

BMJ Open Causes of albuminocytological dissociation and the impact of age-adjusted cerebrospinal fluid protein reference intervals: a retrospective chart review of 2627 samples collected at tertiary care centre

John Alexander Brooks,¹ Christopher McCudden,² Ari Breiner,^{1,3} Pierre R Bourque^{1,3}

To cite: Brooks JA, McCudden C, Breiner A, *et al.* Causes of albuminocytological dissociation and the impact of age-adjusted cerebrospinal fluid protein reference intervals: a retrospective chart review of 2627 samples collected at tertiary care centre. *BMJ Open* 2019;**9**:e025348. doi:10.1136/bmjopen-2018-025348

► Prepublication history for this paper is available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2018-025348>).

Received 11 July 2018
Revised 10 December 2018
Accepted 17 December 2018



© Author(s) (or their employer(s)) 2019. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to

Dr John Alexander Brooks;
jbrooks@toh.ca

ABSTRACT

Objective We set out to test the discriminative power of an age-adjusted upper reference limit for cerebrospinal fluid total protein (CSF-TP) in identifying clinically relevant causes of albuminocytological dissociation (ACD).

Methods We reviewed the charts of 2627 patients who underwent a lumbar puncture at a tertiary care centre over a 20-year period. Samples with CSF-TP above 45 mg/dL (0.45 g/L) were included. Samples with white cell count $>5 \times 10^9/L$, red cell count $>50 \times 10^9/L$ and glucose <2.5 mmol/L (45 mg/dL) were excluded as were samples with incomplete data and those taken from paediatric patients (ie, age <18 years old). Patients with CSF-TP elevated above 45 mg/dL were considered to have 'pseudo' ACD unless their CSF-TP was in excess of age-adjusted norms in which case they were considered to have 'true' ACD. Adjustment for sex was not applied to the age-adjusted norms although the importance of gender has been previously described.

Results The presence of ACD was associated with a broad range of neurological diagnoses. Among all 2627 patients with ACD, a clinical diagnosis explaining CSF-TP elevation was identified in 57% of cases. 'True' ACD was associated with a suitable diagnosis in 75% of cases, whereas patients with 'pseudo' ACD showed an appropriate diagnosis in only 51% of cases. Use of an age-adjusted upper reference limit favoured the detection of polyneuropathy patients (13.5% proportionate increase) and excluded a larger number of patients with isolated headache (10.7% proportionate decrease; $p < 0.0001$).

Conclusions Elevated CSF-TP is a common finding, with a range of underlying causes. Use of an age-adjusted upper reference limit for the CSF-TP value improves diagnostic specificity and helps to avoid overdiagnosis of ACD.

INTRODUCTION

The term 'albuminocytological dissociation' (ACD) was first coined by Sicard and Foix in 1912 to describe the unexpected finding of

Strengths and limitations of this study

- This study is of the largest of its kind, incorporating the results of 2627 retrospectively collected cerebrospinal fluid (CSF) samples over 20 years.
- Charts were thoroughly evaluated for potential sources of albuminocytological dissociation (ACD) including the review consultation notes, neuroimaging and laboratory data.
- Sample size was sufficient to show a clear shift towards clinically relevant diagnoses like polyneuropathy at the exclusion of those without such pathology (eg, benign headache) using age-adjusted CSF-total protein (TP) thresholds in place of a traditional fixed threshold (ie, CSF-TP ≥ 0.45 g/L).
- Charts of those patients with CSF samples demonstrating ACD (2627 of 16 045) were included using strict criteria (white cell count $\leq 5 \times 10^6/L$, red cell count $\leq 50 \times 10^6/L$, glucose ≥ 2.5 mmol/L, age ≥ 18 years, CSF-TP ≥ 0.45 g/L).
- Factors previously described to vary with CSF-TP such as sex, body mass index, CSF sample number or lifestyle factors (smoking, alcohol or physical activity) were not explored as much of these data were unavailable at the time of analysis.

elevated cerebrospinal fluid (CSF) protein without pleocytosis in patients with spinal compression.¹ Four years later, the term became entrenched in the medical literature with the landmark article of Guillain, Barré and Strohl, describing the acute demyelinating polyradiculoneuropathy that now carries their name.²

We recently published CSF total protein (CSF-TP) reference intervals derived from institutional data at the Ottawa Hospital, comprising an initial dataset of 19 591 CSF samples analysed over a period of 20 years.³

After exclusions based on laboratory parameters (white cell count (WCC) $>5 \times 10^9/\text{L}$, red cell count (RCC) $>50 \times 10^9/\text{L}$ and glucose $<2.5 \text{ mmol/L}$) and 60 conditions associated with elevated CSF-TP, we determined age-adjusted continuous reference intervals and suggested that these would be more accurate than a commonly employed cut-off of 0.45 g/L (45 mg/dL).

In the current study, we hypothesised that the implementation of age-adjusted upper reference limits (URLs) would result in a larger proportion of identified patients with expectedly high CSF-TP protein—including those with inflammatory neuropathies. We, therefore, sought to describe the types of clinical diagnoses associated with ACD. Our aim was to distinguish between patients with ‘traditional’ ACD (CSF-TP $>0.45 \text{ g/L}$), ‘true’ ACD (CSF-TP $>$ age-adjusted reference limit) and those with ‘pseudo’ ACD ($0.45 \text{ g/L} < \text{CSF-TP} < \text{age-adjusted reference limit}$)—and to compare the types and frequencies of clinical diagnoses in each group.

