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Measurement of sCD27 in the cerebrospinal fluid identifies patients with neuroinflammatory disease

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ABSTRACT

Background: Laboratory tests to assist in the diagnosis and monitoring of neuroinflammatory diseases are scarce. The soluble form of the CD27 molecule (sCD27) is shed in high concentrations by activated T cells and can be detected in the cerebrospinal fluid. The aim of this study was to investigate whether CSF quantitation of sCD27 could discriminate between inflammatory and non-inflammatory neurological diseases.

Methods: The concentration of sCD27 was measured using a commercially available ELISA in 803 well-defined subjects from a study cohort comprised of 338 patients with neuroinflammatory disease, 338 with non-inflammatory neurological disease and 127 controls without neurological disease.

Results: The median value of cerebrospinal fluid sCD27 was 64 pg/mL (IQR 0–200) in controls, 58 pg/mL (IQR 0–130) in patients with non-inflammatory disease and 740 pg/mL (IQR 230–1800) in patients with inflammatory disease. The likelihood ratio of having an inflammatory disease was 10 (sensitivity 74% and specificity 93%) if the sCD27 concentration was > 250 pg/mL. In patients with a known inflammatory condition, the likelihood ratio of having an infection was 10 (sensitivity 40% and specificity 96%) if the sCD27 concentration was > 2500 pg/mL.

Conclusions: The likelihood of having an inflammatory neurological condition is increased with elevated concentrations of sCD27 in cerebrospinal fluid. Rapid tests of sCD27 should be developed to assist clinicians in diagnosis of neuroinflammatory disease.

1. Introduction

Neuroinflammatory diseases are common and constitute a large part of the patients seen by the general neurologist. Traditional measures to establish and quantitate inflammation in neurological diseases such as white blood cell count and IgG production in the cerebrospinal fluid (CSF) were introduced in the first and middle part of the 20th century. (Greenfield and Carmichael, 1925; Kabat et al., 1948; Karcher et al., 1959) Since then, few laboratory tests for inflammatory processes have been added to the diagnostic arsenal in neurology. This paucity of progress is probably due to the relative success of imaging techniques, such as magnetic resonance tomography. At the same time, the family of neuroinflammatory diseases has grown increasingly complex and reliable biomarkers for diagnosis and monitoring of treatment responses are wanted.

sCD27, the soluble form of the CD27 molecule was investigated as a potential biomarker of inflammation in multiple sclerosis in the 1990s, (Hintzen et al., 1991a; Hintzen et al., 1999) with renewed attention recently.(Komori et al., 2015; van der Vuurst de Vries et al., 2017) CD27 belongs to the tumor necrosis factor receptor superfamily and is expressed as a costimulatory molecule on lymphocytes.(Bremer, 2013) Interaction between CD27 and its ligand CD70 promotes survival and expansion of $CD4^+$ and $CD8^+$ T lymphocytes(Han et al., 2016). After T cell receptor activation, CD27 is shed by T cells in a soluble 32-kD form, which readily can be detected in body fluids.(Hintzen et al., 1991b)

The aim of the present study was to investigate whether quantitation of sCD27 in CSF could discriminate between inflammatory and non-inflammatory neurological diseases.

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Table 1
Concentrations of sCD27 per diagnosis.

