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# Causes of albuminocytologic dissociation: age-adjusted reference limit impact on review of 2,627 CSF samples

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# Submission Details

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CM, PB, JAB

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Dr. Brooks was involved with conceptualization, writing and revision of the current study.

Dr. McCudden was involved with conceptualization, writing and revision of the current study and supervision as it pertained to the current study.

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Extra data can be accessed by e-mailing John Brooks at jobrooks@toh.on.ca.

# Abstract

OBJECTIVE: We set out to test the discriminative power of an age-adjusted upper reference limit (URL) for CSF total protein (CSF-TP) in identifying pathological causes of albuminocytologic dissociation (ACD).

METHODS: We reviewed the charts of 2,627 patients who underwent a lumbar puncture at a tertiary care center over a 20-year period. Samples with CSF-TP above 45 mg/dL (0.45 g/L) were included. Samples with white blood cell count > $5\times10^9$/L, red blood cell count > $50\times10^9$/L, and glucose < 2.5 mmol/L (45 mg/dL) were excluded as were samples with incomplete data and those taken from paediatric patients (i.e. age < 18 years old). Patients with CSF-TP elevated above 45 mg/dL were considered to have 'pseudo' albuminocytologic dissociation (ACD) unless their CSF-TP was in excess of age-adjusted norms in which case they were considered to have 'true' ACD.

RESULTS: The presence of ACD was associated with a broad range of neurological diagnoses. Among all patients with ACD, a pathological source of CSF-TP elevation was identified in 57% (1490/2627) of cases, 51% of those with 'pseudo' ACD, and 75% with 'true' ACD ($p< 0.001$). Use of an age-adjusted upper reference limit favored 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 relatively 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 over-diagnosis of ACD.


## Strengths and limitations of this study

- This study is of the largest of its kind, incorporating the results of 2,627 reterospectively collected CSF samples over 20 years
- Charts were thoroughly evaluated for potential sources of ACD including the review consultation notes, neuroimaging, and laboratory data
- Sample size was sufficient to show a clear shift is demonstrate toward the diagnosis of polyneuropathy at the exclusion of those with benign headache using age adjusted CSF-TP thresholds in place of a traditional fixed threshold (i.e. CSF-TP ≥ 0.45 g/L)
- Charts of those patients with CSF samples demonstrating albuminocytologic dissociation (2,627 of 16,045) were included using strict criteria (WBC ≤ 5 x $10^6$ / L, RBC ≤ 50 x $10^6$ / 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 body mass index, CSF sample number, or lifestyle factors (smoking, alcohol, or physical activity) were not explored due as this data was unavailable at the time of analysis

## Tweet

Albuminocytologic dissociation is seen in a broad range of neurological diagnoses. Our study of 2,627 patients with ACD examines the causes.

# Introduction

The term "albuminocytologic dissociation" (ACD) was first coined by Sicard and Foix in 1912 to describe the unexpected finding of elevated cerebrospinal fluid (CSF) protein without pleocytosis in patients with spinal compression.(1) Four year 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 cerebrospinal fluid total protein (CSF-TP) reference intervals derived from institutional data at the Ottawa Hospital, comprising an initial dataset of 19,591CSF samples analyzed over a period of 20 years.(3) After exclusions based on laboratory parameters (WBC>$5\times10^9$/L, RBC>$50\times10^9$/L, and glucose<2.5 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 cutoff of 0.45g/L (45 mg/dL). Based on this 0.45 g/L limit, our initial dataset had identified 2,627 samples meeting criteria of ACD.

In the current study, we hypothesized that the implementation of age-adjusted upper reference limits (URL) 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 > 0.45g/L), 'true' ACD (CSF-TP > age-adjusted reference limit) and those with 'pseudo' ACD (0.45g/L< CSF-TP < age-adjusted reference limit) – and to compare the types and frequencies of clinical diagnoses in each group.

# Methods

## *Protocol Approvals, Registrations and Study Population*

This study was approved by the Ottawa Hospital Research Institute (OHRI) Ethics Board (protocol #20160863-01H). All data was extracted from the Ottawa Hospital Data Warehouse based on CSF samples collected between Jan 1, 1996 and Dec 31, 2016. Laboratory data obtained from the database included CSF-TP, CSF glucose, CSF WBC, CSF RBC, and serum creatinine and total protein 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 WBC > $5\times10^9$/L, RBC > $50\times10^9$/L, and glucose < 2.5 mmol/L (45 mg/dL) and CSF-TP < 0.45 g/L (< 45 mg/dL). Samples with incomplete clinical or laboratory data, or those performed on pediatric patients were also excluded.

## *Patient and Public Involvement*

The study analysis utilized anonymized 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 did not always reflect the ultimate diagnostic outcome. To ensure accuracy and quality of data, all 2,627 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 lumbar puncture. 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, consensus was reached between the reviewers (JB, PF, PB). Cases in which multiple factors may have contributed to increased CSF-TP were discussed between reviewers (JB, PF, PB), to ensure accuracy of classification. Each patient was subsequently categorized based on the most likely cause of high CSF-TP; if a cause was not found, patients were categorized based on the clinical indication for LP.

### *CSF-TP Analysis*

Technical specifications for the analytic equipment used in CSF analysis have been outlined in the methods section of the 2017 manuscript by McCudden *et al*:(3)

CSF-TP was analyzed on 3 different instruments over the course of the 20 years included in the study as follows: Roche Hitachi 917, January 1, 1996 to Sep- tember 30, 2001; Beckman Lx20, September 30, 2001 to April 1, 2009; and Siemens Vista 1500, April 1, 2009 to December 1, 2016. The Roche method is based on a benzethonium-chloride turbidimetric anal- ysis, 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 manufacturer's directions.

Other laboratory values were measured on different instruments across the 2 decades included in the study. Serum creatinine, total protein, and CSF glucose were measured on the platforms described above for the same time frames; creatinine was measured by the Jaffe method from September 30, 2001 through April 13, 2013 and by the enzymatic method thereafter. CSF WBC and RBC counts were determined using the Beckman Coulter be- tween 1996 and 2009 and the Sysmex XE5000 from 2009 to 2016. All laboratory analyses were determined according to manufacturer's instructions throughout the study in a routine clinical laboratory in an academic medical center (The Ottawa Hospital).

### *Data Analysis*

ACD was defined as 'traditional' ACD if CSF-TP exceeded a typical cutoff 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*. If CSF-TP was between 0.45g/L and the age-adjusted reference limits, the case was labeled '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 (pathological conditions), and those where an increase was *unexpected* (non-pathological conditions).

For each clinical category, the category's share of ACD patients was computed (i.e. the number of patients assigned to a clinical category versus 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 established utility of ACD in polyneuropathy, a subgroup analysis focused on these patients. The mean CSF-TP levels, shown with their 95% confidence intervals, were 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 version 3.3.3 (The R Foundation, Vienna, Austria).

## Results

The range of CSF values among 2,627 patients with albumino-cytologic dissociation over a 20-year timeframe have been plotted in Figure 3. Among all patients with 'traditional' ACD (CSF-TP>0.45g/L), the underlying clinical category/diagnosis was pathological in 57% (1490/2627) of cases. The finding of 'true' ACD was *expected* in 77% (450/597) of cases; whereas in 'pseudo' ACD, ACD was *expected* in only 51% (1040/2030) 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 0.45g/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 relative share of patients with ACD was seen or there was no significant change. The exception to this pattern was inflammatory white matter disease where, similar to clinical categories with *unexpected* CSF-TP elevation, a significant decrease in the relative share of patients 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 $1.37 \pm 0.24$ g/L for inflammatory neuropathy versus $0.59 \pm 0.6$ 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, or 4) sequestration of CSF in spinal compression. Minor elevations of CSF-TP that

are not associated with increased cell counts have also been described in non-pathological conditions. This would include differences due to age, body mass index, and maximal abdominal circumference.(4) Techniques have been proposed to correct for the impact of physiologic variables, such as age, on metrics of blood CSF barrier dysfunction including those more tailored toward such an assessment (e.g. albumin quotient).(5) The results of routine CSF testing however often still leaves clinicians with a need to decide what level of isolated protein elevation may reflect an abnormality requiring further investigation.

Our previous publication used a large dataset of CSF samples, acquired over a 20-year period, to develop age-adjusted upper reference limits for CSF protein values.(3) In the current study, we focused on 2,627 cases that met criteria for ACD based on the traditional 0.45g/L CSF TP reference limit. We aimed to: 1) determine the breakdown of clinical diagnoses associated with ACD, and 2) gauge the effect of applying an age-adjusted reference limit on the types and frequency of cases associated with 'true' ACD.

ACD was a remarkably common CSF finding in diagnostic lumbar puncture at our institution, present in 2,627 of 8,340 specimens (or 31.4%) using the traditional 0.45g/L reference limit, but 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 tumors (8%). We determined that by applying age-adjusted CSF-TP reference limits (rather than a fixed upper reference limit of 0.45g/L), there was a marked reduction in the number of patients meeting criteria for ACD. This was particularly significant for 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 pathological in 75% of cases.

