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Adult CSF total protein: Higher upper reference limits should be considered worldwide. *A web-based survey*

Pierre R. Bourque[a,b,*], Ari Breiner[a,b], David Moher[b,c], John Brooks[a], Harald Hegen[d], Florian Deisenhammer[d], Christopher R. McCudden[e]

[a] Division of Neurology, Department of Medicine, The Ottawa Hospital and University of Ottawa, Ottawa, Ontario, Canada
[b] Ottawa Hospital Research Institute, Ottawa, Ontario, Canada
[c] Ottawa Methods Centre, Ottawa Hospital Research Institute, Ottawa, Canada
[d] Department of Neurology, Neuroimmunology Laboratory, Medical University of Innsbruck, Innsbruck, Austria
[e] Department of Pathology & Laboratory Medicine, The Ottawa Hospital and University of Ottawa, Ontario, Canada

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ABSTRACT

Background: The cerebrospinal fluid total protein level (CSF-TP) is commonly used as a potential marker of infectious or immune disease of the CNS and PNS. Recent laboratory reference studies indicate that the antiquated single upper reference limit of 0.45 g/L commonly used by hospital laboratories and widely quoted in medical literature is a significant underestimation.
Methods: We distributed worldwide a web-based survey comprised of three questions: 1. What is the CSF-TP upper limit used at your institution? 2. What is the source of this upper limit? 3. Do you adjust your upper limit according to age?
Results: A total of 473 unique responses were obtained from North America (37.5%), South America (5.5%), Europe (29.4%), Africa (4%), Asia (21.6%) and Oceania (1.7%). A strong preponderance (86.8%) of institutions reported an upper limit of 0.45 g/L or less. Only 4% reported making age-partitioned adjustments.
Conclusions: Worldwide, a strong majority of hospital laboratories presently use an underestimation of CSF-TP upper reference value, particularly for older adults. Recent well powered laboratory reference studies support higher values with age adjustment.

1. Introduction

An increase in cerebrospinal total protein level (CSF-TP) without an associated pleocytosis is commonly attributed to a suspected disruption of the blood-brain or blood-nerve barriers by infectious or immune disorders of the central or peripheral nervous systems [1,2]. Other potential mechanisms are intrathecal antibody production [3], impaired reabsorption and spinal sequestration. In H. Merritt's 1938 seminal "The Cerebrospinal Fluid" [4], a reference range of 15–45 mg/dL (0.15–0.45 g/L) was reported for lumbar cerebrospinal fluid total protein (CSF-TP). This antiquated range has been widely adopted by clinical laboratories and commonly cited in practice guidelines [5] and medical literature [6,7]. Admittedly, though CSF-TP is the most widely available CSF biochemistry data, it may be much less sensitive and specific than other measures of blood brain barrier permeability such as the albumin quotient or direct measures of intrathecal antibody synthesis.

We recently published rigorously defined CSF-TP reference results from academic institutions in Canada and Austria [8,9]. Both studies found quite similar CSF-TP upper reference limits that steadily increase through adult years, with values over 0.6 g/L by age 50 years. The discrepancy between this recent data and the normative values widely quoted in clinical and laboratory textbooks lead us to investigate the range of reference values in current clinical practice across the globe. We report here results of a web-based survey of CSF-TP upper reference limits from 69 countries. We offer the opinion that a data-driven reappraisal of CSF total protein reference intervals should be considered in hospital clinical laboratories worldwide.

2. Survey methods

The survey methodology was approved by the Research Ethics Board of the Ottawa Hospital Research Institute (protocol #20170816-01H). A web-based survey was distributed by email and consisted of the

* Corresponding author at: The Ottawa Hospital, 1053 Carling Ave, Ottawa K1Y 4E9, Ontario, Canada.
E-mail address: pbourque@toh.ca (P.R. Bourque).

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following three questions (with corresponding answer options): 1) What is the CSF TP upper reference value at your institution for adult patients? (e.g. < 0.45 g/L, < 0.60 g/L, or specify any different value); 2) Do you use age-adjusted reference limits for adults? (yes/no); 3) What is the source of your CSF TP reference intervals? (Published laboratory references, Local/Institutional study data, Unknown). The survey distribution list was targeted at clinical neuroscientists and laboratory medicine specialists.

A distribution list with a quantifiable response rate was derived from email addresses provided by corresponding authors for the period of Jan-Dec 2017 in the scientific journals Neurology (Journal of the American Academy of Neurology, $N = 420$) and the Journal of Neurological Sciences (Publication of the World Federation of Neurology, $N = 339$). After the initial recruitment email, two reminder emails were sent. Responses without a reliably quantifiable reply rate were obtained by posting the survey with the consent of a number of professional associations (Canadian Neurological Sciences Federation, Russian Federation of Laboratory Medicine) as well as online neurology list serves (AAN Consortium of Residents and Fellows, Rick's Real Neuromuscular Friends). Additional individual survey responses were obtained by directly contacting members of the American Association of Clinical Chemistry and through networking with international neurology colleagues. In the event of duplicate laboratory site responses with differing results, we entered the highest value. Similarly, if an age-dependent range of values was reported, we entered the upper limit. Mean CSF-TP upper limit values were compared between tertiary academic centers and community hospitals using an independent samples *t*-test.

