Journal of Neurology (2019) 266:616–624
https://doi.org/10.1007/s00415-018-09174-z

ORIGINAL COMMUNICATION

Adult CSF total protein upper reference limits should be age-partitioned and significantly higher than 0.45 g/L: a systematic review

Ari Breiner[1,2] · David Moher[2,3] · John Brooks[1] · Wei Cheng[2,3] · Harald Hegen[4] · Florian Deisenhammer[4] · Christopher R. McCudden[5] · Pierre R. Bourque[1,2]

Received: 27 November 2018 / Revised: 24 December 2018 / Accepted: 26 December 2018 / Published online: 8 January 2019

Abstract

Background Cerebrospinal fluid total protein (CSF-TP) is measured in the diagnosis of a range of immune or infectious disorders of the nervous system. Most laboratories and the medical literature use an antiquated, age-independent upper limit of 0.45 g/L. Therefore, we performed a systematic review of reference studies in the medical literature, with the primary objective of determining the CSF total protein upper reference limit (URL). Secondary objectives were to assess the effects of age, gender, laboratory methods, and methodological quality.

Methods A pre-planned and peer-reviewed electronic search strategy was used to search Ovid Medline and EMBASE for 1960–2017. All records underwent title/abstract review, and potentially relevant records underwent independent full-text review by two researchers. The remaining studies underwent quality assessment using a modification of the QUADAS2 revised tool. CSF-TP upper reference limits extracted from these studies were used to compute weighted means.

Results Twenty-two articles were retained for qualitative analysis and 20 for quantitative analysis. The weighted average of CSF-TP URL was 0.55 g/L, in studies with high methodological quality. Studies that examined the effect of age reported consistent correlations with advancing age, and CSF-TP URL values incrementally exceeded 0.60 g/L after age 50. There were no meaningful differences according to gender, laboratory method, or quality assessment score.

Conclusions There is concordance in available literature to recommend increasing CSF total protein upper reference limits, and to consider implementing age-adjusted values above 0.60 g/L starting at age 50. This information merits worldwide dissemination, to reduce the risk of over-diagnosis.

Keywords Cerebrospinal fluid · CSF · CSF total protein · Upper reference limits · Systematic review

Introduction

Raised CSF-TP is most commonly caused by disruption of blood–brain and blood-nerve barriers by immune or infectious disorders, but it may also be due to intrathecal immunoglobulin synthesis, impaired reabsorption at the level of arachnoid villi, or spinal sequestration. The quantitative definition of CSF-TP elevation has mostly remained unchallenged in the past 80 years. In H. Merritt's seminal 1938 book, The Cerebrospinal Fluid [1], a reference range of 15–45 mg/dL (0.15–0.45 g/L) was proposed for lumbar CSF-TP. This range has been frequently cited in internal medicine and neurology textbooks [2, 3], widely adopted by hospital laboratories across the world, and quoted in clinical practice guidelines.

We recently published institutional CSF-TP reference results from academic institutions in Canada and Austria

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00415-018-09174-z) contains supplementary material, which is available to authorized users.

✉ Ari Breiner
abreiner@toh.ca

1 Division of Neurology, Department of Medicine, The Ottawa Hospital and University of Ottawa, 1053 Carling Ave., Ottawa, ON K1Y 4E9, Canada

2 Ottawa Hospital Research Institute, Ottawa, ON, Canada

3 Ottawa Methods Centre, Ottawa Hospital Research Institute, Ottawa, Canada

4 Neuroimmunology Laboratory, Department of Neurology, Medical University of Innsbruck, Innsbruck, Austria

5 Department of Pathology and Laboratory Medicine, The Ottawa Hospital and University of Ottawa, Ottawa, ON, Canada

[4, 5]. Both studies found quite similar CSF-TP upper reference limit exceeding 0.6 g/L starting at age 50.

The present study aimed to provide a systematic review of CSF-TP upper reference limit literature, and to compute a weighted mean value for the upper reference limit. We performed this study with the hypothesis that current evidence supports the adoption and dissemination of age-dependent upper limit values significantly higher than the traditional 0.45 g/L value.

Methods

Systematic review

We performed this systematic review according to preferred reporting items for systemic reviews and meta-analyses (PRISMA) guidelines [6]. Our primary objective was to review and synthesize reference data for the CSF-TP upper reference limit. Our secondary objective was to assess the effect of factors, such as age, laboratory method, methodological quality, sample size and gender.

Search strategy

Our search strategy was developed in conjunction with a health informatics specialist. It was subsequently peer reviewed according to peer review of electronic search strategies (PRESS) guidelines [7]. The strategy was used to search Ovid Medline and EMBASE from 1947 until Oct. 18th, 2017 (appendix 1). We also hand-searched the reference lists of included articles to identify additional studies. There were 818 Medline references and 895 Embase references. After the removal of duplicates and addition of individual references, 1148 records were selected for screening (Fig. 1).