Brettschneider *et al*.(6) similarly observed frequencies of particular clinical diagnoses (resulting in higher specificity for pathological 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 a pathological cause of $Q_{alb}$ elevation (including Guillian-Barré syndrome/CIDP, lumbar spinal stenosis, or epileptic seizures, among other diagnoses). While the Brettschneider *et al*. article certainly supports 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 a main source of pathological (*expected*) ACD. Many articles in the medical literature have focused on 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.(7, 8) Non-inflammatory neuropathies often display a more modest degree of blood-nerve barrier dysfunction as evidenced by less extreme elevations in CSF-TP.(8, 9) 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 re-classified using European Federation of Neurological Societies (EFNS) criteria, patients with CIDP had substantially higher mean CSF-TP (1.56g/L) as compared to those without CIDP (0.61g/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 (i.e. the computed estimate of the 97.5$^{th}$ 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 and unnecessary treatment for CIDP.

Other notable clinical categories included headache and inflammatory white matter disease. From examining the data, one may question why benign headache might account for a significant proportion of patients with ACD. We suspect that this reflects the large number of patients who underwent lumbar puncture as screening for subarachnoid hemorrhage or meningitis. Moreover, headache patients were the most likely to be re-classified as 'pseudo' ACD when age adjusted thresholds were applied. Similarly, patients with inflammatory white matter showed a high likelihood of being re-classified 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.(10, 11)

Our study does have several limitations worth mention. First, without a formal chart review of all 16,045 patients (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 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 hemoglobin and thyroid stimulating hormone) may further improve our understanding of CSF-TP variability.(12) Fourth, the age-adjusted CSF-TP upper reference limit and the analysis presented here apply only to those patients with ACD and are not generalizable to those patients who have additional CSF abnormalities (for example – pleocytosis, or high red blood cell count).

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

# Figures

**Figure 1. Flow chart illustrating the exclusion process used to identify patients with ACD.**

CSF: Cerebrospinal fluid; WBC: White blood cell count; RBC: Reb blood cell count; TP: Total Protein.

**Figure 2. Proportions of patients with albuminocytologic dissociation.**

Proportionate breakdowns are shown for 'true' ACD (i.e. CSF-TP value greater than the age-adjusted upper reference limit), 'traditional' ACD (i.e. CSF-TP value greater than 0.45 g/L), and 'pseudo' ACD (i.e. CSF-TP value greater than 0.45 g/L but less than the age-adjusted upper reference limit). Diagnostic categories (reason for LP) not expected to cause ACD are represented in shades of red and organized left to right by descending magnitude of absolute percentage change from all ACD to true ACD. Pathologic categories with a potential expectation for ACD are represented in shades of blue and organized left to right by ascending magnitude of absolute percentage change between all ACD and true ACD. The *other* categories (i.e. "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: albuminocytologic dissociation; CSF-TP: Cerebrospinal fluid total protein; IWMD: Inflammatory white matter disease; T. Encephalopathy: Transient Encephalopathy

**Figure 3. CSF-TP reference interval.**

Points represent post exclusion CSF-TP concentrations with the removal of all but the original point where patients had multiple CSF samples drawn (n = 8,175). Patients were grouped into 5-year bins by their age at the time of lumbar puncture. The resultant 97.5$^{th}$ 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; CSF-TP: Cerebrospinal fluid total protein

# Tables

| Clinical Categories | References |
|---|---|
| Following intrathecal chemotherapy[a] | (13) |
| Following subarachnoid hemorrhage | (14) |
| Infectious / non-infectious encephalitis | (15, 16) |
| Infectious / non-infectious meningitis | (17-19) |
| Intra-axial / extra-axial tumors | (20-22) |
| Inflammatory polyneuropathy | (8, 23) |
| Non-inflammatory polyneuropathy | (24) |
| Hydrocephalus before / after shunt placement | (25, 26) |
| Angiitis of the central nervous system | (27) |
| Inflammatory white matter disease | (28-30) |
| Cerebrovenous sinus occlusion | (31) |
| Optic nerve disease | (32) |
| Optic neuritis | (33) |
| Hypertensive encephalopathy | (34) |
| Structural spinal disorders | (35, 36) |
| Nervous system toxin exposure | (37) |
| Dementia | (38) |
| Seizure | (39) |
| Stroke (Hemorrhagic / Ischemic) | (40) |

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

[a] The underlying condition for which intrathecal chemotherapy was provided in the cited report was related to the Central Nervous System involvement of Systemic Lupus Erythematosus as opposed to predominantly the treatment of a hematologic malignancy in the context of our report.

| Clinical Category | ACD Expected | Proportion with ACD (0.45g/L upper limit) | Proportion with ACD (age-adjusted upper limit) | Change | P-value[a] |
|---|---|---|---|---|---|
| | Y/N | n (%) | n (%) | Δ% | |
| polyneuropathy | Y | 204 (7.8%) | 127 (21.3%) | 13.50% | < 0.0001 |
| tumor | Y | 139 (5.3%) | 47 (7.9%) | 2.60% | 0.019 |
| encephalitis | 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 |
| plexopathy | 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 | Y | 8 (0.3%) | 4 (0.7%) | 0.40% | 0.25 |
| diffuse anoxic-ischemic injury | Y | 17 (0.6%) | 6 (1%) | 0.40% | 0.41 |
| infection (no CNS involvement) | 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 | 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 |
| hemorrhage | 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 | Y | 5 (0.2%) | 1 (0.2%) | 0% | 1 |
| aseptic meningitis | Y | 1 (0%) | 0 (0%) | 0% | 1 |
| IIH | Y | 24 (0.9%) | 5 (0.8%) | -0.10% | 1 |
| hypertensive encephalopathy | Y | 16 (0.6%) | 3 (0.5%) | -0.10% | 1 |
| systemic inflammatory process | Y | 3 (0.1%) | 0 (0%) | -0.10% | 1 |
| cranial neuropathy | Y | 12 (0.5%) | 2 (0.3%) | -0.20% | 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 |
| 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 |
| genetic neurological illness | N | 3 (0.1%) | 2 (0.3%) | 0.20% | 0.23 |
| 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 |
| transient ischemic 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 |

Table 2. Expectation of protein elevation, Number and proportion of patients with a specific clinical category compared to all reported cases with associated percentage change from using an invariant 0.45 g/L CST-TP threshold (all comers with ACD) versus a threshold varying with age ('true' ACD). ACD: Albuminocytologic dissociation; CNS: central nervous system; CSF: cerebrospinal fluid.