METHODS

Registrations and study population

All data were extracted from the Ottawa Hospital Data Warehouse based on CSF samples collected between 1 January 1996 and 31 December 2016. Laboratory data obtained from the database included CSF-TP, CSF glucose, CSF WCC, CSF RCC, and serum creatinine and TP results. In addition, demographics (age and sex) and clinical diagnostic codes (ICD-9/10 codes) were recorded. To identify the subset of patients with ACD, we applied specific inclusion/exclusion criteria (figure 1). Excluded were samples with CSF constituents otherwise outside of established thresholds including WCC $>5 \times 10^9/\text{L}$, RCC $>50 \times 10^9/\text{L}$, glucose $<2.5 \text{ mmol/L}$ (45 mg/dL) and CSF-TP $<0.45 \text{ g/L}$ ($<45 \text{ mg/dL}$). Samples with incomplete clinical or laboratory data, or those performed on paediatric patients were also excluded.

Patient and public involvement

The study analysis used anonymised patient data extracted from the Ottawa Hospital Data Warehouse as described above.

Chart review

Review of our database revealed that diagnostic codes (ICD-9/10 codes) generated at the time of lumbar puncture (LP) did not always reflect the ultimate diagnostic outcome. To ensure accuracy and quality of data, all 2627 clinical charts were reviewed with the goals of identifying: (1) the presence of any clinical condition known or suspected to cause increased CSF-TP and (2) the indication for performing a LP. The reference list of medical conditions believed to be associated with elevated CSF-TP was established based on a thorough search of the medical literature (table 1). Where the literature was unclear as to an expectation of CSF-TP elevation, a consensus was reached between the reviewers (JAB, PF and PRB).

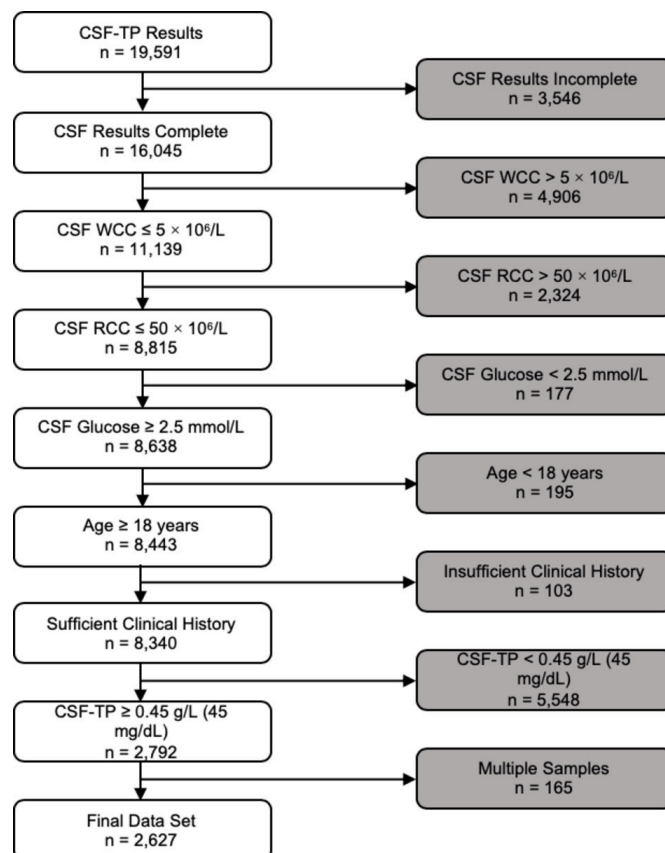


Figure 1 Flow chart illustrating the exclusion process used to identify patients with ACD. ACD, albuminocytological dissociation; CSF, cerebrospinal fluid; RCC, red cell count; TP, total protein; WCC, white cell count.

Cases in which multiple factors may have contributed to increased CSF-TP were discussed between reviewers (JAB, PF and PRB), to ensure the accuracy of classification. Each patient was subsequently categorised based on the most likely cause of high CSF-TP; if a cause was not found, patients were categorised based on the clinical indication for LP.

CSF-TP analysis

Technical specifications for the analytical equipment used in CSF analysis have been outlined in the methods section of the 2017 manuscript by McCudden *et al*.⁸

CSF-TP was analysed on three different instruments over the course of the 20 years included in the study as follows: Roche Hitachi 917, 1 January 1996 to 30 September 2001; Beckman Lx20, 30 September 2001 to 1 April 2009 and Siemens Vista 1500, 1 April 2009 to 1 December 2016. The Roche method is based on a benzethonium chloride turbidimetric analysis, whereas the Beckman and Siemens methods use a pyrogallol red-molybdate complex, which is measured at 600 nm. In all cases, analyses were performed according to the manufacturer's directions.