Study selection, inclusion and exclusion criteria

Title and abstract screening and full-text review were conducted by two researchers (AB, PRB). We included research articles written in English, French, German, Spanish and Portuguese. We excluded papers published prior to 1960. Articles that did not meet all the following criteria were excluded: individual measurement of CSF-TP, specified

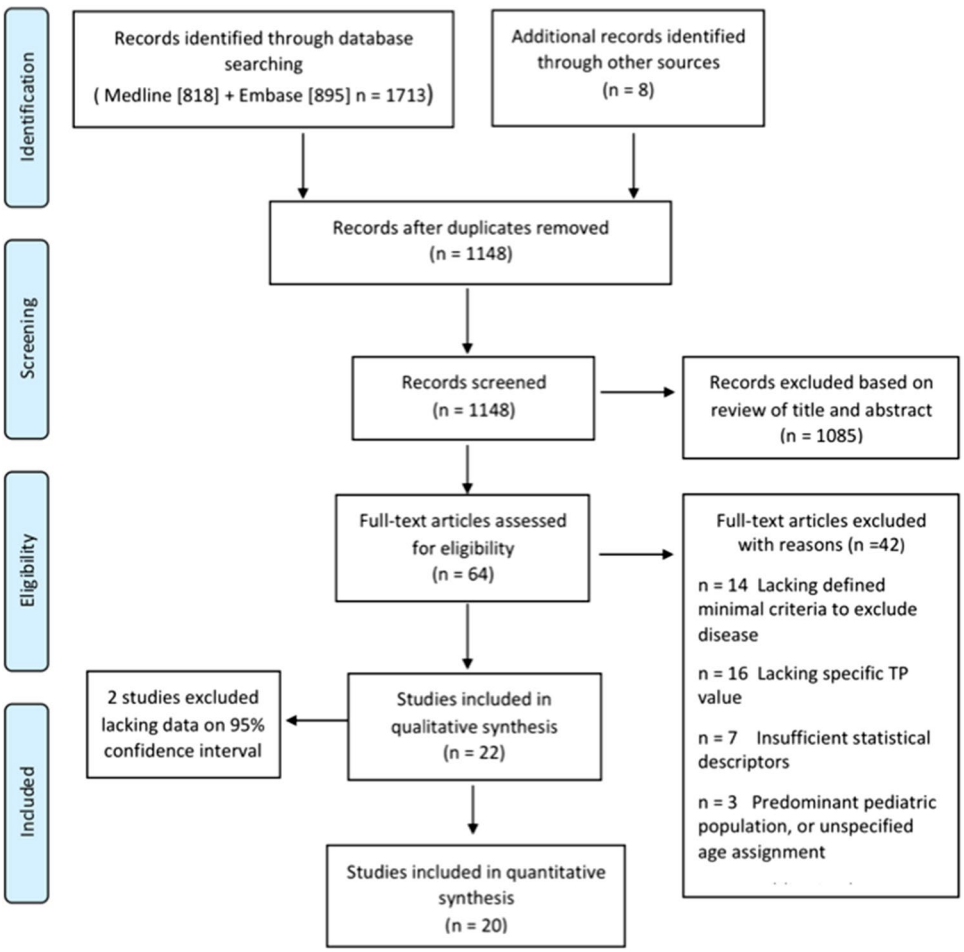

Fig. 1 PRISMA flow diagram of systematic literature review

laboratory technique, inclusion of adult subjects, defined criteria for normal/reference population. These criteria for manuscript evaluation were set out in a protocol that was finalized prior starting the systematic review.

Outcome measure

The primary outcome measure was the CSF-TP upper reference limit (URL). This was either described in the article as the 97.5th percentile of the reference population; or calculated using the formula: URL = mean + 2 × standard deviation (SD).

Quality assessment

We developed a four-item questionnaire based on the QUADAS2 revised tool [8] for the quality assessment of diagnostic accuracy studies. The assessment of quality was based upon the following four criteria: (1) Was the reference cohort representative of the general adult population (e.g. mean age above 30 years, representative sex distribution)? (2) Were there minimal criteria specified to exclude conditions known to elevate CSF-TP (e.g. normal CSF WBC, clinical assessment)? (3) Was the laboratory technique consistent with applicable modern standards? (4) Were there minimal statistical data to calculate a 95% confidence interval? Each question was rated as "satisfactory, unsatisfactory, or inconclusive". The final quality assessment was summarized as S1 (all satisfactory), S2 (one category rated unsatisfactory or inconclusive) or S3 (two or more categories rated unsatisfactory or inconclusive).

Data extraction, analysis and synthesis

Two reviewers extracted data directly to a pre-designed Excel spreadsheet for quantitative analysis. The data collected were: number of reference subjects, mean age, age range, mean CSF-TP (global or by age category), standard deviation, 97.5th or 95th percentile of CSF-TP (if specified by author).

Statistical analysis

For each study which reported results in subgroups, we used the average weighted by sample size and the pooled standard deviation $\sum_{i=1}^{K}\left(n_i-1\right)\times \mathrm{SD}_i^2/\sum_{i=1}^{K}\left(n_i-1\right)$, where K is the number of subgroups. Then we calculated the mean and SD weighted by sample size for the CSF-TP upper limit for the studies of highest methodological quality grade (S1) and for those studies of lesser grade (S2-3). To calculate group averages and generate representative figures we used R version 3.4.3 [9]. Simple weighted average was applied to the upper limit. To test the effect of three factors (year of publication, sample size and methodological quality grade) we applied the covariate-adjusted random effects meta-analysis (Dersimonian-Laird), using the R package "metaphor" version 2.0-0 .