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

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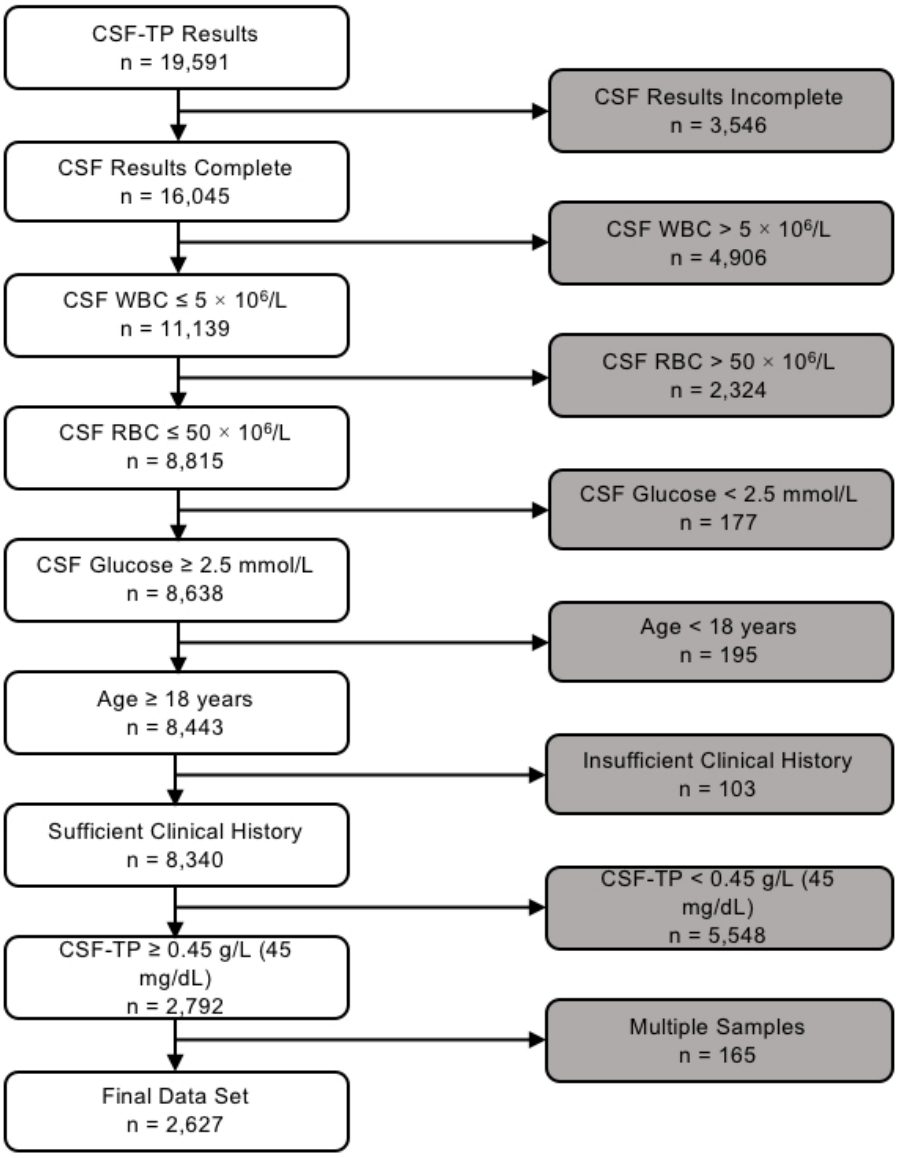
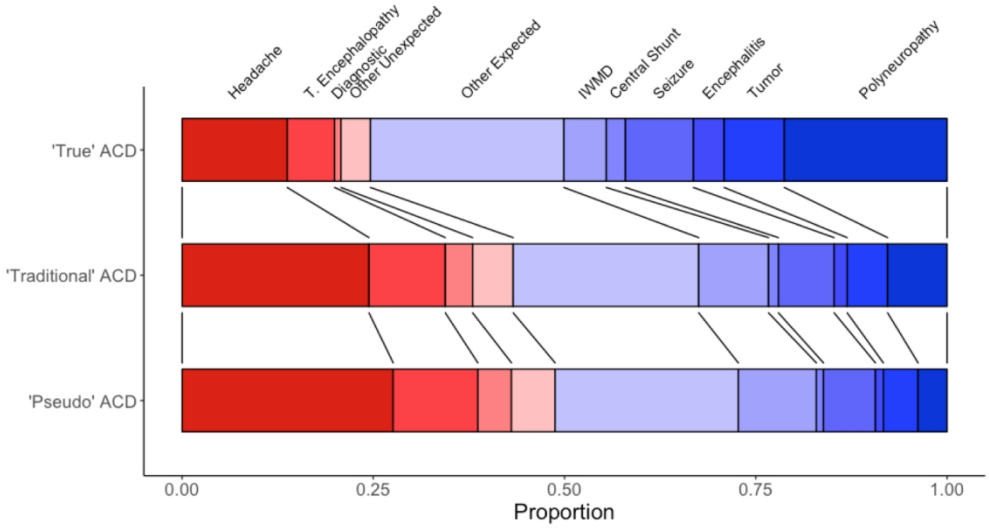


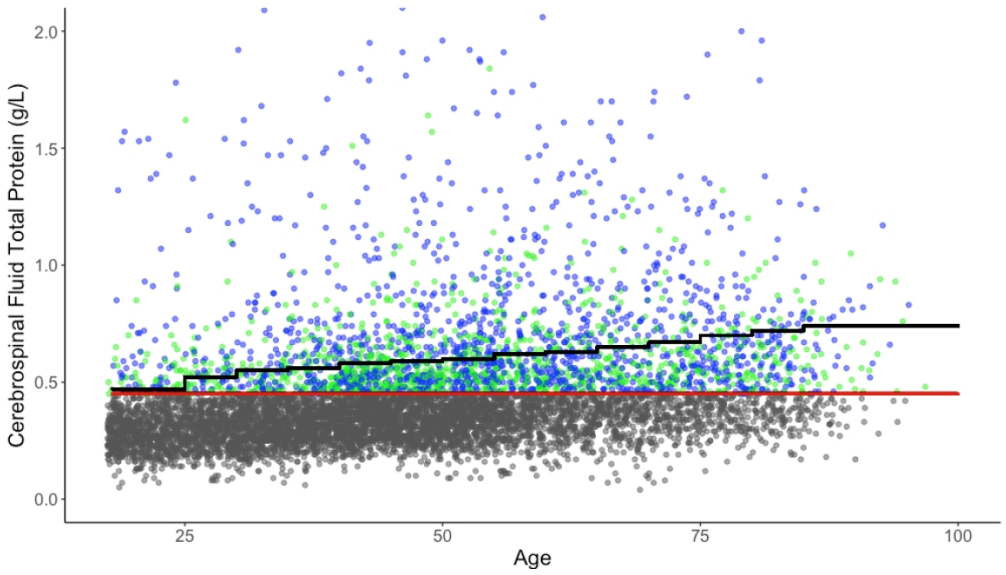

Flow chart illustrating the exclusion process used to identify patients with ACD. CSF: Cerebrospinal fluid; WBC: White blood cell count; RBC: Reb blood cell count; TP: Total Protein.

103x134mm (144 x 144 DPI)




Proportions of patients with albuminocytologic dissociation. Proportionate breakdowns are shown for 'true' ACD (i.e. CSF-TP value greater than the age-adjusted upper reference limit), 'traditional' ACD (i.e. CSF-TP value greater than 0.45 g/L), and 'pseudo' ACD (i.e. CSF-TP value greater than 0.45 g/L but less than the age-adjusted upper reference limit). Diagnostic categories (reason for LP) not expected to cause ACD are represented in shades of red and organized left to right by descending magnitude of absolute percentage change from all ACD to true ACD. Pathologic categories with a potential expectation for ACD are represented in shades of blue and organized left to right by ascending magnitude of absolute percentage change between all ACD and true ACD. The other categories (i.e. "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: albuminocytologic dissociation; CSF-TP: Cerebrospinal fluid total protein; IWMD: Inflammatory white matter disease; T. Encephalopathy: Transient Encephalopathy

274x153mm (144 x 144 DPI)




CSF-TP reference interval. Points represent post exclusion CSF-TP concentrations with the removal of all but the original point where patients had multiple CSF samples drawn (n = 8,175). 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; CSF-TP: Cerebrospinal fluid total protein

350x198mm (144 x 144 DPI)

BMJ Open

# Causes of albuminocytologic dissociation and the impact of age adjusted cerebrospinal fluid protein reference intervals: a retrospective chart review of 2,627 samples collected at tertiary care center



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CM, PB, JAB

Writing and revision:
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**Contributorship Statement:**

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Extra data can be accessed by e-mailing John Brooks at John.brooks@one-mail.on.ca.

# Abstract

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

METHODS: We reviewed the charts of 2,627 patients who underwent a lumbar puncture at a tertiary care center over a 20-year period. Samples with CSF-TP above 45 mg/dL (0.45 g/L) were included. Samples with white blood cell count > $5\times10^9$/L, red blood cell count > $50\times10^9$/L, and glucose < 2.5 mmol/L (45 mg/dL) were excluded as were samples with incomplete data and those taken from paediatric patients (i.e. age < 18 years old). Patients with CSF-TP elevated above 45 mg/dL were considered to have 'pseudo' albuminocytologic dissociation (ACD) unless their CSF-TP was in excess of age-adjusted norms in which case they were considered to have 'true' ACD.

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 favored 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 over-diagnosis of ACD.


## Strengths and limitations of this study

- This study is of the largest of its kind, incorporating the results of 2,627 reterospectively collected CSF samples over 20 years
- Charts were thoroughly evaluated for potential sources of ACD including the review consultation notes, neuroimaging, and laboratory data
- Sample size was sufficient to show a clear shift toward clinically relevant diagnoses like polyneuropathy at the exclusion of those without such pathology (e.g. benign headache) using age adjusted CSF-TP thresholds in place of a traditional fixed threshold (i.e. CSF-TP ≥ 0.45 g/L)
- Charts of those patients with CSF samples demonstrating albuminocytologic dissociation (2,627 of 16,045) were included using strict criteria (WBC ≤ 5 x $10^6$ / L, RBC ≤ 50 x $10^6$ / 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 body mass index, CSF sample number, or lifestyle factors (smoking, alcohol, or physical activity) were not explored as this data was unavailable at the time of analysis

## Tweet

Albuminocytologic dissociation is seen in a broad range of neurological diagnoses. Our study of 2,627 patients with ACD examines the causes.

# Introduction

The term "albuminocytologic dissociation" (ACD) was first coined by Sicard and Foix in 1912 to describe the unexpected finding of elevated cerebrospinal fluid (CSF) protein without pleocytosis in patients with spinal compression.(1) Four year 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 cerebrospinal fluid total protein (CSF-TP) reference intervals derived from institutional data at the Ottawa Hospital, comprising an initial dataset of 19,591CSF samples analyzed over a period of 20 years.(3) After exclusions based on laboratory parameters (WBC>$5\times10^9$/L, RBC>$50\times10^9$/L, and glucose<2.5 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 cutoff of 0.45g/L (45 mg/dL).