Other laboratory values were measured on different instruments across the two decades included in the study. Serum creatinine, TP and CSF glucose were measured on the platforms described above for the same time frames;

Table 1 List of clinical categories for which albuminocytological dissociation or cerebrospinal fluid total protein elevation has been described

Clinical categories	References
Following intrathecal chemotherapy*	14
Following subarachnoid haemorrhage	15
Infectious/non-infectious encephalitis	16 17
Infectious/non-infectious meningitis	18–20
Intra-axial/extra-axial tumours	21–23
Inflammatory polyneuropathy	9 24
Non-inflammatory polyneuropathy	25
Hydrocephalus before/after shunt placement	26 27
Angiitis of the central nervous system	28
Inflammatory white matter disease	29–31
Cerebral venous sinus occlusion	32
Optic nerve disease	33
Optic neuritis	34
Posterior reversible encephalopathy syndrome	35
Structural spinal disorders	36 37
Nervous system toxin exposure	38
Dementia	39
Seizure	40
Stroke (haemorrhagic/ischaemic)	41

*The underlying condition for which intrathecal chemotherapy was provided in the cited report was related to the central nervous system involvement in systemic lupus erythematosus as opposed to predominantly the treatment of a haematological malignancy in the context of our report.

creatinine was measured by the Jaffe method from 30 September 2001 to 13 April 2013 and by the enzymatic method thereafter. CSF WCC and RCC counts were determined using the Beckman Coulter between 1996 and 2009 and the Sysmex XE5000 from 2009 to 2016. All laboratory analyses were determined according to the manufacturer's instructions throughout the study in a routine clinical laboratory in an academic medical centre (The Ottawa Hospital).

Data analysis

ACD was defined as 'traditional' ACD if CSF-TP exceeded a typical cut-off of 0.45 g/L. ACD was defined as 'true' ACD if the CSF-TP exceeded age-adjusted reference limits, as defined in McCudden *et al.* Age-adjusted reference limits were computed using the following formula:

$$URL = 0.124 + 0.0284 \text{ Age} - 7.08 \cdot 10^{-4} \text{ Age}^2 + 8.23 \cdot 10^{-6} \text{ Age}^3 - 3.35 \cdot 10^{-8} \text{ Age}^4 \quad (1)$$

If CSF-TP was between 0.45 g/L and the age-adjusted reference limits, the case was labelled 'pseudo' ACD. Based on the clinical diagnoses/categories and our review of the medical literature, patients were also

divided into those with an expected increase in CSF-TP (patients possessing explanatory underlying conditions), and those where an increase was unexpected (a patient possessing no explanatory condition). For the proportion of patients with 'true' versus 'pseudo' ACD, the expectation and CIs were derived using bootstrap analysis given that all patients where ACD was unexpected were part of the original dataset from which the age-adjusted limits were derived.

For each clinical category, the category's share of ACD patients was computed (ie, the number of patients assigned to a clinical category vs the remainder assigned to all other clinical categories). These proportions were compared for 'traditional' ACD and 'true' ACD using a Fischer's exact test (table 2). Within the 'traditional', 'true' and 'pseudo' ACD groups, the frequencies of the underlying clinical categories were plotted in bar graph format for illustration (figure 2). Given the large number of statistical tests performed (ie, 47 Fischer's exact tests), Bonferroni correction was applied to the threshold p value suggesting statistical significance (ie, $0.0001 = 0.05/47$).

Given the established utility of ACD in polyneuropathy, a subgroup analysis focused on these patients. The median CSF-TP levels, shown with their IQR, were computed and compared with available literature. A Fisher's exact test was also used to compare the relative shifts in ACD classification between inflammatory and non-inflammatory neuropathies when using the 'traditional' versus 'true' definition of ACD. Furthermore, a Mann-Whitney-Wilcoxon test was used to compare the mean CSF-TP of inflammatory and non-inflammatory neuropathies.

All statistical calculations and graphs were generated using R V.3.3.3 (The R Foundation, Vienna, Austria).

RESULTS

The range of CSF values among 2627 patients (1093 female with a median (IQR) age of 54 (25)) with ACD over a 20-year time frame has been plotted in figure 3. Among all patients with 'traditional' ACD (CSF-TP >0.45 g/L), the underlying clinical category/diagnosis was considered sufficiently explanatory in 56% (53%, 59%) (1474/2627) of cases. The finding of 'true' ACD was expected in 75% (0.72, 0.78) (446/597) of cases; whereas in 'pseudo' ACD, ACD was expected in only 51% (48%, 54%) (1028/2030) of cases ($p < 0.001$). The relative number of cases identified and their specific diagnosis are shown in figure 2.

Table 2 lists the clinical categories/diagnoses used to classify patients and demonstrates the effect of using a data-driven age-adjusted reference limit as opposed to a traditional 0.45 g/L on the proportion of patients demonstrating ACD. Where CSF-TP elevation was unexpected, applying age-adjusted reference limits either decreased or did not change the proportion of these patients relative to other clinical categories. The opposite was true for the clinical categories where CSF-TP elevation was expected, as a significant increase in the

Table 2 Expectation of protein elevation, number and proportion of patients with a specific clinical category compared with all reported cases with associated percentage change from using an invariant 0.45 g/L CSF-TP threshold (all comers with ACD) versus a threshold varying with age ('true' ACD)