Results

Results: systematic review

The search strategy yielded 1721 records. After the exclusion of duplicate records, there were 1148 articles for review. During the title and abstract search, 1085 records were excluded, based on the language of publication, non-human studies, and the type of publication (case report, editorial, letter, abstract/conference proceeding with insufficient information, or laboratory calibration technique). There were 64 articles selected for full-text review. We subsequently excluded 42 articles, because they lacked a report of the CSF total protein value ($n = 16$), lacked proper inclusion/exclusion criteria ($n = 14$), lacked sufficient statistical detail ($n = 7$), consisted of a pediatric population or unknown age distribution ($n = 3$), or due to the language of publication ($n = 2$). The PRISMA* flowchart diagram is displayed in Fig. 1.

Study characteristics—included studies

The 22 included studies are listed in Table 1. There was a wide range in the number of reference subjects ($n = 10$–6824), and the mean age varied from 24.5 to 49 years. Variations of the Biuret method were the most commonly used laboratory method ($n = 15$), followed by protein precipitation ($n = 5$) and spectrophotometry ($n = 2$).

Of these 22 studies, ten comprised less than 50 participants [11, 12, 14, 18, 21, 24–27, 29], five included 50–100 participants [13, 15, 19, 20, 28], four included 100–200 subjects [10, 22, 23, 30] and only three included more than 200 participants [4, 5, 17]. Four studies prospectively enrolled healthy volunteers [11, 13, 18, 25]. All four had small sample sizes (10–51 subjects) and two sought to limit their recruitment to younger participants (mean age in the mid-twenties) [11, 25]. As required in our systematic review protocol, all 22 studies selected for qualitative analysis had to list inclusion/exclusion criteria. Only half [4, 5, 15, 17, 20, 22–24, 26, 28, 30] of the studies specifically stated the exclusion of abnormally elevated CSF white blood cell count. There was much variability in the documentation of the clinical selection criteria. Some authors simply mentioned that "neurological or CNS disease was excluded". Other publications documented specific exclusion diagnoses (e.g., abscess, ALS, benign tumors, Guillain–Barré syndrome, etc)

Table 1 Summary of 22 studies selected for qualitative analysis

Author, year	# Of subjects	Age in years Mean (range)	Clinical inclusion/exclusion criteria	CSF WBC exclusion criterion	Lab method	Quality tool score[g]
McCudden, 2017 [5]	6524	44.9 (18–94)	Laboratory and clinical exclusion of relevant neurological disease	Yes	Benzethonimum-chlor pyrogallol red	S1
Hegen et al., 2016 [4]	332	42.1 (18–80)	Exhaustive exclusion of relevant neurological disorders	Yes	Protein precip	S1
Lott and Warren, 1989 [10]	46	27–90[a]	Exclusion neurological disease, lab exclusions	NS	Biuret	S2
	75	NS	Exclusion neurological disease, lab exclusions		Protein precip	
	68	NS	Exclusion neurological disease, lab exclusions		Modif. reverse Biuret	
Ben Menachem et al., 1989 [11]	10	28 (23–42)	Normal volunteers	NS	Biuret	S3
Hirohata et al., 1984 [12]	30	(33–35)	Spinal anesthesia, no neurological disease	NS	Bradford	S2
Merril and Harrington, 1984[b] [13]	51	41	Normal volunteers	NS	Biuret	S3
Wikkelso et al., 1981 [14]	13	49[c]	No relevant neurological disease or transient ischemic attack	NS	Biuret	S2
Eeg-Olofsson et al., 1981 [15]	62[d]	16–30	Tension headache ,dizziness, normal clinical examination	Yes	Biuret	S2
Ahonen et al., 1978 [16]	46	39 (19–55)	Back pain with negative myelography	NS	Biuret	S2
Bernhardt et al., 1978 [17]	290	43.8 (21–80)	No neurological disease	Yes	Biuret	S1
	139	38	No neurological disease, normal laboratory assessments	Yes	Gel filtration protein precip	S1
Stibler, 1978[b] [18]	32	34	Normal volunteers	NS	Biuret	S2
Thomsen et al., 1978 [19]	98	18–72	No evidence of relevant neurological disease	NS	Biuret	S2
Tibbling et al., 1977 [20]	93	17–77	Dizziness, benign headache mild psychoneurotic disorders	Yes	Biuret	S1
Takeoka et al., 1976 [21]	27	(41)	LP for spinal analgesia, no neurological disease	NS	Protein precip	S2
Jung et al., 1973 [22]	119	34.2	No relevant neurological disease	Yes	Biuret	S1
Mertin et al., 1971 [23]	140	16–74	No neurological illness other than benign headache, vertigo, tremor, or depression	Yes	Biuret	S1
Igou, 1967 [24]	23	(17–72)	LP for spinal analgesia, no neurological disease	NS	Gel filtration-spectrophotometry	S2
Gilland, 1967 [25]	15	24.5 (21–32)	Normal volunteers	Yes	Biuret	S2
Arnold, 1966 [26]	35	~(21–72)[e]	"Certainly normal" based on clinical assessment	Yes	Biuret	S1
Brackenridge, 1962 [27]	20	33 (13–66)	No relevant neurological disease	NS	Protein Precip	S2
Rieder and Wuthrich, 1962 [28]	63	12–62[f]	CNS disease excluded	Yes	Biuret	S1