Diagnosis	n	Inflammatory	M/F	Median age (Range)	sCD27 Mean values ± SD (95% CI)	sCD27 Median values (Range)
Controls	127					
Healthy volunteers	47	No	18/29	37 (18–74)	50 ± 88 (95% CI 24–76)	16 (0–428)
Investigated without disease	38	No	9/29	41 (19–85)	45 ± 60 (95% CI 25–64)	13 (0–214)
Urologic controls	42	No	40/2	68 (45–85)	280 ± 130 (95% CI 239–321)	252 (105–727)
Dementias	195					
Alzheimer's disease	41	No	14/27	71 (51–85)	53 ± 75 (95% CI 30–77)	22 (0 − 321)
Binswanger's disease	4	No	0/4	64 (61–68)	56 ± 91 (95% CI -88-200)	17 (0–191)
Dementia NUD	5	No	4/1	73 (70–84)	186 ± 179 (95% CI -37-409)	166 (0–459)
Dementia with Lewy bodies	1	No	0/1	64 (64–64)	109	109 (109–109)
Frontotemporal dementia	9	No	3/6	67 (55–76)	148 ± 62 (95% CI 101–196)	148 (73–245)
Mild cognitive impairment	53	No	31/22	67 (44–78)	71 ± 181 (95% CI 21–121)	27 (0 − 1303)
Normal pressure hydrocephalus	82	No	50/32	74 (50–88)	106 ± 148 (95% CI 74–139)	83 (0–931)
Headaches	16					
Migraine	4	No	0/4	45 (21–66)	54 ± 45 (95% CI -17-126)	53 (13 − 100)
Other headaches	12	No	6/6	40 (21–72)	60 ± 84 (95% CI 6–113)	28 (0–273)
Infectious CNS disease	130					
Borrelia burgdorferi	68	Yes	38/30	53 (3–80)	4104 ± 4053 (95% CI 3123–5085)	2590 (341–20,294)
Herpes Simplex Type 1 Encephalitis	5	Yes	2/3	71 (6–83)	955 ± 705 (95% CI 79–1830)	621 (523–2196)
Herpes Simplex Type 2 Encephalitis	19	Yes	5/14	36 (0–69)	1712 ± 1064 (95% CI 1199–2225)	1605 (77–3691)
Varicella Zoster Encephalitis	38	Yes	22/16	47 (4–95)	1864 ± 1373 (95% CI 1412–2315)	1573 (206–4857)
Movement disorders	57					
Huntington's disease: asymptomatic carrier	12	No	7/5	35 (19–56)	136 ± 104 (95% CI 70–202)	167 (0–289)
Huntington's disease: manifest	12	No	7/5	51 (30–72)	251 ± 116 (95% CI 177–325)	271 (40–447)
Multiple system atrophy	14	No	6/8	68 (58–77)	65 ± 63 (95% CI 29–101)	49 (0–188)
Parkinsons's disease	5	No	2/3	70 (60–81)	240 ± 286 (95% CI -115-595)	95 (0–679)
Progressive supranuclear palsy	5	No	2/3	74 (69–77)	37 ± 39 (95% CI -11-86)	35 (0–89)
Other movement disorders	9	No	3/6	63 (46–87)	183 ± 468 (95% CI -177-543)	0 (0–1426)
Neuromuscular disease	103					
Acute inflammatory demyelinating polyneuropathy	35	Yes	17/18	57 (23–88)	496 ± 822 (95% CI 214–778)	263 (0–4304)
Chronic inflammatory neuropathies	43	Yes	35/8	58 (29–90)	337 ± 421 (95% CI 208–466)	226 (0–1839)
Non-inflammatory neuropathies	4	No	3/1	67 (48–73)	22 ± 21 (95% CI -11-55)	25 (0–40)
Motor neuron disease	21	No	11/10	63 (39–79)	98 ± 74 (95% CI 64–131)	104 (0–244)
Non-infectious inflammatory CNS disease	130					
Idiopathic myelitis	7	Yes	3/4	33 (26–50)	758 ± 948 (95% CI -119-1636)	442 (26–2784)
Neuromyelitis optica	2	Yes	0/2	52 (29–74)	142 ± 155 (95% CI -1250-1533)	142 (32–251)
Isolated optic neuritis	3	Yes	0/3	32 (26–50)	121 ± 95 (95% CI -115-356)	70 (62–230)
Primary progressive multiple sclerosis	11	Yes	5/6	50 (39–73)	925 ± 720 (95% CI 441–1408)	887 (99–2444)
Relapsing-remitting multiple sclerosis	64	Yes	20/44	37 (18–76)	729 ± 710 (95% CI 551–906)	530 (0–3048)
Secondary progressive multiple sclerosis	36	Yes	13/23	54 (29–68)	656 ± 677 (95% CI 427–885)	449 (38–3190)
Susac's syndrome	2	Yes	0/2	41 (41–42)	158 ± 32 (95% CI -128-443)	158 (135–180)
Other non-infectious inflammatory CNS disease	5	Yes	1/4	36 (24–76)	1116 ± 1059 (95% CI -199-2430)	703 (225–2871)
Other diseases	45					
CADASIL	1	No	1/0	39 (39–39)	271	271 (271–271)
Epilepsy	1	No	1/0	70 (70–70)	13	13 (13 − 13)
Hydrocephalus	6	No	2/4	58 (45–75)	146 ± 240 (95% -106-398)	63 (0–622)
Idiopathic intracranial hypertension	9	No	3/6	29 (21–59)	42 ± 52 (95% CI 1.6–82)	26 (0–164)
Kleine-Levin syndrome	4	No	2/2	23 (22–27)	229 ± 320 (95% -280-737)	106 (0–702)
Narcolepsy	7	No	2/5	44 (17–71)	17 ± 33 (95% -13-47)	0 (0–86)
Non-arteritic anterior ischemic optic neuropathy	2	No	2/0	53 (46–59)	4.5 ± 6.4 (95% -53-62)	4.5 (0–9)
Orthopedic spinal disease	4	No	3/1	62 (57–70)	226 ± 356 (95% -341-792)	55 (34–759)
Pain	10	No	4/6	43 (31–51)	87 ± 69 (95% 38–136)	89 (0 − 203)
Papilledema	1	No	1/0	34 (34–34)	0	0 (0–0)