In the current study, we hypothesized that the implementation of age-adjusted upper reference limits (URL) 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 > 0.45g/L), 'true' ACD (CSF-TP > age-adjusted reference limit) and those with 'pseudo' ACD (0.45g/L< CSF-TP < age-adjusted reference limit) – and to compare the types and frequencies of clinical diagnoses in each group.

# Methods

## *Protocol Approvals, Registrations and Study Population*

This study was approved by the Ottawa Hospital Research Institute (OHRI) Ethics Board (protocol #20160863-01H). All data was extracted from the Ottawa Hospital Data Warehouse based on CSF samples collected between Jan 1, 1996 and Dec 31, 2016. Laboratory data obtained from the database included CSF-TP, CSF glucose, CSF WBC, CSF RBC, and serum creatinine and total protein 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 WBC > $5\times10^9$/L, RBC > $50\times10^9$/L, and glucose < 2.5 mmol/L (45 mg/dL) and CSF-TP < 0.45 g/L (< 45 mg/dL). Samples with incomplete clinical or laboratory data, or those performed on pediatric patients were also excluded.

## *Patient and Public Involvement*

The study analysis utilized anonymized 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 did not always reflect the ultimate diagnostic outcome. To ensure accuracy and quality of data, all 2,627 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 lumbar puncture. 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, consensus was reached between the reviewers (JB, PF, PB). Cases in which multiple factors may have contributed to increased CSF-TP were discussed between reviewers (JB, PF, PB), to ensure accuracy of classification. Each patient was subsequently categorized based on the most likely cause of high CSF-TP; if a cause was not found, patients were categorized based on the clinical indication for LP.

## CSF-TP Analysis

Technical specifications for the analytic equipment used in CSF analysis have been outlined in the methods section of the 2017 manuscript by McCudden *et al*:(3)

CSF-TP was analyzed on 3 different instruments over the course of the 20 years included in the study as follows: Roche Hitachi 917, January 1, 1996 to September 30, 2001; Beckman Lx20, September 30, 2001 to April 1, 2009; and Siemens Vista 1500, April 1, 2009 to December 1, 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 manufacturer's directions.

Other laboratory values were measured on different instruments across the 2 decades included in the study. Serum creatinine, total protein, and CSF glucose were measured on the platforms described above for the same time frames; creatinine was measured by the Jaffe method from September 30, 2001 through April 13, 2013 and by the enzymatic method thereafter. CSF WBC and RBC counts were determined using the Beckman Coulter be- tween 1996 and 2009 and the Sysmex XE5000 from 2009 to 2016. All laboratory analyses were determined according to manufacturer's instructions throughout the study in a routine clinical laboratory in an academic medical center (The Ottawa Hospital).

## Data Analysis

ACD was defined as 'traditional' ACD if CSF-TP exceeded a typical cutoff 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:

$$URL = 0.124 + 0.0284 \times Age - 7.08 \times 10^{-4} \times Age^2 + 8.23 \times 10^{-6} \times Age^3 - 3. \times Age^4 \quad (1)$$

If CSF-TP was between 0.45g/L and the age-adjusted reference limits, the case was labeled '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 confidence intervals 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 (i.e. the number of patients assigned to a clinical category versus 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**Figure 2). Given the large number of statistical tests performed (i.e. 47 Fischer's exact tests) Bonferroni correction was applied to the threshold p-value suggesting statistical significance (i.e. 0.001 = 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 interquartile range, 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 version 3.3.3 (The R Foundation, Vienna, Austria).

# Results

The range of CSF values among 2,627 patients (1,093 female with a median [interquartile range] age of 54 [25]) with albumino-cytologic dissociation over a 20-year timeframe have been plotted in Figure 3. Among all patients with 'traditional' ACD (CSF-TP>0.45g/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 0.45g/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 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).

Additional data can be obtained by emailing the corresponding author, John Brooks, at John.brooks@one-mail.on.ca.

# 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 physiologic variables, such as age, on metrics of blood CSF barrier dysfunction including those more tailored toward such an assessment (e.g. albumin quotient).(5) The results of routine CSF testing however often still leaves clinicians with a need to decide what level of isolated protein elevation may reflect an abnormality requiring further investigation.

ACD was a remarkably common CSF finding in diagnostic lumbar puncture at our institution, present in 2,627 of 8,340 specimens (or 31.4%) using the traditional 0.45g/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 tumors (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 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 Guillian-Barré syndrome/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 a main source of clinically relevant (*expected*) ACD. Many articles in the medical literature have focused on 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 re-classified using European Federation of Neurological Societies (EFNS) criteria, patients with CIDP had a substantially higher mean CSF-TP (1.56g/L) as compared to those without CIDP (0.61g/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 (i.e. the computed estimate of the 97.5$^{th}$ 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 headache and inflammatory white matter disease. From examining the data, one may question why 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 lumbar puncture as screening for subarachnoid hemorrhage 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 re-classified as 'pseudo' ACD when age adjusted thresholds were applied. Similarly, patients with inflammatory white matter showed a high likelihood of being re-classified 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 16,045 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 hemoglobin 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 19,591 samples, only 2,627 samples were included in the analysis after eliminating repeat and incomplete sampling as well as those with biochemical and cytologic measures outside of established norms and thus are not generalizable to those patients who have additional CSF abnormalities (for example – pleocytosis, hypoglycorrhachia or high red blood cell count).

Apart from the above limitations, we believe that our study successfully presents the relevant clinical diagnoses associated with 'true' ACD, above the age-adjusted upper reference limit. 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 over-emphasize the importance of a finding of ACD, particularly given that common conditions such as lumbar stenosis may be the cause. To maximize the insight gained from CSF-TP levels, future study evaluating the effects of additional factors on values within the 'true' ACD range is warranted.

# Figures

**Figure 1. Flow chart illustrating the exclusion process used to identify patients with ACD.**

CSF: Cerebrospinal fluid; WBC: White blood cell count; RBC: Reb blood cell count; TP: Total Protein.

**Figure 2. Proportions of patients with albuminocytologic dissociation.**

Proportionate breakdowns are shown for 'true' ACD (i.e. CSF-TP value greater than the age-adjusted upper reference limit), 'traditional' ACD (i.e. CSF-TP value greater than 0.45 g/L), and 'pseudo' ACD (i.e. CSF-TP value greater than 0.45 g/L but less than the age-adjusted upper reference limit). Diagnostic categories (reason for LP) not expected to cause ACD are represented in shades of red and organized left to right by descending magnitude of absolute percentage change from all ACD to true ACD. Pathologic categories with a potential expectation for ACD are represented in shades of blue and organized left to right by ascending magnitude of absolute percentage change between all ACD and true ACD. The *other* categories (i.e. "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: albuminocytologic dissociation; CSF-TP: Cerebrospinal fluid total protein; IWMD: Inflammatory white matter disease; T. Encephalopathy: Transient Encephalopathy

**Figure 3. CSF-TP reference interval.**

Points represent post exclusion CSF-TP concentrations with the removal of all but the original point where patients had multiple CSF samples drawn (n = 8,175). Patients were grouped into 5-year bins by their age at the time of lumbar puncture. The resultant 97.5$^{th}$ 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; CSF-TP: Cerebrospinal fluid total protein

# Tables

| Clinical Categories | References |
|---|---|
| Following intrathecal chemotherapy[a] | (14) |
| Following subarachnoid hemorrhage | (15) |
| Infectious / non-infectious encephalitis | (16, 17) |
| Infectious / non-infectious meningitis | (18-20) |
| Intra-axial / extra-axial tumors | (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) |
| Cerebrovenous sinus occlusion | (32) |
| Optic nerve disease | (33) |
| Optic neuritis | (34) |
| Posterior Reversible Encephalopathy Syndrome (PRES) | (35) |
| Structural spinal disorders | (36, 37) |
| Nervous system toxin exposure | (38) |
| Dementia | (39) |
| Seizure | (40) |
| Stroke (Hemorrhagic / Ischemic) | (41) |

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

[a] The underlying condition for which intrathecal chemotherapy was provided in the cited report was related to the Central Nervous System involvement of Systemic Lupus Erythematosus as opposed to predominantly the treatment of a hematologic malignancy in the context of our report.

| Clinical Category | ACD Expected | Traditional ACD - Proportion with ACD (0.45g/L upper limit) | True ACD - Proportion with ACD (age-adjusted upper limit) | Change | P-value[a] |
|---|---|---|---|---|---|
| | Y/N | n (%) | n (%) | Δ% | |
| **polyneuropathy** | **Y** | **204 (7.8%)** | **127 (21.3%)** | **13.50%** | **< 0.0001** |
| tumor | 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 (e.g. post neurosurgery, diffuse axonal injury, etc.) | Y | 8 (0.3%) | 4 (0.7%) | 0.40% | 0.25 |
| diffuse anoxic-ischemic injury | Y | 17 (0.6%) | 6 (1%) | 0.40% | 0.41 |
| infection (no CNS involvement e.g. 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 (e.g. carcinomatosis, IgG4 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 |
| Hemorrhage within 3 months (e.g. 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 e.g. 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 PRES | 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 |
| 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 ischemic 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 | | 2,627 (100%) | 597 (100%) | | |

Table 2. Expectation of protein elevation, Number and proportion of patients with a specific clinical category compared to all reported cases with associated percentage change from using an invariant 0.45 g/L CST-TP threshold (all comers with ACD) versus a threshold varying with age ('true' ACD). 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. ACD: Albuminocytologic dissociation; All Cause Major Stroke: includes thromboembolic disease, vasculitis of the CNS causing stroke and reversible cerebrovascular constriction syndrome; CNS: central nervous system; CSF: cerebrospinal fluid. Inflammatory white matter disease: includes multiple sclerosis, Neuromyelitis Optica, Acute Demyelinating Encephalomyelitis.