Table 1 (continued)

NS not specified, *LP* lumbar puncture, *WBC* white blood cells, *CNS* central nervous system

[a]Age distribution obtained from Fig. 3 of publication

[b]Excluded from quantitative analysis: upper limit of CSF total protein was stated only as highest value in sample

[c]Pooled average calculated from mean and SD of 2 subgroups

[d]Combining 20 subjects aged 16–20 years and 42 subjects aged 21–30 years

[e]Age distribution obtained from Table 2 of publication

[f]Only 6 subjects age 12–16 years in Fig. 3 of publication

[g]See methods for description of quality tool scores S1-3

[10] or referred to published consensus guidelines for CSF biomarker [31] or other laboratory reference guidelines. There was also a wide range of clinical indication for lumbar puncture. The most common inclusion categories were: benign headache, vertigo, tremor, depression, psychoneurotic disease, and back pain with negative neuroimaging. Only a few reports [4, 5, 15, 17, 28] stated that there was longitudinal follow-up data to document the lack of clinically relevant neurological disease.

For the quality assessment criteria, studies were predominantly downgraded to category S2 (one deficiency) or S3 (two deficiencies) based on the following two methodological criteria: lack of CSF WBC exclusion criteria [10–14, 16, 18, 19, 21, 24, 27] and over-representation of young patients [11, 13, 15, 25].

Study characteristics—excluded studies

The reasons for exclusion of studies upon full-text review are listed in Fig. 1. Despite its large sample size ($n = 1192$) we excluded the study of Dufour-Rainfray [32], because it only used CSF laboratory exclusion criteria (white blood cell count, glucose) without any exclusion based on a review of clinical diagnosis, and without providing data on standard distribution. We also excluded two studies that listed predetermined CSF TP limits in their selection criteria [33, 34], thereby artificially narrowing the reference range. One study was excluded because the authors did not specifically state the CSF-TP value, although it provided separate measurements of albumin and Gamma globulin [35]. We excluded from quantitative analysis two studies that only stated the highest CSF TP value in their cohort [13, 18] without providing either a 97.5th percentile, or the mean and standard deviation. Thus there were 20 studies remaining for the primary outcome and subgroup analyses.

Primary outcome—CSF-TP upper reference limit

The upper reference limits for CSF-TP are displayed in Fig. 2, and ranged from 0.336 to 0.829 g/L. The weighted average for the CSF-TP upper limit, for the entire adult age span, was calculated at 0.550 g/L for studies with the highest methodological quality grade (S1) and 0.613 g/L for studies with a lower quality grade (S2 and S3). Fifteen of twenty studies had an upper limit above the 0.45 g/L value.

Subgroup analyses—effect of patient age, sample size, study year and gender

All three of the studies that included more than 300 participants [4, 5, 17] and one smaller study of 93 participants [20] reported a statistically significant correlation of higher CSF TP with advancing age (Table 2). Two other studies of small sample size found no age correlation. In a reference group of 62 patients, Eeg Olofsson [15] did not find a correlation with age, but the two groups compared were both very young (16–20 vs 21–30 years). Breebaart [30] did not find a significant difference in CSF TP comparing 50 patients aged 20–40 years (pooled mean CSF-TP of 0.32 g/L) and 55 patients older than 40 years (pooled mean CSF-TP of 0.33 g/L).

Allowing for the uneven distribution of the number of published studies and their respective sample sizes, our systematic review showed that CSF-TP did not differ significantly when comparing CSF-TP values in reports of less or more than 100 participants (Table 3). Similarly, we did not find a statistically significant impact of year of publication (before or after 2016) or the assessed grade of methodological quality (S1 versus S2-3).

Few studies have compared CSF TP in males and females. Two small reports of less than 50 reference participants found no sex difference [10, 13]. Five other small cohorts [18, 19, 23, 29, 30] showed a higher value in males in the range of 0.03–0.11 g/L. The much larger reference study of McCudden [5] found a higher CSF TP in males that was highly statistically significant ($p < 0.00001$) but of small magnitude (0.06 g/L). This difference is not likely to be relevant in clinical practice.