2. Methods

2.1. Ethics approval

The study was approved by the Regional Ethical Board of Uppsala (Dnr 2008/182, Dnr2013/278, Dnr 2015/462).

2.2. Subjects

The study cohort contained 676 patients with a neurological diagnosis and 127 controls without neurological disease (Table 1). The controls were healthy volunteers (n = 47); patients who were investigated for suspected neurological disease where a neurological diagnosis was ruled out (n = 38); and patients who underwent spinal anaesthesia for minor urological surgery, with no prior history of a neurological disease (n = 42). In the latter group comorbidities were common, including arterial hypertension (n = 22); urinary bladder cancer in situ (n = 15); ischemic heart disease (n = 11); diabetes type 2 (n = 8); and benign prostate hyperplasia (n = 5).

Of the 676 patients with a neurological diagnosis, 345 came from in-house biobanks at the Department of Neurology, Uppsala University Hospital. In these patients, the investigators had full access to medical records and laboratory results made in routine health care. The remaining patient samples were acquired from other biobanks containing samples from well-defined patient cohorts with specific diagnoses. Those biobanks were located at Uppsala University Hospital, Karolinska University Hospital and the University Hospital of Umeå. These latter samples were anonymised and only data on age, gender and diagnosis were available.

2.3. Classification of subjects

Patients were diagnosed according to commonly used diagnostic criteria (for details see Appendix). The different diagnoses were then classified as 'inflammatory' or 'non-inflammatory'. The following diseases and conditions were classified as 'inflammatory': CNS infections, acute and chronic inflammatory polyneuropathies, multiple sclerosis, optic neuritis, myelitis, Susac's syndrome and systemic inflammatory diseases with CNS involvement. The remaining diseases and conditions were classified as 'non-inflammatory', e.g. dementias, migraine and movement disorders (see Table 1 for details).

2.4. CSF handling and storage

The samples were collected between 2003 and 2016. A total of 246 samples were collected at lumbar puncture made in routine health care. Patients were asked to donate 3 mL for research purposes, if consent was given the samples were brought to the laboratory within 30 min and then entered a semi-automatic process of spinning, aliquoting and freezing. The samples were centrifuged at 250*g* for five minutes, stored in polypropylene tubes in aliquots of 240 µL at −80 °C until analysed. The remaining samples were collected and stored according to previously published guidelines(Teunissen et al., 2009) with one notable exception. The samples from patients with inflammatory neuropathies were stored in glass tubes.

2.5. Quantification of sCD27

The sCD27 assay was performed by a technician blinded to the clinical diagnosis. Human CD27/TNFRSF7 was analysed by a commercial sandwich ELISA kit, (DY382, R&D Systems, Minneapolis, MN, USA) and all measurements were performed with the same batch. A monoclonal antibody specific for the peptide was coated onto microtiter plates. After blocking the wells with bovine serum albumin, standards and samples were pipetted into the wells and the peptide was bound to the immobilized antibodies. A biotinylated anti-peptide antibody was added to the wells after washing, a streptavidine-HRP conjugate was then added after another incubation and washing cycle. Finally, after incubation and washing, a substrate solution was added. The development was stopped and the absorbance was measured in a SpectraMax 250 (Molecular Devices, Sunnyvale, CA, USA). The peptide concentrations in the samples were determined by comparing the optical density of the sample with the standard curve. The assay's limit of detection was 40 pg/mL and the highest standard point was 8000 pg/mL. If sCD27 > 8000 pg/mL, the concentration of sCD27 was determined by repeat analysis of diluted samples. The assays were calibrated against highly purified recombinant human CD27. The pooled CV of the assay was approximately 5%.