Clinical category	ACD expected	Traditional ACD – proportion with ACD (0.45 g/L upper limit)	True ACD – proportion with ACD (age-adjusted upper limit)	Change	P value*
	Y/N	n (%)	n (%)	Δ%	
Polyneuropathy	Y	204 (7.8)	127 (21.3)	13.50	<0.0001
Tumour	Y	139 (5.3)	47 (7.9)	2.60	0.019
Encephalitis (infectious, paraneoplastic or autoimmune)	Y	45 (1.7)	24 (4)	2.30	0.0014
Seizure	Y	191 (7.3)	53 (8.9)	1.60	0.20
Central shunt	Y	34 (1.3)	15 (2.5)	1.20	0.039
CNS structural anomaly	Y	7 (0.3)	4 (0.7)	0.40	0.13
Myelopathy	Y	47 (1.8)	13 (2.2)	0.40	0.50
Hydrocephalus	Y	34 (1.3)	10 (1.7)	0.40	0.44
Trauma (eg, postneurosurgery, diffuse axonal injury, etc)	Y	8 (0.3)	4 (0.7)	0.40	0.25
Diffuse anoxic–ischaemic injury	Y	17 (0.6)	6 (1)	0.40	0.41
Infection (no CNS involvement, eg, meningitis)	Y	67 (2.6)	17 (2.8)	0.20	0.67
CNS vasculitis	Y	19 (0.7)	6 (1)	0.30	0.44
Neuroinflammation	Y	28 (1.1)	8 (1.3)	0.20	0.52
Cerebral venous occlusion	Y	11 (0.4)	4 (0.7)	0.30	0.50
Meningeal disease/process (eg, carcinomatosis, IgG ₄ disease, etc)	Y	16 (0.6)	5 (0.8)	0.20	0.57
CSF leak	Y	3 (0.1)	2 (0.3)	0.20	0.23
Unresolved encephalopathy	Y	79 (3)	19 (3.2)	0.20	0.79
Haemorrhage within 3 months (eg, subarachnoid, intraparenchymal, etc)	Y	19 (0.7)	5 (0.8)	0.10	0.79
Mononeuropathy multiplex (inflammatory)	Y	7 (0.3)	2 (0.3)	0	0.68
Neurotoxicity (toxin causing CNS damage, eg, heroin inhalation)	Y	5 (0.2)	1 (0.2)	0	1
Aseptic meningitis	Y	1 (0)	0 (0)	0	1
Idiopathic intracranial hypertension	Y	24 (0.9)	5 (0.8)	−0.10	1
Hypertensive encephalopathy including posterior reversible encephalopathy syndrome	Y	16 (0.6)	3 (0.5)	−0.10	1
Systemic inflammatory process	Y	3 (0.1)	0 (0)	−0.10	1
Spinal disease	Y	12 (0.5)	2 (0.3)	−0.20	1
Unresolved neurological symptoms	Y	4 (0.2)	0 (0)	−0.20	1
Prior intrathecal chemotherapy	Y	23 (0.9)	4 (0.7)	−0.20	0.80
Neurodegenerative	Y	24 (0.9)	4 (0.7)	−0.20	0.81
Optic nerve disease	Y	35 (1.3)	4 (0.7)	−0.60	0.22
All-cause major stroke	Y	112 (4.3)	19 (3.2)	−1.10	0.25
Inflammatory white matter disease	Y	240 (9.1)	33 (5.5)	−3.60	0.0033
Plexopathy	N	7 (0.3)	4 (0.7)	0.40	0.13
Genetic neurological illness	N	3 (0.1)	2 (0.3)	0.20	0.23

Continued

Table 2 Continued

Clinical category	ACD expected	Traditional ACD—proportion with ACD (0.45 g/L upper limit)	True ACD—proportion with ACD (age-adjusted upper limit)	Change	P value*
	Y/N	n (%)	n (%)	Δ%	
First dose prophylactic intrathecal chemotherapy	N	2 (0.1)	1 (0.2)	0.10	0.46
Motor neuron disease	N	7 (0.3)	2 (0.3)	0	0.68
Cerebrovascular disease (vasculopathy)	N	9 (0.3)	2 (0.3)	0	1
Pain benign syndromes	N	5 (0.2)	1 (0.2)	0	1
Neuropathy (focal)	N	1 (0)	0 (0)	0	1
Myopathy	N	6 (0.2)	1 (0.2)	0	1
Psychiatric/psychogenic symptoms	N	17 (0.6)	3 (0.5)	−0.10	1
Ocular disease	N	5 (0.2)	0 (0)	−0.20	1
Cranial neuropathy	N	12 (0.5)	2 (0.3)	−0.20	1
Transient ischaemic attack	N	9 (0.3)	0 (0)	−0.30	0.38
Transient neurological symptoms	N	72 (2.7)	9 (1.5)	−1.20	0.11
Diagnostic testing	N	94 (3.6)	5 (0.8)	−2.80	0.0001
Transient encephalopathy	N	262 (10)	37 (6.2)	−3.80	0.0037
Benign headache	N	642 (24.4)	82 (13.7)	−10.70	<0.0001
Totals		2627 (100)	597 (100)		

Samples were considered to have 'ACD expected' after evaluating the available literature or consensus between authors where expectation of ACD was unclear from the literature review.

All-cause major stroke: includes thromboembolic disease, vasculitis of the CNS causing stroke and reversible cerebrovascular constriction syndrome.

Inflammatory white matter disease: includes multiple sclerosis, neuromyelitis optica, acute demyelinating encephalomyelitis.

Results in bold showed a significant proportionate change when age-adjusted thresholds were applied

*P values compare the change in proportion for the specific diagnostic category versus all other categories when assessing pseudo versus 'true' ACD.

ACD, albuminocytological dissociation; CNS, central nervous system; CSF, cerebrospinal fluid; TP, total protein.

relative share of patients with ACD was seen or there was no significant change. A notable exception to this pattern was inflammatory white matter disease where a large but non-significant decrease in the relative share of patients who would have been traditionally classified with ACD was seen.