Discussion

This systematic review of the laboratory reference literature for CSF-TP shows several important points. First, the widely used CSF-TP URL of 0.45 g/L (which is based on

Table 2 CSF TP Mean and upper reference limit in the four larger studies that compared results by decade of age

Age group (years)	Tibbling et al. (1977) [20]		Bernhardt et al. (1978) [17]		Hegen et al. 2016 [4]		McCudden et al. 2017 [5]	
	# Subj	Mean (URL) (g/L)	# Subj	Mean (URL) (g/L)	# Subj	Mean (95th percentile) (g/L)[a]	# Subj	Mean (URL) (g/L)
18–29	21	0.37 (0.49)	49	0.38 (0.52)[b]	69	0.32 (0.53)	1158	0.30 (0.49)
30–39	18	0.36 (0.48)	92	0.39 (0.55)	81	0.36 (0.62)	1204	0.32 (0.55)
40–49	22	0.43 (0.59)	68	0.41 (0.53)	89	0.39 (0.60)	1355	0.36 (0.58)
50–59	21	0.48 (0.66)	47	0.44 (0.58)	58	0.44 (0.69)	1040	0.37 (0.60)
60–69	11	0.53 (0.79)	24	0.42 (0.55)	27	0.46 (0.62)	598	0.38 (0.64)
70–79			10	0.42 (0.55)			446	0.40 (0.68)

All four studies reported a statistically significant age correlation with advancing age (Tibbling: $r=0.54$, p not specified; Bernhardt: $r=0.319$, $p<0.001$; Hegen: $r=0.3$, $p<0.001$; McCudden: $r^2=0.1$, $p<0.00001$). URL: 97.5% upper reference limit (calculated as mean + 2 × SD, except for Hegen et al)

y: years of age

[a]95th percentile as reported by Hegen et al. (2016) [4]

[b]Mean and URL for age 21–30 as reported by Bernhardt et al. (1978) [17]. URL: upper reference limit

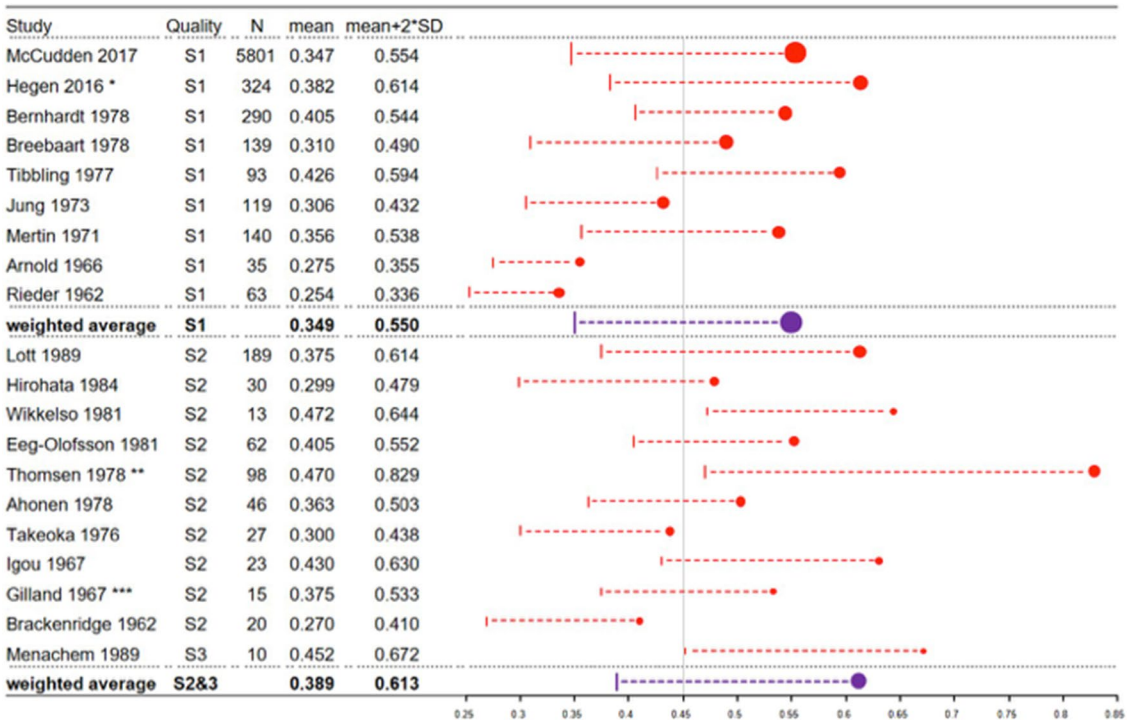

Fig. 2 Summary plot of CSF Total protein mean (short vertical line) and 97.5% upper limit (dot) in 20 studies selected for quantitative analysis. Dot size is proportional to log10 of study sample size. Weighted averages were calculated for the 9 studies assessed to have higher methodological quality (S1) and the 11 studies with lower quality score (S2 or 3). A vertical line is set at the current widespread 0.45 g/L reference standard. Annotations: Hegen (2016)* The SD per age group was calculated assuming (95th percentile − 5th percentile)/2 = 1.644854 * SD. We used the reported median as mean per age group and calculated the pooled mean and SD. Thomsen (1978)** The SD was calculated assuming (95th percentile − 5th percentile)/2 = 1.644854 * SD. We used the reported median as mean. The 95th percentile reported by authors was 0.88 g/L, larger than our estimated mean + 2*SD (0.829 g/L). Gilland (1967)*** The 97.5th percentile reported by authors was 0.541 g/L, larger than mean + 2*SD (0.533) we calculated from reported mean and SD

H. Merritt's seminal 1938 publication), is not supported by more recent studies using modern laboratory techniques. Second, the rise CSF-TP URL with increasing age must be taken into account, as demonstrated in 4 larger reference studies. Third, this systematic review showed a weighted mean of 0.55 g/L, in those studies of the highest