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

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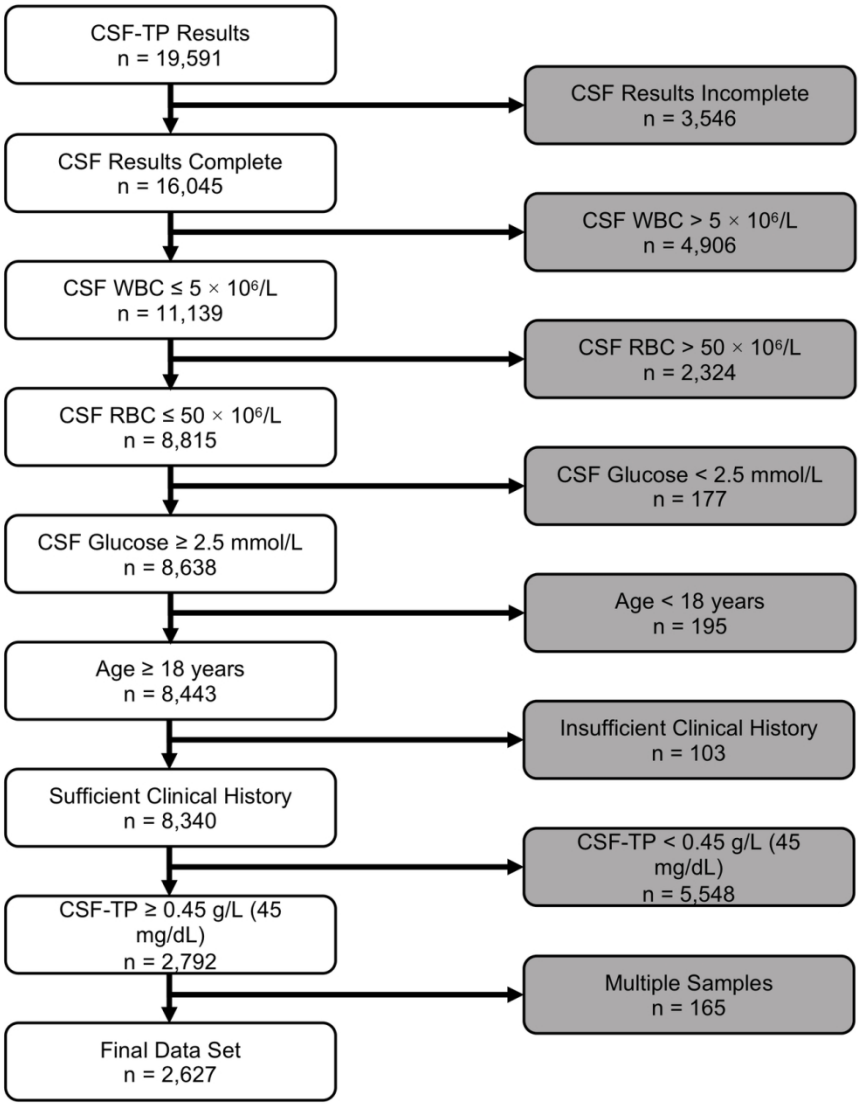
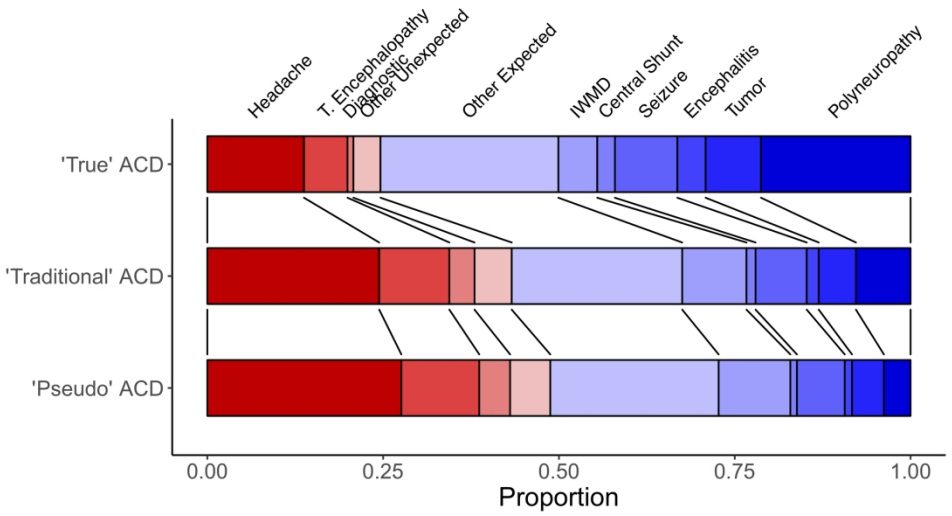



Flow chart illustrating the exclusion process used to identify patients with ACD.

CSF: Cerebrospinal fluid; WBC: White blood cell count; RBC: Reb blood cell count; TP: Total Protein.

120x153mm (300 x 300 DPI)

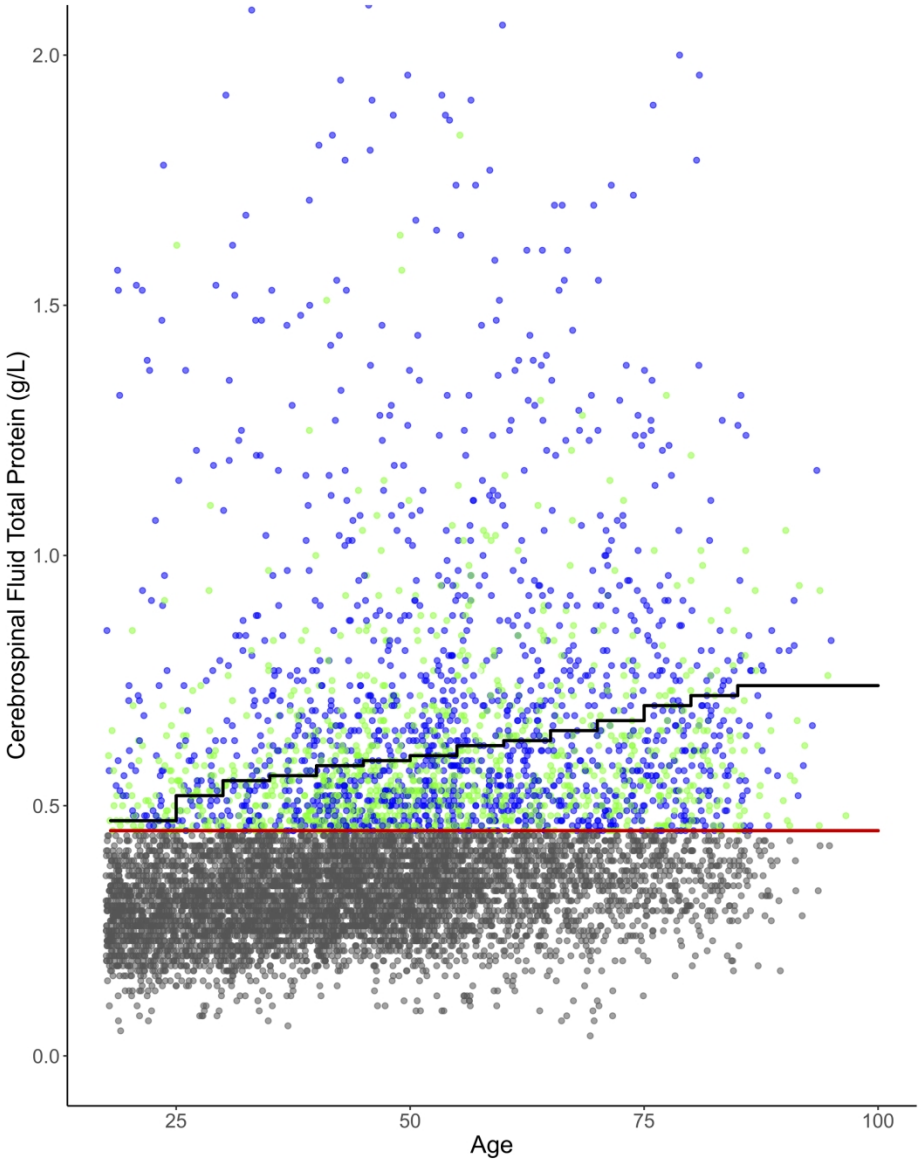



Proportions of patients with albuminocytologic dissociation.