3. Survey results

For the main survey, distributed through emails of corresponding authors in Neurology and the Journal of Neurological Sciences, the response rate was 36.9% (280/759) and the completion rate was 98%. The full survey result dataset, including additional results from other sources and after the exclusion of 124 duplicate survey entries, was comprised of 473 unique hospital laboratory responses from 69 countries. The geographical distribution was as follows: North America 177 (37.5%), South America 26 (5.5%), Europe 139 (29.4%), Africa 20 (4%), Asia 102 (21.6%) and Oceania 8 (1.7%) (Fig. 1). The mean CSF-TP upper limit was 0.46 g/L, with a range of 0.20–0.79 g/L. The single value of 0.45 g/L was the most commonly stated upper reference limit (74%), while 86.8% of responders reported a value of 0.45 g/L or less.

The stated source of the CSF TP upper reference limit were: published reference values in 233 (49.4%), unknown source in 215 (45.6%) and local/ institutional data in 24 (5%). Age-adjusted reference limits were reported for adults in only 19/473 (4%) of hospital centers. Although not requested in the survey, 17 (3.6%) responders specified that they used a different reference value for newborns.

The independent samples *t*-test did not show a significant difference between mean CSF-TP upper limits reported from tertiary and community hospitals.

4. Discussion

Cerebrospinal fluid analysis remains a key investigation in the assessment of infectious, neoplastic, paraneoplastic, structural and inflammatory disorders of the CNS and PNS. Even though several tertiary centers offer more sophisticated and accurate measures of blood-brain or blood-nerve barrier permeability (such as the serum/CSF albumin quotient) [1], the simple measurement of CSF-TP is still widely used in clinical practice and most commonly cited in medical publications. With little variation across continents, 86.8% of clinicians in our survey report using a limit at or below 0.45 g/L. Most clinicians (49.4%) correctly assumed that their institutional reference range was taken from published reference data, or stated they did not know how this value was obtained (45.6%). The 5% response rate for “local institutional reference study” is possibly an overestimate, judging from the scarcity of publications on this topic. Few institutions are likely to have established normative limits based on a systematic laboratory and chart review of their patient data. It is remarkable that only 4% of survey responders reported using age- adjusted reference limits in adults.

Our results are consistent with a national survey of hospital laboratories from the United Kingdom, with a 42% response rate (84/200), where only 7% of laboratories used a CSF TP reference value > 0.45 g/L and none reported age adjustment in adults [10]. Our survey population was limited to neurologists and laboratory medicine specialists, who were felt to be most likely to frequently interpret CSF total protein measurements. The response rate of 36.9% for journal corresponding authors is in line with what can be expected for web-based surveys [11]. A limitation of our survey is the over-representation of North-America (37.5%) and Europe (29.4%) relative to their respective demographic weights. This is likely a function of the geographic density of university hospitals, as physicians were contacted mostly through an academic affiliation (medical journal authorship, or academic websites.

Defining laboratory reference ranges for CSF analysis poses a unique challenge, because of the discomfort, logistics and risks associated with lumbar puncture. We found only four published reports studying normal volunteers [12–15]. All four studies enrolled < 50 subjects and were strongly skewed towards young adults (mean age of < 30 years). The more feasible strategy to define a population for CSF reference protein limits is a retrospective analysis of laboratory results, with strict clinical and laboratory criteria designed to exclude specimens from patients when there can be an expectation of relevant neurological disease. The most important laboratory exclusion criteria is the presence of any concurrent abnormality of CSF nucleated cells, red blood cells or glucose. A chart review must also be performed to exclude a long list of disorders where abnormal CSF protein can be expected, such as the wide range of inflammatory, infectious, neoplastic or structural diseases of the PNS and CNS. Over the past 50 years, most published studies in this category had relied on small data sets: 11 studies with 10–99 subjects [16–26] and 4 studies with 100–200 subjects [27–30]. Only three had > 200 samples, including the recent Austrian and Canadian data of the authors [8,9,31]. Our Table 1 shows how the CSF-TP upper limit is likely above 0.6 g/L after age 50 years. It is important to partition reference values by advancing decades of life. The study of McCudden analyzed 6524 CSF results over a period of 20 years, with three sequential laboratory methods [8]. The impact of laboratory method was statistically significant but of low magnitude, in the range of 0.04 g/L. The effect of gender was also statistically significant but similarly small (0.06 g/L higher in males).