2.6. Statistical analyses

Statistical analyses were done with GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA). Data were summarized using frequencies for categorical variables and as medians for continuous variables. To determine statistically significant differences between two groups, the Mann-Whitney test was used; for statistical significance between three or more groups the Kruskal-Wallis test. Dunn's multiple comparison test was used for post hoc analysis. Correlations were described with Spearman's rank correlation coefficient. A two-tailed p value of < 0.05 was considered significant.

3. Results

The levels of sCD27 in patients and controls are described in Table 1. Age did not correlate with the levels of sCD27 in any of the control groups (healthy volunteers Spearman r = 0.16, p = .27; investigated without disease Spearman r = −0.0016, p = .92; urologic controls Spearman r = 0.04, p = .80); and the levels of sCD27 were similar between males and females. The levels of sCD27 were similar between healthy volunteers and patients who were investigated for suspected neurological disease, whereas the urologic controls had higher sCD27 values than the other two control groups (p < .0001). Of the 676 patients in the cohort, 338 were classified as 'inflammatory' and 338 as 'non-inflammatory'.

Data on albumin ratio (CSF albumin:plasma albumin) were available in 192 patients. sCD27 did not correlate with the albumin ratio in these (Spearman r = 0.12, p = .083). Data on mononuclear cells in CSF were available in 294 patients and in those patients sCD27 correlated moderately with the number of mononuclear cells (Spearman r = 0.48, p < .0001).

3.1. Increased levels of sCD27 is highly indicative of an inflammatory condition

The median value of cerebrospinal fluid sCD27 was 64 pg/mL (IQR 0–200) in controls, 58 pg/mL (IQR 0–130) in patients with non-inflammatory condition and 740 pg/mL (IQR 230–1800) in patients with an inflammatory condition (Fig. 1A). A ROC analysis comparing non-inflammatory conditions versus inflammatory conditions was made (Fig. 1B). The area under the ROC curve was 0.89 (95% CI 0.86–0.91). Using a cut-off at 250 pg/mL, 24/338 of patients with non-inflammatory conditions and 250/338 of patients with an inflammatory

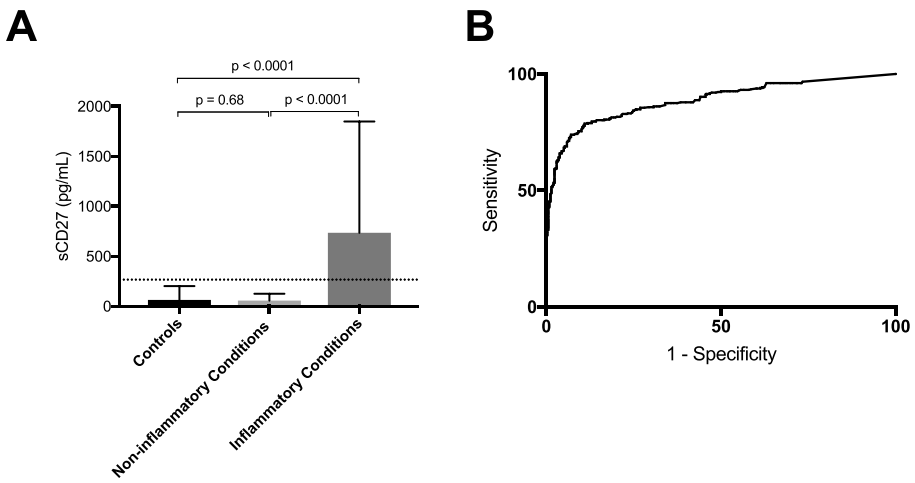

Fig. 1. CSF concentrations of sCD27 in inflammatory and non-inflammatory neurological diseases.
(A) Bars represent medians and whiskers IQR. Patients with non-inflammatory diseases and controls had similar levels of sCD27. In contrast, patients with inflammatory diseases had on average much higher concentrations of sCD27 in the cerebrospinal fluid. Choosing a cut-off at 250 pg/mL (dotted line), the sensitivity was 74%, the specificity 93% and the likelihood ratio was 10 to identify an inflammatory condition. (B) ROC curve illustrating the relationship between sensitivity and specificity at different cut-offs.