When the subgroup of patients with polyneuropathy was examined, the effect of applying an age-adjusted URL tended to be more pronounced in those patients with non-inflammatory neuropathies (although $p=0.25$, thus, statistical significance was not reached). When the age-adjusted URL was applied, the number of non-inflammatory neuropathy patients exhibiting ACD decreased by 65% (from 17 to 6), in contrast, those with inflammatory neuropathy showed only a 35% reduction in cases (from 187 to 121). Moreover, the mean CSF-TP showed significant difference, measuring 1.05 g/L (0.85 g/L) for inflammatory neuropathy versus 0.57 g/L (0.16 g/L) for non-inflammatory neuropathy ($p<0.001$).

DISCUSSION

ACD has been described in a large number of peripheral and central nervous system disorders. Several disease-specific mechanisms have been proposed, including: (1) the intrathecal production or liberation of proteins such as IgG and myelin basic protein, (2) blood–brain barrier dysfunction in meningeal or parameningeal inflammation, (3) blood–nerve barrier dysfunction in neuropathy, (4) sequestration of CSF in spinal compression or (5) decreased CSF flow. Minor elevations of CSF-TP that are not associated with increased cell counts have also been linked to various attributes. This would include differences due to sex, age, body mass index and maximal abdominal circumference.⁴ Techniques have been proposed to correct for the impact of physiological variables, such as age, on metrics of blood CSF barrier dysfunction including those more tailored towards such an assessment (eg, albumin quotient).⁵ The result of routine CSF testing however often still leaves clinicians with a need to decide

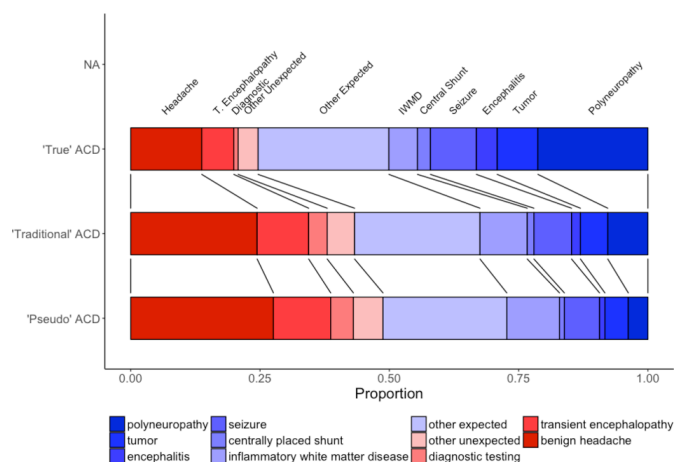


Figure 2 Proportions of patients with ACD. Proportionate breakdowns are shown for 'true' ACD (ie, CSF-TP value greater than the age-adjusted upper reference limit), 'traditional' ACD (ie, CSF-TP value greater than 0.45 g/L) and 'pseudo' ACD (ie, CSF-TP value greater than 0.45 g/L but less than the age-adjusted upper reference limit). Diagnostic categories (reason for lumbar puncture) not expected to cause ACD are represented in shades of red and organised left to right by descending magnitude of absolute percentage change from all ACD to true ACD. Pathological categories with a potential expectation for ACD are represented in shades of blue and organised left to right by ascending magnitude of absolute percentage change between all ACD and true ACD. The other categories (ie, 'other expected' and 'other unexpected') represent an amalgamation of those diagnostic groups where the absolute percentage change from all ACD to true ACD was not statistically significant. ACD, albuminocytological dissociation; CSF-TP, cerebrospinal fluid total protein; IWMD, inflammatory white matter disease; T, Encephalopathy, Transient Encephalopathy.

what level of isolated protein elevation may reflect an abnormality requiring further investigation.

ACD was a remarkably common CSF finding in diagnostic LP at our institution, present in 2627 of 8340 specimens (or 31.4%) using the traditional 0.45 g/L reference limit. This was proportion was similar to that observed in a publication by Hegen *et al* where CSF TP elevation was present in 31.8% of samples.⁶ We found however that ACD was only present in 597 (or 7%) with age-adjusted institutional reference limits. Of those patients with 'true' ACD, the most frequently associated clinical diagnoses were polyneuropathy (21%), benign headache (14%), seizures (9%) and intra-axial/extra-axial tumours (8%). There was, therefore, a marked reduction in the number of patients meeting criteria for ACD particularly in patients with clinical diagnoses not expected to be associated with ACD (benign headaches, transient encephalopathy and others), who often exhibited 'pseudo' ACD. Conversely, reductions in ACD frequency were less prominent in diagnostic categories where ACD has been well described, such as inflammatory polyneuropathy. Moreover, in those patients with 'true' ACD, the underlying clinical diagnosis was