Table 3 Summary of CSF TP studies grouped by quality (S1 and S2–S3), year of publication (2016–2017 and 1962–1989), and number of subjects (> 100 and ≤ 100)

Subgroups	No. of Studies	Average (weighted by sample size)			Covariate-adjusted meta-analysis		
		Mean	SD	Mean + 2 × SD	Mean	Δ Of mean	*p* value
Quality							
S1	9	0.349	0.100	0.550	0.340		
S2 and S3	11	0.389	0.112	0.613	0.379	0.039	0.080
Year							
2016–2017	2	0.349	0.104	0.557	0.364		
1962–1989	18	0.368	0.088	0.544	0.361	−0.003	0.942
No. of subjects							
> 100	7	0.350	0.102	0.554	0.354		
≤100	13	0.377	0.091	0.560	0.364	0.010	0.679

Central panel: average of mean and mean + 2 × SD weighted by sample sizes of studies. Right panel: Mean by subgroup estimated from covariate-adjusted random effects meta-analysis (Dersimonian-Laird) for one factor at a time. If we assume SD to be constant across subgroups, testing whether the pooled mean differ across subgroups can reflect the significance of difference in the upper limit (mean + 2 × SD)

methodological quality. Finally, the findings from this review would support incremental upper reference limit values over 0.6 g/L, starting at age 50 years.

CSF analysis remains particularly relevant to clinical practice for the assessment of a wide range of autoimmune disorders of the PNS and CNS, including the spectrum of Guillain–Barré syndrome and CIDP, and an ever-increasing number of antibody-mediated encephalitides. The data reviewed here shows that using a CSF-TP cut-off value of 0.45 g/L would lead to false positive determinations in the range of 25% for *neurologically healthy* individuals in later decades of life (Fig. 3). This may lead to both overdiagnosis and overuse, for which recent literature has raised concerns of psychologic stress and overtreatment. An unfounded concern about CSF-TP could trigger unnecessary neurological investigations, such as gadolinium-enhanced MRI and repeat lumbar puncture, or erroneously support a diagnosis of central or peripheral neuro-inflammatory disease. Increasing the CSF-TP upper reference limit would impact reported sensitivity and sensitivity in a wide range of disorders. Innumerable illustrations can be offered. For example, over-reliance on the finding of "mild or moderate" protein elevation was specifically cited as an important pitfall in the diagnosis of CIDP [36]. In that review, mild albuminocytologic dissociation (CSF-TP mean value 0.61 g/L) had been used as supportive diagnostic data in 50% of patients who were subsequently deemed not to fulfill criteria CIDP. In a different study on the posterior reversible encephalopathy syndrome (PRES), 85% of patients were found to have an elevated CSF-TP, based on a "normal" value of < 0.35 g/L

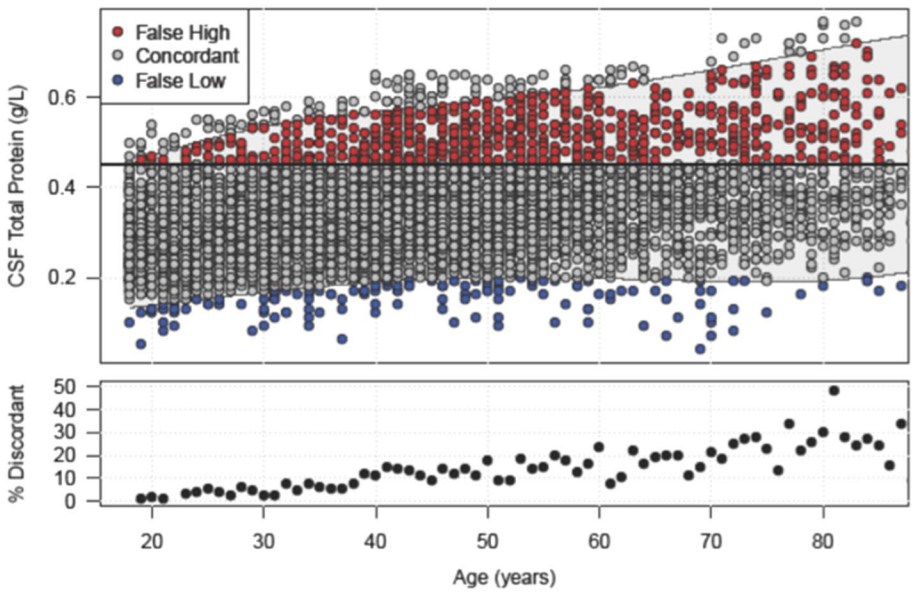

Fig. 3 Effect of a 0.45 g/L cut-off on a large study of CSF-TP reference limits. The top panel depicts the CSF-TP values of 6068 unique patients included in the reference interval study of McCudden et al. (Figure modified from McCudden et al. [5]). Using the customary value of 0.45 g/L (solid black line), all cases depicted by red dots would be false positives. The lower panel plots by age the percentage of patients which would be false-positives, or could be erroneously labelled "albuminocytologic dissociation", using a 0.45 g/L upper reference limit. This is in the range of 15% at age 50, 17% at age 60, 25% at age 70

[37]. However, the median CSF-TP value in patients with PRES was 0.58 g/L, which would mostly fall within the age-adjusted reference ranges proposed here. In a cohort of patients with limbic encephalitis associated with voltage-gated potassium channel antibodies, with a median age of 61 years, the CSF-TP exceeded the quoted reference limit of 0.45 g/L in 47%, but it exceeded a limit of 0.6 g/L in only 6% [38]. A similar discrepancy was found in a report on limbic encephalitis associated with AMPA receptor antibodies.