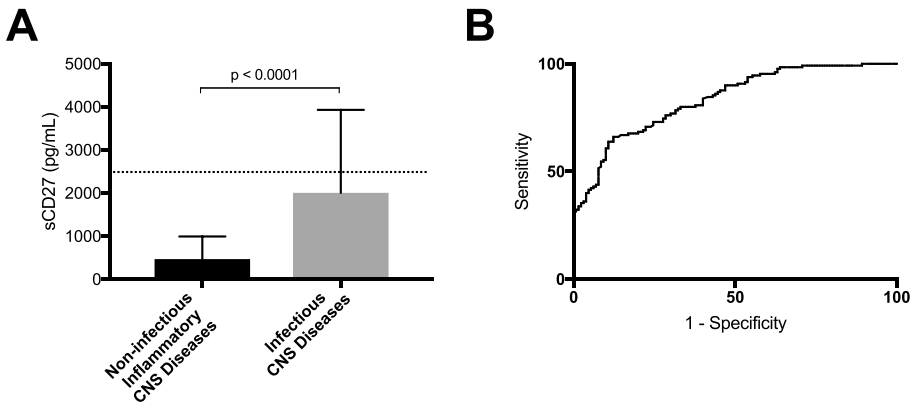

Fig. 2. CSF concentrations of sCD27 in inflammatory diseases of the central nervous system.
(A) Bars represent medians and whiskers IQR. Patients with infectious CNS diseases had higher concentrations of sCD27 in the cerebrospinal fluid than patients with other inflammatory CNS diseases. Choosing a cut-off for sCD27 at 2500 pg/mL (dotted line), the sensitivity was 40%, the specificity 96% and the likelihood ratio was 10 to identify an infectious disease. (B) ROC curve illustrating the relationship between sensitivity and specificity at different cut-offs.

condition had sCD27 concentrations above the cut-off. This yielded a sensitivity of 74% (95% CI 69–79), a specificity of 93% (95% CI 90–95) and a likelihood ratio (LR) of 10.

3.2. High levels of sCD27 is highly indicative of an infection

Patients with an inflammatory condition were further subdivided into patients with a sterile inflammation of the CNS and those with infectious disease and a comparison between these two groups was made. The median value of sCD27 was 470 pg/mL (IQR 160–990) in patients with sterile inflammation and 2000 pg/mL (IQR 940–3900) in patients with infectious disease (Fig. 2A). A ROC analysis comparing these conditions was made (Fig. 2B). The area under the ROC curve was 0.84 (95% CI 0.79–0.88). Using a cut-off at 2500 pg/mL, 5/130 of patients with sterile inflammation and 51/130 of patients with infectious disease had sCD27 concentrations above the cut-off. This yielded a sensitivity of 40% (95% CI 32–49), a specificity of 96% (95% CI 91–99) and a likelihood ratio (LR) of 10. Patients with neuroborreliosis had significantly higher concentrations of sCD27 in comparison to those with viral encephalitides (Fig. 3D). Of note, sCD27 concentrations > 5000 pg/mL were only seen in patients with a Borrelia Burgdorferi infection.

3.3. sCD27 in inflammatory diseases of the central and peripheral nervous system

A comparison was made between inflammatory diseases of the peripheral nervous system (PNS) and non-infectious inflammatory diseases of the CNS. On average CNS disease had higher concentrations than PNS disease, but both were significantly higher than the control group (Fig. 3A). There was no statistically significant difference between the concentrations of sCD27 in acute inflammatory demyelinating neuropathies (Guillain-Barré syndrome) and chronic inflammatory demyelinating neuropathies (including paraproteinemic demyelinating neuropathy, multifocal motor neuropathy, chronic inflammatory demyelinating neuropathy) (Fig. 3B) and similarly no difference between different types of demyelinating disease in the CNS (Fig. 3C), although the number of patients with optic neuritis were too few to impact the analysis.