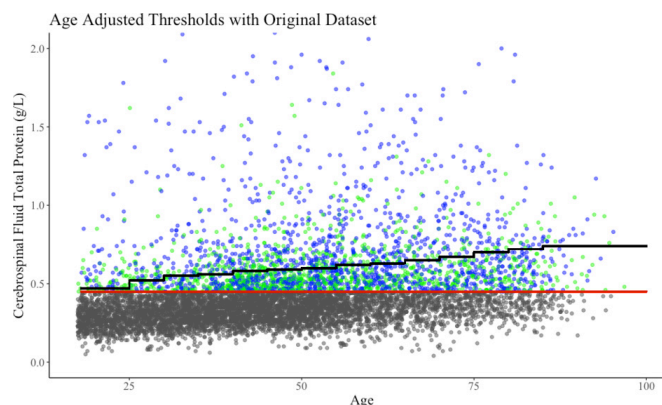


Figure 3 CSF-TP reference interval. Points represent postexclusion CSF-TP concentrations with the removal of all but the original point where patients had multiple CSF samples drawn (n=8175). Patients were grouped into 5-year bins by their age at the time of lumbar puncture. The resultant 97.5th percentile is delineated in black. The commonly used threshold of 0.45 g/L or 45 mg/dL is marked by a red line. Cases above the red line were reviewed for inclusion in percentile computation. Cases represented by blue circles were anticipated to have elevated CSF-TP and those in green were not. Cases below the red line did not undergo chart review and are represented in grey. CSF, cerebrospinal fluid; TP, total protein.

considered to be the potential cause of the protein elevation in 75% (72%, 78%) of cases.

Brettschneider *et al*⁷ similarly observed frequencies of particular clinical diagnoses (resulting in higher specificity for apparently causal conditions), when age-adjusted reference limits were applied, though their study used the serum albumin quotient (Q_{alb}) rather than CSF-TP. Similar to our findings, they observed that in patients with what we qualify in our report as 'true' ACD, 73% had an explanatory cause of Q_{alb} elevation (including Guillain-Barré syndrome (GBS)/Chronic Inflammatory Demyelinating Polyneuropathy (CIDP), lumbar spinal stenosis or epileptic seizures, among other diagnoses). While the Brettschneider *et al* article aligns with our findings, their sample size was significantly smaller (only 367 patients with ACD were studied).

In our dataset and the Brettschneider *et al* study, patients with polyneuropathy were found to be the main source of clinically relevant (expected) ACD. Many articles in the medical literature have focused on the detection of ACD in polyneuropathy, for the purpose of identifying those patients with immune/demyelinating neuropathies. In inflammatory neuropathies (including GBS and CIDP), ACD is considered one of the cardinal diagnostic features, with mean CSF-TP levels in excess of 1.0 g/L (100 mg/dL) in some reports.^{8,9} Non-inflammatory neuropathies often display a more modest degree of blood-nerve barrier dysfunction as evidenced by less extreme elevations in CSF-TP.^{9,10} Providing CSF-TP thresholds that consider age adjustment may explain some element of the mild

elevation seen in non-inflammatory neuropathy and therefore aid in distinguishing them from their inflammatory counterparts. The significance of this has been highlighted in the study by Allen *et al* which examined the diagnosis and misdiagnosis of CIDP in 59 consecutive patients. They showed that over-reliance on mild elevations of CSF-TP was often a source of false CIDP diagnoses. Moreover, they showed that once reclassified using European Federation of Neurological Societies criteria, patients with CIDP had a substantially higher mean CSF-TP (1.56 g/L) as compared with those without CIDP (0.61 g/L). To put this roughly into the context of our previously derived population norms, the median age of those falsely diagnosed with CIDP was 49.8 years for which our estimates suggest 0.59 g/L (59 mg/dL) as a more appropriate threshold for the CSF-TP URL (ie, the computed estimate of the 97.5th percentile) than a more traditional 0.45 mg/dL.³ This paper by Allen *et al*, therefore, underscores the need to explore techniques like age-adjusting CSF-TP URLs as a potential means to reduce misdiagnosis of CIDP.

Other notable clinical categories included a headache and inflammatory white matter disease. From examining the data, one may question why a benign headache might be so prominently represented in a sample of patients with ACD. We suspect that this reflects the volume of patients who underwent LP as screening for subarachnoid haemorrhage or meningitis to investigate a common and non-specific symptom, namely headache, in the context of an overly sensitive age-invariant threshold. To that point, headache patients were the most likely to be reclassified as 'pseudo' ACD when age-adjusted thresholds were applied. Similarly, patients with inflammatory white matter showed a high likelihood of being reclassified as 'pseudo' ACD when age-adjusted thresholds were applied. We suspect that this relates to the mild degree of CSF-TP elevation noted in multiple sclerosis—likely as a result of less aggressive and more chronic blood–brain barrier dysfunction.^{11 12}

Our study does have several limitations worth mention. First, without a formal chart review of all 16045 patients with complete laboratory data (especially those with CSF-TP <0.45), we are unable to formally quantify the sensitivity and specificity of CSF analysis for particular diagnoses. Second, we did not take into account the effect of sex, body mass index, CSF sample number or lifestyle factors (smoking, alcohol or physical activity) on CSF-TP levels.⁴ Third, we believe that a proportion of CSF-TP variability remains unexplained and prospective data collection (including additional laboratory values such as glycosylated haemoglobin and thyroid stimulating hormone) may further improve our understanding of CSF-TP variability.¹³ Fourth, three different instruments were used to measure CSF-TP over the course of the study. Although 95% CIs for age-and-instrument-partitioned intervals overlapped for ages <65 years; for >65 years, a modest but statistically significant difference in CSF-TP was found between devices as outlined in

our previous paper. This raises the importance of device calibration and the potential impact on the interpretation of borderline CSF-TP levels.³ Fifth, although our median estimates of CSF-TP in inflammatory and non-inflammatory neuropathy appear to align with previously reported values, they represent a biased sample where those with CSF-TP <0.45 were excluded. Sixth, out of 19591 samples, only 2627 samples were included in the analysis after eliminating repeat and incomplete sampling as well as those with biochemical and cytological measures outside of established norms and thus are not generalisable to those patients who have additional CSF abnormalities (eg, pleocytosis, hypoglycorrhachia or high RCC).