The invasive nature of lumbar puncture poses a unique challenge in trying to define reference ranges, compared to most other laboratory values. Large numbers of asymptomatic volunteers cannot be easily recruited, because of the fear and pain associated with the procedure, and the risk of post-puncture headache. In analyzing hospital data banks, researchers must contend with the limitation that the mere performance of a lumbar puncture often implies an *a priori* suspicion of neurologic disease. With the help of ancillary laboratory criteria (normal CSF cell count and glucose) and the review of longitudinal clinical, neuroimaging and lab data, it is, however, possible to define a suitable *a posteriori* reference population [31]. It has been calculated that 200 participants are required to capture 99% of the middle 95% of non-parametric data [39], whereas a sample of 100 participants provides only a 75% confidence level for this reference range. Only three studies we reviewed met this 200-participant requirement [4, 5, 17].

Through our systematic review, we have shown that the CSF-TP limit of 0.45 g/L (45 mg/dL) may only apply to individuals less than 30 years of age, while incremental values over 0.60 g/L (60 mg/dL) are necessary above the age of 50 years. There is a strong support for age-adjusted limits throughout adulthood, separate from the well documented transient peak in CSF-TP in the neonatal period [40]. Reference values for individuals over the age of 80 are less well defined, because of small sample sizes, but the literature we reviewed favors an upper limit above 0.7 g/L. Ironically, such age-partitioned limits are reminiscent of an old 'rule of thumb' that proposed an upper CSF-TP limit equivalent to the patient's age expressed in mg/dL. It should be noted that the CSF-TP reference data analysis presented in our quantitative review does not constitute a formal data meta-analysis, as the value of interest was the 97.5% upper reference limit, not the mean CSF-TP. Meta-analysis could not be performed given that the 97.5% upper reference limit does not have a standard error of measurement associated with it. Therefore, weighted averages were calculated (Fig. 2).

Although many laboratory techniques have acceptable reproducibility ranges of up to 10%, we feel it is unlikely that laboratory methodologies account for significant discrepancies across institutions and published reference intervals. Two studies [5, 10], each comparing three different techniques and manufacturer equipment, found that the choice of analytical method may have an impact in the range of 0.04 g/L. In addition, our quality assessment tool did take the laboratory methods into account. Even those studies with lower methodologic quality (S2 and S3) had a very similar CSF-TP upper reference limit to those with the highest methodological quality—thus implying that the laboratory equipment used does not have significant impact.

In most clinical settings, it must be emphasized that the CSF-TP is not interpreted in isolation, but rather with conjunction additional CSF indices such as cell count, glucose, microbiology, cytology and more importantly the clinical syndrome, neuroimaging and electrophysiological data. The present review also does not address the important contribution of additional CSF studies that may provide information about blood–brain and blood-nerve permeability. The CSF/Serum Albumin quotient has been well studied and may greatly enhance the interpretation of CSF protein elevation, though it is not yet widely used across the globe. Other specific CSF protein measurements are directly relevant to special clinical syndromes: detection of intrathecal immunoglobulin production (CSF IgG index, oligoclonal bands), or the detection of specific CSF antibodies (e.g. anti-LGi 1, anti-NMDA) and potential biomarker proteins (e.g. tau, amyloid beta, synuclein, and TDP-43).

In conclusion, this systematic review supports the view that the medical literature, hospital laboratories, and clinicians worldwide have underestimated the CSF-TP upper reference limit, particularly for older adults. It would not be feasible to recommend institution-specific CSF reference data, but our evidence synthesis suggests that updated ranges should be adopted, with age-partitioned adjustments.

Author contributions AB, PB: conception of the work, data acquisition, data interpretation, drafting of manuscript. DM, WC: data analysis, revision of manuscript for critically important intellectual content. JB, HH, FD, CM: contributions to study design, data interpretation, and revisions for critically important intellectual content.

Compliance with ethical standards

Conflicts of interest No relevant conflicts or competing interests.

