients with a definite, probable or possible diagnosis of neurosarcoidosis according to current diagnostic criteria (4,5). We looked for cases for whom samples of serum and CSF had been collected simultaneously and stored at -20°C. We enrolled nine neurosarcoidosis patients (mean age 46.2 years, range 16-61 years, M/F 5/4). Diagnosis was definite in one case, probable in six and possible in two. We also studied medical records of patients monitored at Siena Neurology and Neurophysiology Unit, enrolling nine patients with a chronic neurodegenerative disease, i.e. amyotrophic lateral sclerosis (mean age 53.1 years, range 37-65 years, M/F 5/4) and nine patients with a chronic demyelinating disease, i.e. multiple sclerosis (mean age 46.3 years, range 18-65 years, M/F 5/4). Unfortunately, serum from the former was not available. Serum samples from nine healthy controls were collected for KL-6 assay. 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 or other immunosuppressants. 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 and radiological features were obtained from medical records and entered in an electronic database for statistical analysis.

All patients gave their written informed consent to participation in the study/use of their data/

Table 1. Demographic, clinical, radiological and immunological data about NS patients

Patients	Sex	Age (yrs)	Scadding (stage)	FVC (%)	FEV1 (%)	DLCO (%)	Serum ACE (U/l)	Serum chitotriosidase (nmol/ml/h)	ACE CSF (U/l)	KL-6 CSF (U/ml)	CNS localization	NS diagnosis
01	F	16	III	N.A.	N.A.	57	63	64	13	N.D.	Brain meninges	Probable
02	F	46	II	N.A.	N.A.	N.A.	48	98	27.8	7	Brain and spinal meninges, brain and spinal cord parenchyma, hypophysis	Probable
03	M	61	0	N.A.	N.A.	N.A.	33	109	14.3	2	Spinal cord parenchyma	Definite
04	M	53	III	118.2	108.2	95	64	133	12	2	Brain and spinal cord parenchyma	Possible
05	F	51	II	99	100	65	72	260	3.2	10	Spinal roots	Probable
06	M	47	II	N.A.	N.A.	N.A.	68	96.5	7	N.D.	Spinal meninges	Probable
07	M	31	0	114.5	111.8	N.A.	59	60	N.D.	5	Brain parenchyma and meninges	Possible
08	F	58	II	90.9	77.3	56.5	4	43.3	N.D.	2	Brain and spinal meninges, brain parenchyma	Probable
09	M	53	IV	69	56	52	38	101	6.5	15	Brain arteries	Probable

Abbreviations: N.A., not available; N.D., not detectable

biological material, which was approved by our local ethics committee (CEAVSE 18712; Markerlung 17431).

Table 1 shows the demographic, clinical, radiological, immunological and functional data of our neurosarcoidosis patients. Two patients had negative chest radiology at the time of diagnosis: in one, isolated neurosarcoidosis was confirmed by detection of granulomatous inflammation in a spinal cord biopsy; in the other, lung involvement only emerged at follow-up. Patients often had normal lung volumes and diffusing capacity. Serum ACE was also often normal, whereas serum chitotriosidase exceeded 100 nmol/ml/h in 4/9 patients. CNS localization of granulomatous lesions was heterogeneous, including brain and/or spinal meninges in five patients, brain and/or spinal cord parenchyma in five patients and hypophysis, spinal roots and brain arteries in one patient respectively; 4/9 patients had more than one concomitant CNS localization. No patient had clinical manifestations suggesting concomitant neuromuscular sarcoidosis.

Measurable CSF concentrations of KL-6 were detected in 7/9 neurosarcoidosis patients but in no ND or DM patients. No significant differences in CSF concentrations of ACE were observed between the three groups (p=0.0819).

In neurosarcoidosis patients, CSF concentrations of KL-6 were directly correlated with CSF albumin index (r=0.98; p<0.0001), albumin (r=0.979, p=0.0001), IgG (r=0.928, p=0.0009) and total protein concentrations (r=0.945, p=0.0004). No significant correlation between CSF and serum concentrations of KL-6 was observed in neurosarcoidosis patients. Serum KL-6 concentrations were higher in neurosarcoidosis and DM patients than in healthy controls (median (IQR), 324 (276-366) and 320 (174-458) vs 297 (179-419), p=0.7703).

The present study compared KL-6 levels in CSF and serum of neurosarcoidosis, DM and ND patients. To our knowledge, this protein and its alterations have never been studied in neurosarcoidosis.

Our research group recently demonstrated elevated serum and BAL KL-6 levels in sarcoidosis patients with fibrotic lung disease (1) with good sensitivity even during chronic steroid therapy. KL-6 concentrations seemed correlated with extrapulmonary localizations.

Here we detected KL-6 (200kDa) in CSF of 7/9 neurosarcoidosis patients but not in the DM and ND control groups. Albumin (69 kDa) and IgG (108 kDa) concentrations were also increased in CSF of our neurosarcoidosis population, and a direct correlation between KL-6 concentrations and albumin index was found.

Since KL-6 is a high molecular weight protein, under physiological conditions it is unlikely to cross the blood-brain barrier. However, when the barrier is damaged due to pathological conditions, as in neurosarcoidosis, KL-6 produced by affected lungs could cross the barrier together with albumin and IgG, as confirmed by the direct correlation.

Our finding of KL-6 in CSF of neurosarcoidosis patients but not in ND and DM patients suggests that this protein could be a specific diagnostic marker of neurosarcoidosis, particularly useful in cases with little thoracic involvement and non-diagnostic serum concentrations of KL-6.

Since no biomarkers have yet been approved to distinguish neurosarcoidosis, our findings suggest that simple, mini-invasive and inexpensive KL-6 assay in CSF may discriminate this specific phenotype of sarcoidosis with 81.2% sensitivity and 100% specificity. More than ACE (also elevated in CSF of ND and DM patients), assay of KL-6 in CSF could be worthy of further research.

The main limitations of our study were retrospective design, monocentric data collection and limited sample size, though the latter is justified by the rarity of neurosarcoidosis.

In conclusion, we found KL-6 in CSF from neurosarcoidosis and not from ND and DM patients. The finding sustains the specificity of changes in KL-6 in this granulomatous disease, suggesting it as a candidate biomarker for recognition of neurosarcoidosis.

Ethics Statement: The studies involving human participants were reviewed and approved by Comitato Etico Area Vasta Sud Est (CEAVSE). Patients/participants provided written informed consent to participation in the study/use of their data.

Conflicts of Interest: The authors declare that the research was conducted without commercial or financial relationships that could be construed as a potential conflict of interest.

Study funding: none

Author contributions: Silvia Bocci: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data. Miriana d'Alessandro: Drafting/revision of the manuscript for content; Major role

in the acquisition of data; Study concept or design; Analysis or interpretation of data. Laura Bergantini: Drafting/revision of the manuscript for content; Analysis or interpretation of data. Alfonso Cerase: Drafting/revision of the manuscript for content; Analysis or interpretation of data. Monica Ulivelli: Drafting/revision of the manuscript for content; Analysis or interpretation of data. Paola Rottoli: Drafting/revision of the manuscript for content; Analysis or interpretation of data. Elena Bargagli: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data. Fabio Giannini: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data. All authors read and approved the final version of the manuscript.

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