Apart from the above limitations, we believe that our study successfully presents the relevant clinical diagnoses associated with 'true' ACD, above the age-adjusted URL. In addition, our analysis highlights that the use of age-adjusted CSF-TP thresholds seems to increase the specificity for clinically relevant (expected) conditions. We would, however, caution clinicians not to overemphasise the importance of a finding of ACD, particularly given that common conditions such as lumbar stenosis may be the cause. To maximise the insight gained from CSF-TP levels, a future study evaluating the effects of additional factors on values within the 'true' ACD range is warranted.

Author affiliations

¹Department of Medicine, Division of Neurology, University of Ottawa, Ottawa, Ontario, Canada

²Department of Pathology and Laboratory Medicine, Division of Biochemistry, University of Ottawa, Ottawa, Ontario, Canada

³The Ottawa Hospital Research Institute, Ottawa, Ontario, Canada

Acknowledgements Priya Figurado (Department of Medicine, Division of Neurology, University of Ottawa, Ottawa, Canada) who performed data collection; Michel Shamy (Department of Medicine, Division of Neurology, University of Ottawa, Ottawa, Canada; The Ottawa Hospital Research Institute, Ottawa, Canada) who performed substantive editing.

Contributors Conceptualisation, methodology: CM, PRB and JAB. Writing and revision: JAB, AB, PRB and CM. Supervision: PRB and CM. JAB was involved with conceptualisation, writing and revision of the current study. CM was involved with conceptualisation, writing and revision of the current study and supervision as it pertained to the current study. AB writing and revision of the current study. PRB was involved with conceptualisation, writing and revision of the current study and supervision as it pertained to the current study.

Funding JAB currently receives fellowship funding from the Canadian Network of MS Clinics which is supported through contributions from Biogen Idec Canada, EMD Serono, Sanofi Genzyme, Novartis Pharmaceuticals Canada., Hoffmann-La Roche and Teva Canada Innovation. AB reports grant funding from the GBS-CIDP Foundation International and Grifols.

Disclaimer This study did not receive any direct financial support. CM reports no disclosures. PRB reports no disclosures.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study was approved by the Ottawa Hospital Research Institute (OHRI) Ethics Board (protocol #20160863–01H).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Additional data regarding disease specific cerebrospinal total protein levels can be accessed by emailing John Brooks at John.brooks@one-mail.on.ca.

Author note Albuminocytologic dissociation is seen in a broad range of neurological diagnoses. Our study of 2627 patients with ACD examines the causes.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