Proportionate breakdowns are shown for 'true' ACD (i.e. CSF-TP value greater than the age-adjusted upper reference limit), 'traditional' ACD (i.e. CSF-TP value greater than 0.45 g/L), and 'pseudo' ACD (i.e. CSF-TP value greater than 0.45 g/L but less than the age-adjusted upper reference limit). Diagnostic categories (reason for LP) not expected to cause ACD are represented in shades of red and organized left to right by descending magnitude of absolute percentage change from all ACD to true ACD. Pathologic categories with a potential expectation for ACD are represented in shades of blue and organized left to right by ascending magnitude of absolute percentage change between all ACD and true ACD. The other categories (i.e. "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: albuminocytologic dissociation; CSF-TP: Cerebrospinal fluid total protein; IWMD: Inflammatory white matter disease; T. Encephalopathy: Transient Encephalopathy

215x177mm (300 x 300 DPI)




CSF-TP reference interval.

Points represent post exclusion CSF-TP concentrations with the removal of all but the original point where patients had multiple CSF samples drawn (n = 8,175). 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; CSF-TP: Cerebrospinal fluid total protein

215x279mm (300 x 300 DPI)

STROBE Checklist

| | |
|---|---|
| 1 a | Pg 1 |
| 1 b | Pg 3 |
| 2 | Pg 4 |
| 3 | Pg 4 |
| 4 | Pg 4 |
| 5 | Pg 4 |
| 6 | Pg 4 |
| 7 | Pg 4 – 6 |
| 8 | Pg 4 – 6 |
| 9 | Pg 4 – 6 |
| 10 | Pg 4 – 6 |
| 11 | Pg 4 – 6 |
| 12 a | Pg 5 – 6 |
| 12 b | Pg 5 – 6 |
| 12 c | Pg 4 |
| 12 d | NA |
| 12 e | Pg 5 – 6 |
| 13 a | Pg 6 |
| 13 b | Pg 6 |
| 13 c | Pg 6 |
| 14 a | Pg 6 |
| 14 b | Pg 6 |
| 14 c | Pg 6 |
| 15 | Pg 6 |
| 16 a | Pg 6 |
| 16 b | Pg 6 |
| 16 c | NA |
| 17 | Pg 6 |
| 18 | Pg 9 |
| 19 | Pg 9 – 10 |
| 20 | Pg 9 |
| 21 | Pg 10 |
| 22 | Included separately |

BMJ Open

# Causes of albuminocytologic dissociation and the impact of age adjusted cerebrospinal fluid protein reference intervals: a retrospective chart review of 2,627 samples collected at tertiary care center



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**Title:** Causes of albuminocytologic dissociation and the impact of age adjusted cerebrospinal fluid protein reference intervals: a retrospective chart review of 2,627 samples collected at tertiary care center

**Running head:** Causes of albuminocytologic dissociation

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Dr. Brooks was involved with conceptualization, writing and revision of the current study.

Dr. McCudden was involved with conceptualization, writing and revision of the current study and supervision as it pertained to the current study.

Dr. Breiner writing and revision of the current study.

Dr. Bourque was involved with conceptualization, writing and revision of the current study and supervision as it pertained to the current study.

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Additional data regarding disease specific cerebrospinal total protein levels can be accessed by e-mailing John Brooks at jobrooks@toh.ca.

# Abstract

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

METHODS: We reviewed the charts of 2,627 patients who underwent a lumbar puncture at a tertiary care center over a 20-year period. Samples with CSF-TP above 45 mg/dL (0.45 g/L) were included. Samples with white blood cell count > $5\times10^9$/L, red blood cell count > $50\times10^9$/L, and glucose < 2.5 mmol/L (45 mg/dL) were excluded as were samples with incomplete data and those taken from paediatric patients (i.e. age < 18 years old). Patients with CSF-TP elevated above 45 mg/dL were considered to have 'pseudo' albuminocytologic dissociation (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 favored 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 over-diagnosis of ACD.


## Strengths and limitations of this study

- This study is of the largest of its kind, incorporating the results of 2,627 reterospectively collected CSF samples over 20 years
- Charts were thoroughly evaluated for potential sources of ACD including the review consultation notes, neuroimaging, and laboratory data
- Sample size was sufficient to show a clear shift toward clinically relevant diagnoses like polyneuropathy at the exclusion of those without such pathology (e.g. benign headache) using age adjusted CSF-TP thresholds in place of a traditional fixed threshold (i.e. CSF-TP ≥ 0.45 g/L)
- Charts of those patients with CSF samples demonstrating albuminocytologic dissociation (2,627 of 16,045) were included using strict criteria (WBC ≤ 5 x $10^6$ / L, RBC ≤ 50 x $10^6$ / 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 this data was unavailable at the time of analysis

## Tweet

Albuminocytologic dissociation is seen in a broad range of neurological diagnoses. Our study of 2,627 patients with ACD examines the causes.

# Introduction

The term "albuminocytologic dissociation" (ACD) was first coined by Sicard and Foix in 1912 to describe the unexpected finding of elevated cerebrospinal fluid (CSF) protein without pleocytosis in patients with spinal compression.(1) Four year 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 cerebrospinal fluid total protein (CSF-TP) reference intervals derived from institutional data at the Ottawa Hospital, comprising an initial dataset of 19,591CSF samples analyzed over a period of 20 years.(3) After exclusions based on laboratory parameters (WBC>$5\times10^9$/L, RBC>$50\times10^9$/L, and glucose<2.5 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 cutoff of 0.45g/L (45 mg/dL).

In the current study, we hypothesized that the implementation of age-adjusted upper reference limits (URL) 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 > 0.45g/L), 'true' ACD (CSF-TP > age-adjusted reference limit) and those with 'pseudo' ACD (0.45g/L< CSF-TP < age-adjusted reference limit) – and to compare the types and frequencies of clinical diagnoses in each group.

# Methods

## *Protocol Approvals, Registrations and Study Population*

This study was approved by the Ottawa Hospital Research Institute (OHRI) Ethics Board (protocol #20160863-01H). All data was extracted from the Ottawa Hospital Data Warehouse based on CSF samples collected between Jan 1, 1996 and Dec 31, 2016. Laboratory data obtained from the database included CSF-TP, CSF glucose, CSF WBC, CSF RBC, and serum creatinine and total protein 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 WBC > $5\times10^9$/L, RBC > $50\times10^9$/L, and glucose < 2.5 mmol/L (45 mg/dL) and CSF-TP < 0.45 g/L (< 45 mg/dL). Samples with incomplete clinical or laboratory data, or those performed on pediatric patients were also excluded.

## *Patient and Public Involvement*

The study analysis utilized anonymized 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 did not always reflect the ultimate diagnostic outcome. To ensure accuracy and quality of data, all 2,627 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 lumbar puncture. 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, consensus was reached between the reviewers (JB, PF, PB). Cases in which multiple factors may have contributed to increased CSF-TP were discussed between reviewers (JB, PF, PB), to ensure accuracy of classification. Each patient was subsequently categorized based on the most likely cause of high CSF-TP; if a cause was not found, patients were categorized based on the clinical indication for LP.

## CSF-TP Analysis

Technical specifications for the analytic equipment used in CSF analysis have been outlined in the methods section of the 2017 manuscript by McCudden *et al*:(3)

CSF-TP was analyzed on 3 different instruments over the course of the 20 years included in the study as follows: Roche Hitachi 917, January 1, 1996 to September 30, 2001; Beckman Lx20, September 30, 2001 to April 1, 2009; and Siemens Vista 1500, April 1, 2009 to December 1, 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 manufacturer's directions.

Other laboratory values were measured on different instruments across the 2 decades included in the study. Serum creatinine, total protein, and CSF glucose were measured on the platforms described above for the same time frames; creatinine was measured by the Jaffe method from September 30, 2001 through April 13, 2013 and by the enzymatic method thereafter. CSF WBC and RBC counts were determined using the Beckman Coulter be- tween 1996 and 2009 and the Sysmex XE5000 from 2009 to 2016. All laboratory analyses were determined according to manufacturer's instructions throughout the study in a routine clinical laboratory in an academic medical center (The Ottawa Hospital).