An incorrect conclusion of albuminocytologic dissociation could trigger unwarranted repeat lumbar punctures, electrophysiologic studies, neuroimaging and serum/CSF auto-antibody assays for a wide range of infectious, neoplastic, paraneoplastic or autoimmune disorders of the PNS and CNS. Using data from the largest published reference study of CSF-TP an arbitrary cut-off of 0.45 g/L could lead to a false positive determination in roughly 25% of individuals without relevant neurological disease over the age of 65 [8]. Updating reference CSF-TP limits will impact its diagnostic sensitivity and specificity. For example, an elevated CSF-TP above 0.45 g/L has been listed as a supportive criterion (level A recommendation) in the European Federation of Neurological Societies/Peripheral Nerve Society guidelines on the diagnosis and management of CIDP [32]. However, over-reliance on modest CSF-TP elevation was subsequently reported as an important pitfall in the diagnosis of CIDP when critically reviewed in a tertiary referral clinic [33]. A similar risk of overdiagnosis from incorrect CSF-TP interpretation can be expected in the workup of autoimmune encephalitis, particularly when MRI or specific auto-antibody tests are equivocal or negative.

Worldwide, a strong majority of hospital laboratories presently use an underestimation of CSF-TP upper reference value, particularly for

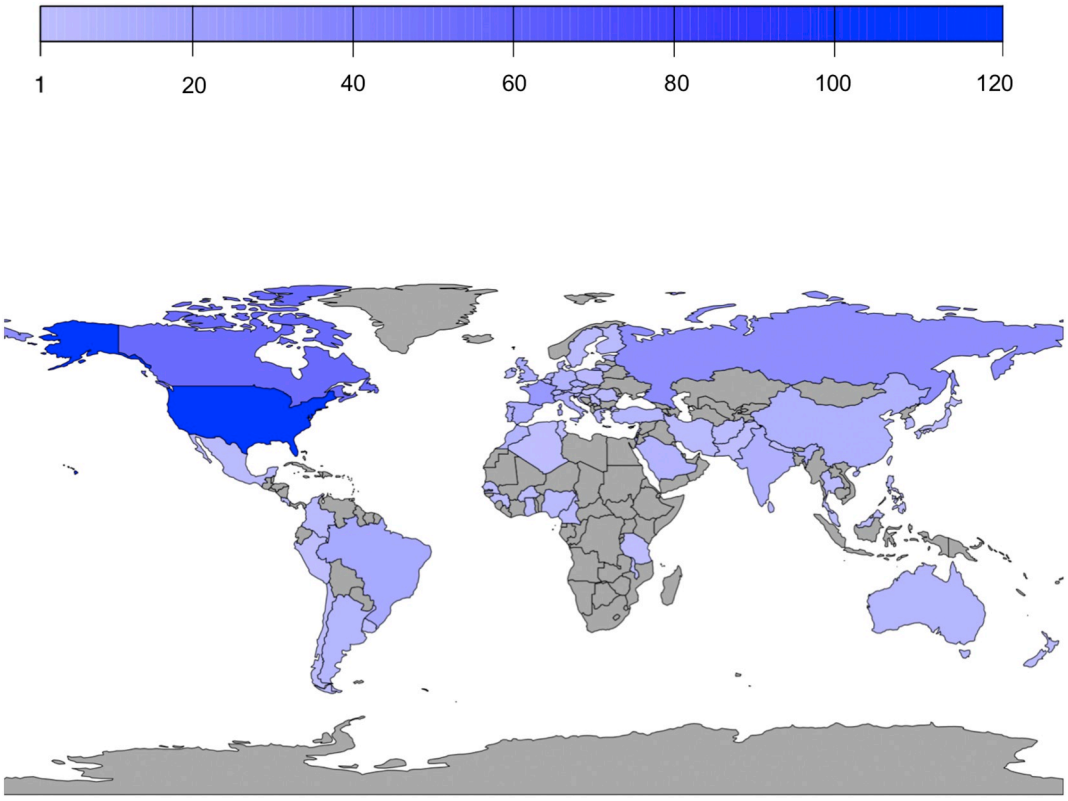

Fig. 1. Geolocation map of CSF Total Protein upper reference limit survey responses.
The frequency distribution of the number of survey responses is represented by country with a blue color density scale ranging from 1 to 120. There were no survey results from countries in grey.

Table 1
Summary of recent published upper reference limit values for CSF Total Protein, by decade of age.

	Bernhardt 1978[28]		Hegen 2016[9]		McCudden 2017[8]	
Age group	**# Subj.**	**URL (g/L)**	**# Subj.**	**URL (g/L)**	**# Subj.**	**URL (g/L)**
18–29 y	49	0.52	69	0.53	1158	0.49
30–39 y	92	0.55	81	0.62	1204	0.55
40–49 y	68	0.53	89	0.60	1355	0.58
50–59 y	47	0.58	58	0.69	1040	0.60
60–69 y	24	0.55	27	0.62	598	0.64
70–79 y	10	0.55	ND	ND	446	0.68

Data was extracted from the last three published reports that included at least 200 subjects, and provided sufficient data for age stratification.
Abbreviations: # Subj: Number of study subjects; URL: Upper reference limit; ND: No data.

older adults. Recent well powered laboratory reference studies support higher values with age adjustment.

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