REFERENCES

- Caplan LR. Charles Foix--the first modern stroke neurologist. *Stroke* 1990;21:348–56.
- Guillain G, Barré JA, Strohl A. [Radiculoneuritis syndrome with hyperalbuminosis of cerebrospinal fluid without cellular reaction. Notes on clinical features and graphs of tendon reflexes. 1916]. *Ann Med Interne* 1999;150:24–32.
- McCudden CR, Brooks J, Figurodo P, et al. Cerebrospinal fluid total protein reference intervals derived from 20 years of patient data. *Clin Chem* 2017;63:1856–65.
- Seyfert S, Kunzmann V, Schwertfeger N, et al. Determinants of lumbar CSF protein concentration. *J Neurol* 2002;249:1021–6.
- Reiber H. Knowledge-base for interpretation of cerebrospinal fluid data patterns. Essentials in neurology and psychiatry. *Arq Neuropsiquiatr* 2016;74:501–12.
- Hegen H, Auer M, Zeileis A, et al. Upper reference limits for cerebrospinal fluid total protein and albumin quotient based on a large cohort of control patients: implications for increased clinical specificity. *Clin Chem Lab Med* 2016;54:285–92.
- Bretschneider J, Claus A, Kassubek J, et al. Isolated blood-cerebrospinal fluid barrier dysfunction: prevalence and associated diseases. *J Neurol* 2005;252:1067–73.
- Mateen FJ, Cornblath DR, Jafari H, et al. Guillain-Barré Syndrome in India: population-based validation of the Brighton criteria. *Vaccine* 2011;29:9697–701.
- Allen JA, Lewis RA. CIDP diagnostic pitfalls and perception of treatment benefit. *Neurology* 2015;85:498–504.
- Irani DN. *Neuromuscular Disease*. Philadelphia: Saunders, 2009:121–6.
- Pohl D, Rostasy K, Reiber H, et al. CSF characteristics in early-onset multiple sclerosis. *Neurology* 2004;63:1966–7.
- Freedman MS, Thompson EJ, Deisenhammer F, et al. Recommended standard of cerebrospinal fluid analysis in the diagnosis of multiple sclerosis: a consensus statement. *Arch Neurol* 2005;62:865–70.
- Deisenhammer F, Bartos A, Egg R, et al. Guidelines on routine cerebrospinal fluid analysis. Report from an EFNS task force. *Eur J Neurol* 2006;13:913–22.
- Dong Y, Zhang X, Tang F, et al. Intrathecal injection with methotrexate plus dexamethasone in the treatment of central nervous system involvement in systemic lupus erythematosus. *Chin Med J* 2001;114:764–6.
- dos Reis-Filho JB, Ribeiro SB, Juliano Y. [CSF total proteins in the prognosis of patients with subarachnoid hemorrhage]. *Arq Neuropsiquiatr* 1995;53:69–74.
- Gultekin SH, Rosenfeld MR, Voltz R, et al. Paraneoplastic limbic encephalitis: neurological symptoms, immunological findings and tumour association in 50 patients. *Brain* 2000;123:1481–94.
- Saraya AW, Wacharapuesadee S, Petcharat S, et al. Normocellular CSF in herpes simplex encephalitis. *BMC Res Notes* 2016;9:95.
- Sakayama K, Kidani T, Matsuda Y, et al. Subdural spinal granuloma resulting from *Candida albicans* without immunosufficiency: case report. *Spine* 2002;27:E356–E360.
- Rosin VS. [Echinococcosis of the spinal canal]. *Klin Med* 1990;68:60–2.
- Motta LP, Costa MA, Gouvea MB, et al. Postmalaria neurological syndrome: a case report. *Rev Soc Bras Med Trop* 2011;44:787–8.
- Rogg JM, Ahn SH, Tung GA, et al. Prevalence of hydrocephalus in 157 patients with vestibular schwannoma. *Neuroradiology* 2005;47:344–51.
- Liu J, Jia H, Yang Y, et al. Cerebrospinal fluid cytology and clinical analysis of 34 cases with leptomeningeal carcinomatosis. *J Int Med Res* 2009;37:1913–20.
- Shim Y, Gwak HS, Kim S, et al. Retrospective analysis of cerebrospinal fluid profiles in 228 patients with leptomeningeal carcinomatosis: differences according to the sampling site, symptoms, and systemic factors. *J Korean Neurosurg Soc* 2016;59:570–6.
- Illes Z, Blaabjerg M. Cerebrospinal fluid findings in Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathies. *Handb Clin Neurol* 2017;146:125–38.
- Li J, Li Y, Chen H, et al. Autonomic neuropathy and albuminocytologic dissociation in cerebrospinal fluid as the presenting features of primary amyloidosis: a case report. *Front Neurol* 2017;8:368.
- Tullberg M, Blennow K, Månsson JE, et al. Cerebrospinal fluid markers before and after shunting in patients with secondary and idiopathic normal pressure hydrocephalus. *Cerebrospinal Fluid Res* 2008;5:9.
- Wikkelsø C, Blomstrand C. Cerebrospinal fluid proteins and cells in normal-pressure hydrocephalus. *J Neurol* 1982;228:171–80.
- Geri G, Saadoun D, Guillemin R, et al. Central nervous system angiitis: a series of 31 patients. *Clin Rheumatol* 2014;33:105–10.
- Kuzume D, Sajima K, Kon-no Y, et al. [A case of acute disseminated encephalomyelitis (ADEM) with an anti-galactocerebroside antibody]. *Rinsho Shinkeigaku* 2015;55:550–4.
- Aimard G, Devic M, Bourgeay M, et al. [Albumino-cytologic dissociation associated with multiple sclerosis syndrome]. *J Med Lyon* 1968;49:1479–88.
- Avsar T, Korkmaz D, Tütüncü M, et al. Protein biomarkers for multiple sclerosis: semi-quantitative analysis of cerebrospinal fluid candidate protein biomarkers in different forms of multiple sclerosis. *Mult Scler* 2012;18:1081–91.
- Wang X, Sun X, Liu H. Clinical analysis and misdiagnosis of cerebral venous thrombosis. *Exp Ther Med* 2012;4:923–7.
- Saracco JB, Genevet J, Mouly A. [Optic atrophy and albuminocytologic dissociation]. *Bull Soc Ophtalmol Fr* 1971;71(5-6):637–41.
- Rolak LA, Beck RW, Paty DW, et al. Cerebrospinal fluid in acute optic neuritis: experience of the optic neuritis treatment trial. *Neurology* 1996;46:368–72.
- Datar S, Singh TD, Fugate JE, et al. Albuminocytologic dissociation in posterior reversible encephalopathy syndrome. *Mayo Clin Proc* 2015;90:1366–71.
- Bonelli C. [Physiopathology of the radicular complex in relation to the pathogenesis and albumino-cytologic dissociation]. *Rass Neuropsichiatri* 1951;5:305–28.
- Kalichman L, Cole R, Kim DH, et al. Spinal stenosis prevalence and association with symptoms: the Framingham Study. *Spine J* 2009;9:545–50.
- Rahman SS, Kadakia S, Balsam L, et al. Autonomic dysfunction as a delayed sequelae of acute ethylene glycol ingestion: a case report and review of the literature. *J Med Toxicol* 2012;8:124–9.
- Wikkelsø C, Blomstrand C, Rönnbäck L. Cerebrospinal fluid specific proteins in multi-infarct and senile dementia. *J Neurol Sci* 1981;49:293–303.
- Chatzikonstantinou A, Ebert AD, Hennerici MG. Cerebrospinal fluid findings after epileptic seizures. *Epileptic Disord* 2015;17:453–9.
- Lee MC, Heaney LM, Jacobson RL, et al. Cerebrospinal fluid in cerebral hemorrhage and infarction. *Stroke* 1975;6:638–41.