## Data Analysis

ACD was defined as 'traditional' ACD if CSF-TP exceeded a typical cutoff 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:

$$URL = 0.124 + 0.0284 \times Age - 7.08 \times 10^{-4} \times Age^2 + 8.23 \times 10^{-6} \times Age^3 - 3. \times Age^4 \quad (1)$$

If CSF-TP was between 0.45g/L and the age-adjusted reference limits, the case was labeled '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 confidence intervals 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 (i.e. the number of patients assigned to a clinical category versus 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**Figure 2). Given the large number of statistical tests performed (i.e. 47 Fischer's exact tests) Bonferroni correction was applied to the threshold p-value suggesting statistical significance (i.e. 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 interquartile range, 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 version 3.3.3 (The R Foundation, Vienna, Austria).

# Results

The range of CSF values among 2,627 patients (1,093 female with a median [interquartile range] age of 54 [25]) with albumino-cytologic dissociation over a 20-year timeframe have been plotted in Figure 3. Among all patients with 'traditional' ACD (CSF-TP>0.45g/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 0.45g/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 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).

Additional data regarding disease specific cerebrospinal total protein levels can be accessed by e-mailing John Brooks at jobrooks@toh.ca.

# 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 physiologic variables, such as age, on metrics of blood CSF barrier dysfunction including those more tailored toward such an assessment (e.g. albumin quotient).(5) The results of routine CSF testing however often still leaves clinicians with a need to decide what level of isolated protein elevation may reflect an abnormality requiring further investigation.

ACD was a remarkably common CSF finding in diagnostic lumbar puncture at our institution, present in 2,627 of 8,340 specimens (or 31.4%) using the traditional 0.45g/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 tumors (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 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 Guillian-Barré syndrome/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 a main source of clinically relevant (*expected*) ACD. Many articles in the medical literature have focused on 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 re-classified using European Federation of Neurological Societies (EFNS) criteria, patients with CIDP had a substantially higher mean CSF-TP (1.56g/L) as compared to those without CIDP (0.61g/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 (i.e. the computed estimate of the 97.5$^{th}$ 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 headache and inflammatory white matter disease. From examining the data, one may question why 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 lumbar puncture as screening for subarachnoid hemorrhage 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 re-classified as 'pseudo' ACD when age adjusted thresholds were applied. Similarly, patients with inflammatory white matter showed a high likelihood of being re-classified 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 16,045 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 hemoglobin 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 19,591 samples, only 2,627 samples were included in the analysis after eliminating repeat and incomplete sampling as well as those with biochemical and cytologic measures outside of established norms and thus are not generalizable to those patients who have additional CSF abnormalities (for example – pleocytosis, hypoglycorrhachia or high red blood cell count).

Apart from the above limitations, we believe that our study successfully presents the relevant clinical diagnoses associated with 'true' ACD, above the age-adjusted upper reference limit. 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 over-emphasize the importance of a finding of ACD, particularly given that common conditions such as lumbar stenosis may be the cause. To maximize the insight gained from CSF-TP levels, future study evaluating the effects of additional factors on values within the 'true' ACD range is warranted.

# Figures

**Figure 1. Flow chart illustrating the exclusion process used to identify patients with ACD.**

CSF: Cerebrospinal fluid; WBC: White blood cell count; RBC: Reb blood cell count; TP: Total Protein.

**Figure 2. Proportions of patients with albuminocytologic dissociation.**

Proportionate breakdowns are shown for 'true' ACD (i.e. CSF-TP value greater than the age-adjusted upper reference limit), 'traditional' ACD (i.e. CSF-TP value greater than 0.45 g/L), and 'pseudo' ACD (i.e. CSF-TP value greater than 0.45 g/L but less than the age-adjusted upper reference limit). Diagnostic categories (reason for LP) not expected to cause ACD are represented in shades of red and organized left to right by descending magnitude of absolute percentage change from all ACD to true ACD. Pathologic categories with a potential expectation for ACD are represented in shades of blue and organized left to right by ascending magnitude of absolute percentage change between all ACD and true ACD. The *other* categories (i.e. "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: albuminocytologic dissociation; CSF-TP: Cerebrospinal fluid total protein; IWMD: Inflammatory white matter disease; T. Encephalopathy: Transient Encephalopathy

**Figure 3. CSF-TP reference interval.**

Points represent post exclusion CSF-TP concentrations with the removal of all but the original point where patients had multiple CSF samples drawn (n = 8,175). Patients were grouped into 5-year bins by their age at the time of lumbar puncture. The resultant 97.5$^{th}$ 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; CSF-TP: Cerebrospinal fluid total protein

# Tables

| Clinical Categories | References |
|---|---|
| Following intrathecal chemotherapy[a] | (14) |
| Following subarachnoid hemorrhage | (15) |
| Infectious / non-infectious encephalitis | (16, 17) |
| Infectious / non-infectious meningitis | (18-20) |
| Intra-axial / extra-axial tumors | (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) |
| Cerebrovenous sinus occlusion | (32) |
| Optic nerve disease | (33) |
| Optic neuritis | (34) |
| Posterior Reversible Encephalopathy Syndrome (PRES) | (35) |
| Structural spinal disorders | (36, 37) |
| Nervous system toxin exposure | (38) |
| Dementia | (39) |
| Seizure | (40) |
| Stroke (Hemorrhagic / Ischemic) | (41) |

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

[a] The underlying condition for which intrathecal chemotherapy was provided in the cited report was related to the Central Nervous System involvement of Systemic Lupus Erythematosus as opposed to predominantly the treatment of a hematologic malignancy in the context of our report.

| Clinical Category | ACD Expected | Traditional ACD - Proportion with ACD (0.45g/L upper limit) | True ACD - Proportion with ACD (age-adjusted upper limit) | Change | P-value[a] |
|---|---|---|---|---|---|
| | Y/N | n (%) | n (%) | Δ% | |
| **polyneuropathy** | **Y** | **204 (7.8%)** | **127 (21.3%)** | **13.50%** | **< 0.0001** |
| tumor | 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 (e.g. post neurosurgery, diffuse axonal injury, etc.) | Y | 8 (0.3%) | 4 (0.7%) | 0.40% | 0.25 |
| diffuse anoxic-ischemic injury | Y | 17 (0.6%) | 6 (1%) | 0.40% | 0.41 |
| infection (no CNS involvement e.g. 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 (e.g. carcinomatosis, IgG4 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 |
| Hemorrhage within 3 months (e.g. 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 e.g. 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 PRES | 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 |
| 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 ischemic 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 | | 2,627 (100%) | 597 (100%) | | |

Table 2. Expectation of protein elevation, Number and proportion of patients with a specific clinical category compared to all reported cases with associated percentage change from using an invariant 0.45 g/L CST-TP threshold (all comers with ACD) versus a threshold varying with age ('true' ACD). 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. ACD: Albuminocytologic dissociation; All Cause Major Stroke: includes thromboembolic disease, vasculitis of the CNS causing stroke and reversible cerebrovascular constriction syndrome; CNS: central nervous system; CSF: cerebrospinal fluid. Inflammatory white matter disease: includes multiple sclerosis, Neuromyelitis Optica, Acute Demyelinating Encephalomyelitis.