4. Discussion

The aim of this study was to investigate if CSF measurements of sCD27 could be used to identify patients with inflammatory neurological diseases. Our results suggest that this is possible. The likelihood of having an inflammatory disease was increased tenfold if the concentration of sCD27 was higher than 250 pg/mL. Furthermore, sCD27 values higher than 2500 pg/mL was highly suggestive of a CNS infection.

We strived to include as many neurological diagnoses as possible to create a 'catalogue' of reference values for different diseases. The dynamic range of this test was very broad with values ranging from 0 to > 20,000 pg/mL. We used three control groups in the study, mirroring different clinical scenarios. The first group consisted of healthy volunteers, without any known disease. These subjects had very low levels of sCD27, suggesting that basal turnover of sCD27 in CSF is negligible and consistent with the fact that there are very few T cells present in the healthy brain. The second group consisted of individuals investigated for possible neurological disease, where no disease was found after extensive work-up. These included patients with commonly encountered symptoms at an outpatient clinic, such as paresthesia and dizziness. These patients had similar levels to the healthy controls. The third control group was a heterogeneous group of patients who underwent minor urological surgery for various reasons. This group was included to reflect individuals with comorbidities. Their sCD27 concentrations were slightly higher than the other controls, implying that intermediate sCD27 values have to be interpreted with caution in individuals with concomitant diseases.

The highest values of sCD27 were seen in patients with infectious disease, which should come as no surprise and is in agreement with a previous study on a few cases of HTLV-1 associated myelopathy. (Hintzen et al., 1999) Exceptionally high values were seen with *B burgdorferi* infections. Another biomarker that has seen increasing use in the diagnostic work-up of Lyme neuroborreliosis is CXCL13. It is a B cell attracting chemokine, which increases even before *B burgdorferi* specific antibodies can be detected in the CSF.(Schmidt et al., 2011) Sensitivity and specificity of CXCL13 have been described as excellent in this context and a formal comparison between CXCL13 and sCD27 should be made to investigate if quantitation of sCD27 adds additional information.

Previous studies have mainly focused on sCD27 as a biomarker in multiple sclerosis (MS) and lymphoproliferative disorders.(Hintzen et al., 1991a; Hintzen et al., 1999; Komori et al., 2015; van der Vuurst de Vries et al., 2017; Mondria et al., 2008; Kersten et al., 1996; Murase et al., 2000; Komori et al., 2016) In our study, MS patients had higher concentrations of sCD27 in comparison to controls and in agreement with previous studies, there were no statistically significant differences between patients with relapsing-remitting MS, secondary progressive MS or primary progressive MS.(Komori et al., 2015; van der Vuurst de Vries et al., 2017) We did not have access to CSF from patients with malignant CNS diseases, but previous studies have shown that sCD27 can be used as a tumor marker of lymphoid malignancy in the CNS as a complementary element to morphological analysis and the determination of cancer load.(Kersten et al., 1996; Murase et al., 2000) In a previous study, the levels of sCD27 were fairly similar between patients with primary CNS lymphoma and patients with CNS infection, which suggests that measurement of sCD27 is not helpful in discriminating lymphoid malignant disease from inflammatory CNS disease.(Murase et al., 2000)