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

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

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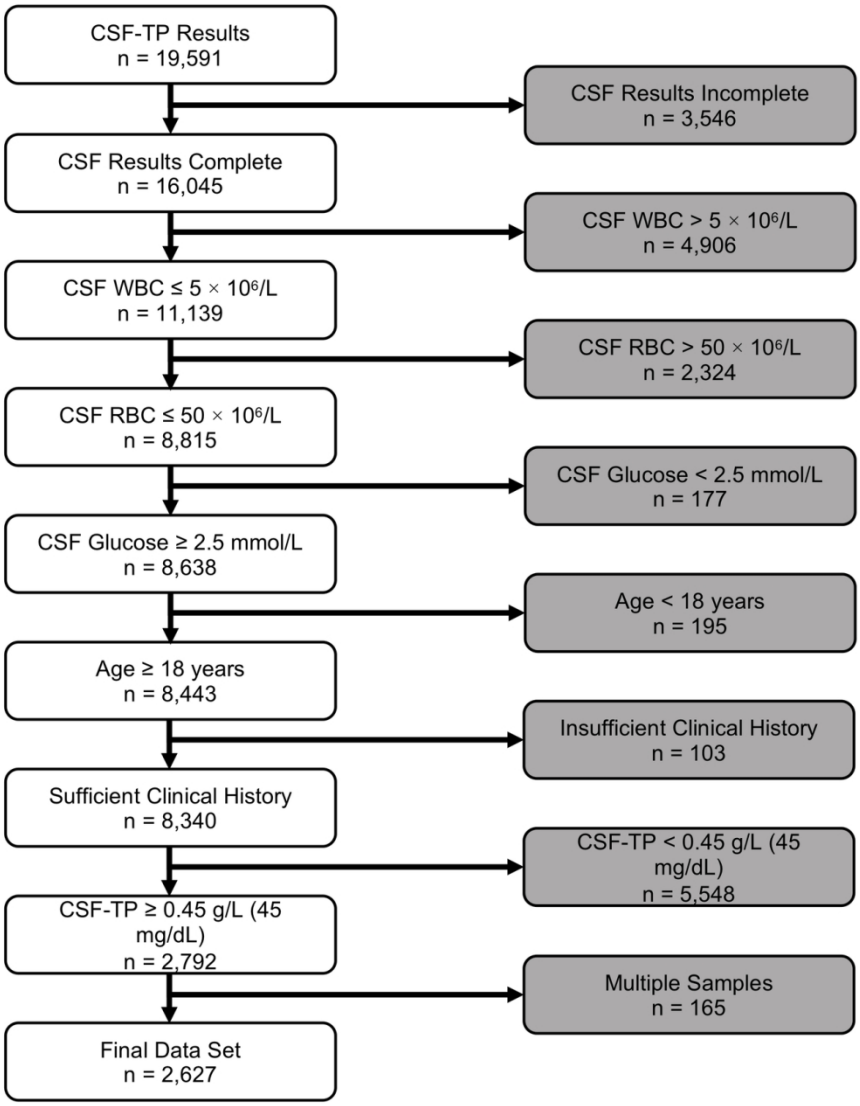
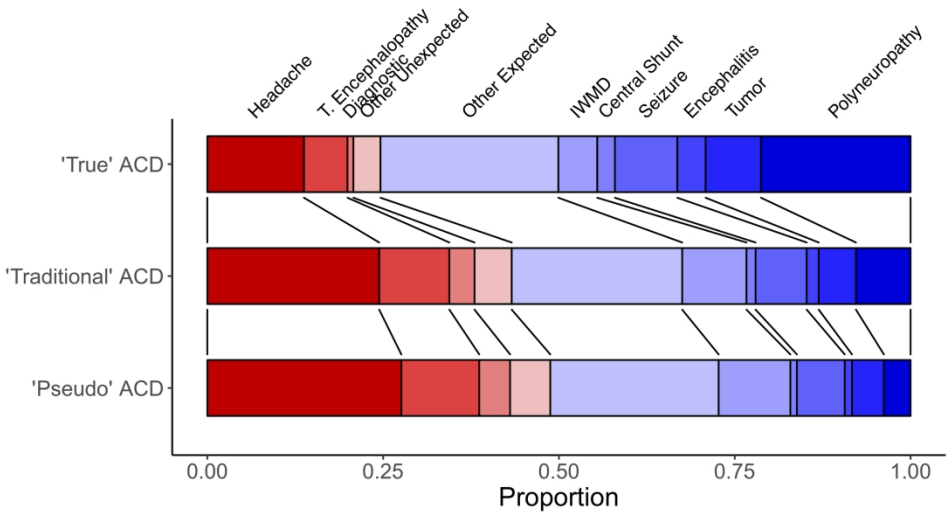



Flow chart illustrating the exclusion process used to identify patients with ACD.

CSF: Cerebrospinal fluid; WBC: White blood cell count; RBC: Reb blood cell count; TP: Total Protein.

120x153mm (300 x 300 DPI)

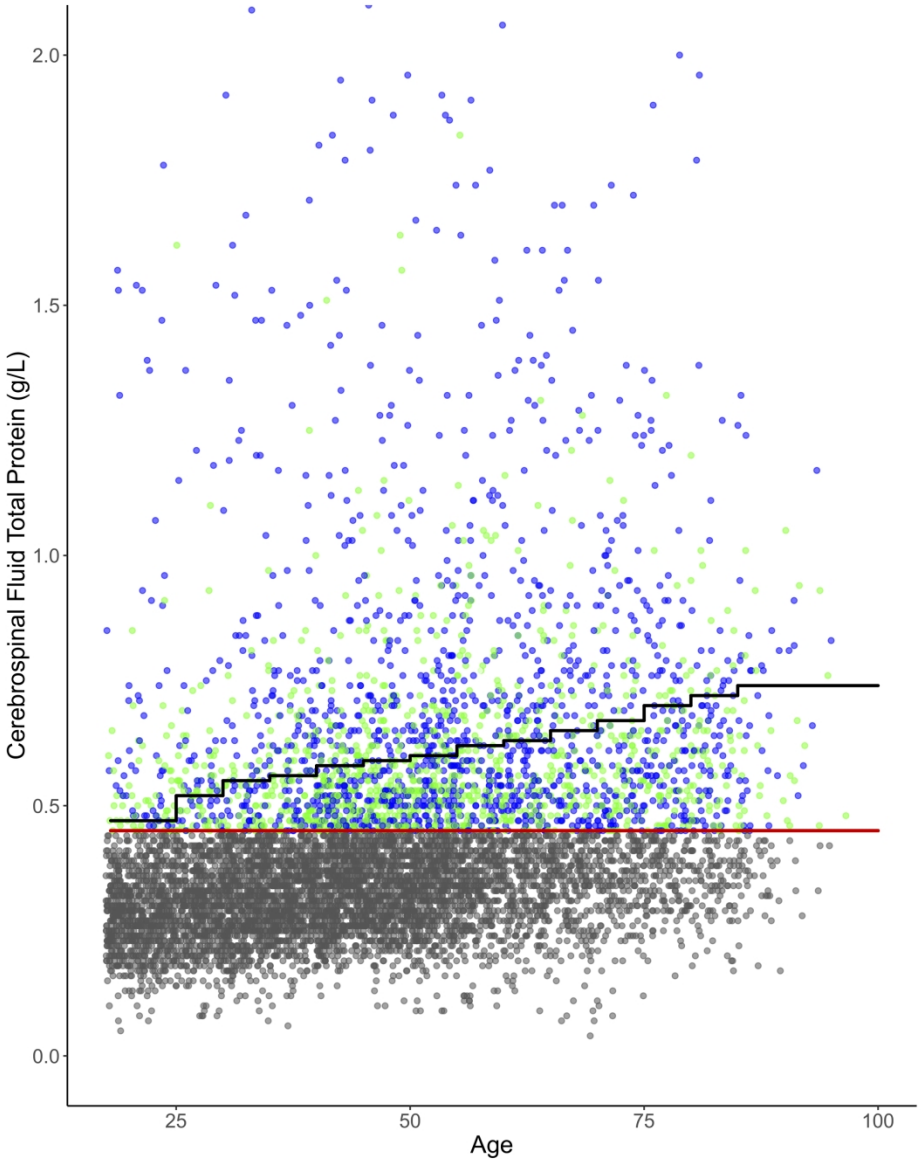



Proportions of patients with albuminocytologic dissociation.

Proportionate breakdowns are shown for 'true' ACD (i.e. CSF-TP value greater than the age-adjusted upper reference limit), 'traditional' ACD (i.e. CSF-TP value greater than 0.45 g/L), and 'pseudo' ACD (i.e. CSF-TP value greater than 0.45 g/L but less than the age-adjusted upper reference limit). Diagnostic categories (reason for LP) not expected to cause ACD are represented in shades of red and organized left to right by descending magnitude of absolute percentage change from all ACD to true ACD. Pathologic categories with a potential expectation for ACD are represented in shades of blue and organized left to right by ascending magnitude of absolute percentage change between all ACD and true ACD. The other categories (i.e. "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: albuminocytologic dissociation; CSF-TP: Cerebrospinal fluid total protein; IWMD: Inflammatory white matter disease; T. Encephalopathy: Transient Encephalopathy

215x177mm (300 x 300 DPI)




CSF-TP reference interval.

Points represent post exclusion CSF-TP concentrations with the removal of all but the original point where patients had multiple CSF samples drawn (n = 8,175). 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; CSF-TP: Cerebrospinal fluid total protein

215x279mm (300 x 300 DPI)

STROBE Checklist

| | |
|---|---|
| 1 a | Pg 1 |
| 1 b | Pg 3 |
| 2 | Pg 4 |
| 3 | Pg 4 |
| 4 | Pg 4 |
| 5 | Pg 4 |
| 6 | Pg 4 |
| 7 | Pg 4 – 6 |
| 8 | Pg 4 – 6 |
| 9 | Pg 4 – 6 |
| 10 | Pg 4 – 6 |
| 11 | Pg 4 – 6 |
| 12 a | Pg 5 – 6 |
| 12 b | Pg 5 – 6 |
| 12 c | Pg 4 |
| 12 d | NA |
| 12 e | Pg 5 – 6 |
| 13 a | Pg 6 |
| 13 b | Pg 6 |
| 13 c | Pg 6 |
| 14 a | Pg 6 |
| 14 b | Pg 6 |
| 14 c | Pg 6 |
| 15 | Pg 6 |
| 16 a | Pg 6 |
| 16 b | Pg 6 |
| 16 c | NA |
| 17 | Pg 6 |
| 18 | Pg 9 |
| 19 | Pg 9 – 10 |
| 20 | Pg 9 |
| 21 | Pg 10 |
| 22 | Included separately |