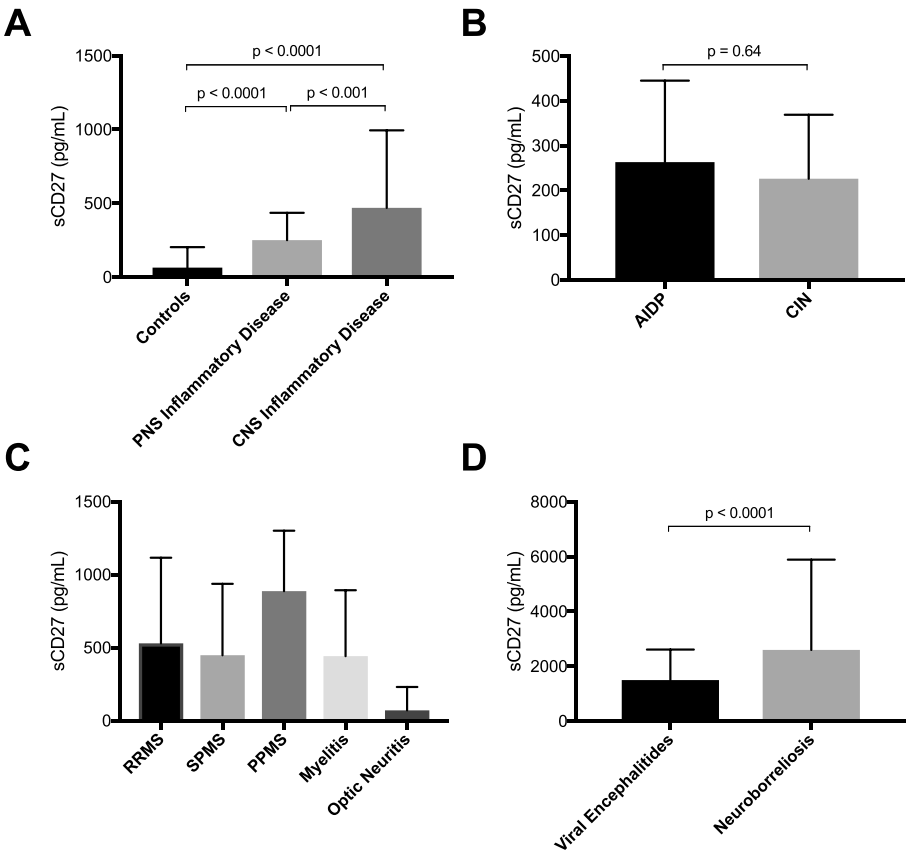

Fig. 3. CSF concentrations of sCD27 in specific diagnoses.
Bars represent medians and whiskers IQR. (A) Comparison of non-infectious inflammatory diseases of the peripheral (PNS) and central (CNS) nervous system. (B) Comparison of sCD27 concentrations in acute inflammatory demyelinating polyneuropathy (AIDP) and chronic forms of inflammatory demyelinating neuropathies (CIN). (C) Levels of sCD27 in different types of central nervous system demyelinating diseases including relapsing-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS) and primary progressive multiple sclerosis (PPMS). (D) Comparison between two main types of central nervous system infections.

The analysis of CSF samples of patients affected by inflammatory peripheral neuropathies has to our knowledge never been performed previously. The diagnostic procedure of polyneuropathies can be particularly challenging. Peripheral nerve biopsy in order to detect inflammation and/or demyelination is an invasive method and is usually not immediately available in the diagnostic work-up of patients. Radiological methods to assess peripheral nervous system (PNS) inflammation are virtually absent.(Ohana et al., 2014) Our results indicate that CSF sCD27 is elevated in inflammatory peripheral neuropathies. In the clinical setting the potential use of sCD27 is of interest since no reliable CSF biomarkers of peripheral nerve inflammation currently exist and we are currently planning a more extensive study of sCD27 in these diseases.

Our results suggest that sCD27 is a sensitive marker of CNS inflammation, that may fill a similar role in the management of inflammatory neurological diseases, as the well-known and widely used analysis of C-reactive protein (CRP) have in systemic disease. The dynamic range of these tests are broad, with a very low basal production and large increases in response to infection, with non-infectious inflammatory conditions falling in between. Importantly, we could also demonstrate that the levels of sCD27 were very low in a vast majority of controls and patients with non-inflammatory conditions. CRP has proven to be a simple and valuable blood test, aiding clinicians in diagnostic processes, determining disease progress and estimating the effectiveness of treatments in systemic inflammatory disease. sCD27 is almost exclusively produced by T cells(Komori et al., 2015) and provides a more direct approach to quantitate inflammation in an enclosed compartment such as the nervous system.

The validity of our results hinges on a correct classification of subjects into patients with ‘inflammatory’ and ‘non-inflammatory’ diseases. On the surface, this may seem like a straight-forward process, but certainly reality is more complex than this simple dichotomization. In MS, perhaps the most well-known inflammatory CNS disease, there is currently a lively debate whether primary and secondary progressive MS is caused by inflammation or neurodegeneration.(Mahad et al., 2015) sCD27 was equally high in all subtypes of MS, which lend support to the proposition that inflammation is still very much important in progressive forms of MS. Type 1 narcolepsy is a disease considered to be caused by the severe loss of neurons that produce the orexin neuropeptides.(Scammell, 2015) Although hardly proven, it has been hypothesized that this is due to a monophasic autoimmune process (i.e. inflammatory) but the symptoms of narcolepsy often emerge later when inflammation may have abated. Such considerations led to the classification of narcolepsy as a non-inflammatory disease. Acute demyelinating neuropathies are also considered to be caused by monophasic autoimmune processes. In contrast to narcolepsy, these often have a rapid course, are diagnosed early and are proven to be inflammatory. Accordingly, these were classified as inflammatory. Increasing lines of evidence also point to immune activation being important in conditions traditionally viewed as neurodegenerative, such as Alzheimer's disease and other dementias.(Schain and Kreisl, 2017) In the present study, patients with frontotemporal dementia had somewhat higher concentrations of sCD27 than patients with Alzheimer's disease. Increased concentrations of cerebrospinal fluid cytokines (notably TNF-α) have previously been demonstrated in frontotemporal dementia (Sjogren et al., 2004) and brain microglial activation is also an early feature of frontotemporal dementia,(Radford et al., 2015) suggesting that inflammation may be important to the pathogenesis of this disease. Even if we do not understand the role of sCD27 and adaptive immunity in frontotemporal dementia, analysis of sCD27 may prove to be a valuable aid in diagnosis and discrimination between different neurodegenerative diseases, not traditionally considered as inflammatory disorders. This needs to be explored in future studies.

This was a cross-sectional study and an obvious weakness is that we did not have access to more than one sample from each individual and no data on when the sample was collected in relation to disease onset.

Thus, we were not able to determine the kinetics of the sCD27 response, which is probably of importance, not least in the assessment of infections. We will also need more information on whether sCD27 responds to therapeutic interventions in neuroinflammatory diseases such as MS and if such a response is related to long-term prognosis. Another limitation of this study is that we did not have access to data on lymphocyte counts, albumin ratio, IgG index and IgG oligoclonal band status for all patients, which would have provided for an analysis of the additional value of sCD27 vis-à-vis measurement of these traditional variables alone. Moreover, a large majority of the available data on IgG index and IgG oligoclonal band status came from patients with MS, which put a specific limitation on how those could be generalized to the other diagnoses of the cohort. The study contained a large number of patients, with contributors from several biobanks and different hospitals. Therefore, it is hardly surprising that sample handling and storage were somewhat variable. This may have affected the precision in the estimation of sCD27 values of some diagnoses (notably peripheral neuropathies), however it is unlikely that the it impacted the main results. Finally, the results from the control group undergoing minor urologic surgery indicate that non-neurological comorbidities in some instances may be associated with increased sCD27 levels in the CSF. This needs to be confirmed in other studies and investigated in cohorts with other types of comorbidities.

We believe that analysis of CSF sCD27 is ready to be implemented in clinical practice in order to identify neuroinflammatory conditions. sCD27 has already been used as a surrogate endpoint in clinical trials (Komori et al., 2016; Komori et al., 2017) and further studies should be made to establish if sCD27 has prognostic value. Some obstacles and unresolved issues remain, before sCD27 can be used in routine health care. The kinetics of the sCD27 response in acute and chronic inflammation has not been sufficiently characterized. At present the quantitation of sCD27 is made with ELISA, with long turnaround times. A further development of an analysis that could be applied on one of the frequently used random access instruments in the hospital laboratories would be highly appreciated. This would reduce turnaround times from weeks to hours and increase the clinical usefulness of this test considerably.

Contributors

The study was outlined and organized by JB and AL. JB, AS and JC collected samples from healthy controls. JB and AS collected samples from MS patients. IN collected samples from patients with movement disorders and motor neuron disease. VN collected samples from patients with Huntington's disease. MI and KK provided samples from patients with dementias. KN provided samples from patients with infectious disease. TG provided samples from urologic controls. RP and IK provided samples from patients with inflammatory neuropathies. AL made the analysis of sCD27 in CSF. JB and AF analysed the data. JB, AF and AL wrote the draft. All authors critically read the manuscript and approved of the final content.

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Competing interests

No competing interest were reported by the authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2019.03.015.

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