References

1. Merritt Houston, H F-SF (1937) The cerebrospinal fluid. W.B. Saunders, Philadelphia
2. Daroff RB, Bradley WG, Jankovic J, Mazziotta JC, Pomeroy SL, Elsevier (2016) Bradley's neurology in clinical practice, vol 2. Elsevier, London [etc.]
3. Goldman L, Schafer AI (2016) Goldman-cecil medicine, 25th edn. Elsevier/Saunders, Philadelphia, PA, USA
4. Hegen H, Auer M, Zeileis A, Deisenhammer F (2016) Upper reference limits for cerebrospinal fluid total protein and albumin quotient based on a large cohort of control patients: implications for increased clinical specificity. Clin Chem Lab Med 54:285–292

5. McCudden CR, Brooks J, Figurado P, Bourque PR (2017) Cerebrospinal fluid total protein reference intervals derived from 20 years of patient data. Clin Chem 63(12):1856–1865
6. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D (2009) The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med 6:e1000100
7. McGowan J, Sampson M, Salzwedel DM, Cogo E, Foerster V, Lefebvre C (2016) PRESS peer review of electronic search strategies: 2015 guideline statement. J Clin Epidemiol 75:40–46
8. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM (2011) QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 155:529–536
9. R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/
10. Lott JA, Warren P (1989) Estimation of reference intervals for total protein in cerebrospinal fluid. Clin Chem 35:1766–1770
11. Ben Menachem E, Persson L, Schechter PJ, Haegele KD, Huebert N, Hardenberg J (1989) Cerebrospinal fluid parameters in healthy volunteers during serial lumbar punctures. J Neurochem 52:632–635
12. Hirohata S, Inoue T, Yamada A, Hirose S, Miyamoto T (1984) Quantitation of IgG, IgA and IgM in the cerebrospinal fluid by a solid-phase enzyme-immunoassay. Establishment of normal control values. J Neurol Sci 63:101–110
13. Merril CR, Harrington MG (1984) 'Ultrasensitive' silver stains: their use exemplified in the study of normal human cerebrospinal fluid proteins separated by two-dimensional electrophoresis. Clin Chem 30:1938–1942
14. Wikkelso C, Blomstrand C, Ronnback L (1981) Cerebrospinal fluid specific proteins in multiinfarct and senile dementia. J Neurol Sci 49:293–303
15. Eeg-Olofsson O, Link H, Wigertz A (1981) Concentrations of CSF proteins as a measure of blood brain barrier function and synthesis of IgG within the CNS in 'normal' subjects from the age of 6 months to 30 years. Acta Paediatr Scand 70:167–170
16. Ahonen A, Myllyla VV, Hokkanen E (1978) Measurement of reference values for certain proteins in cerebrospinal fluid. Acta Neurol Scand 58:358–365
17. Bernhardt W, Weisner B, Rehpenning W (1978) Laboratory findings in cerebrospinal fluid in relation to age: trivariate evaluation of albumin and immunoglobulin G concentrations. J Clin Chem Clin Biochem 16:435–439 **(author's transl)**
18. Stibler H (1978) The normal cerebrospinal fluid proteins identified by means of thin-layer isoelectric focusing and crossed immunoelectrofocusing. J Neurol Sci 36:273–288
19. Thomsen J, Bech P, Nielsen OS (1978) CSF total protein: normal values. A re-appraisal and discussion of its value in diagnosis of acoustic neuromas. Acta Oto-Laryngol 86:359–365
20. Tibbling G, Link H, Ohman S (1977) Principles of albumin and IgG analyses in neurological disorders. I. Establishment of reference values. Scand J Clin Lab Investig 37:385–390
21. Takeoka T, Gotoh F, Furumi K, Mori K (1976) Polyacrylamide-gel disc electrophoresis of native cerebrospinal fluid proteins with special reference to immunoglobulins and some clinical applications. J Neurol Sci 29:213–239
22. Jung PR, Rieder HP, Jeltsch C (1973) Total protein levels in lumbar cerebrospinal fluid of normal subjects. Klinische Wochenschrift 51:810–816
23. Mertin J, Wisser H, Doerr P (1971) Studies on the normal concentration range of total protein and the total protein fractions of the cerebrospinal fluid by electrophoresis on cellulose acetate. Zeitschrift fur klinische Chemie klinische Biochemie 9:337–340 **(author's transl)**
24. Igou PC (1967) Normal values for cellulose acetate electrophoresis of spinal fluid proteins. Amer J Med Technol 33:501–504
25. Gilland O (1967) Lumbar cerebrospinal fluid total protein in healthy subjects. Acta Neurol Scand 43:526–529
26. Arnold F (1966) Cerebrosplnal liquid examinations in healthy individuals and in patients with multiple sclerosis. Schweizerische Medizinische Wochenschrift 96:1098–1103
27. Brackenridge CJ (1962) Cerebrospinal fluid protein fractions in health and disease. J Clin Pathol 15:206–210
28. Rieder HP, Wuthrich R (1962) The cu-folin method for determining the total proteinsin the csf. KljnWschr 40:241–255
29. Ahonen A, Myllyla VV, Hokkanen E (1979) Cerebrospinal fluid protein findings in various lower back pain syndromes. Acta Neurol Scand 60:93–99
30. Breebaart K, Becker H, Jongebloed FA (1978) Investigation of reference values of components of cerebrospinal fluid. J Clin Chem Clin Biochem 16:561–565
31. Teunissen C, Menge T, Altintas A, Alvarez-Cermeno JC, Bertolotto A, Berven FS, Brundin L, Comabella M, Degn M, Deisenhammer F, Fazekas F, Franciotta D, Frederiksen JL, Galimberti D, Gnanapavan S, Hegen H, Hemmer B, Hintzen R, Hughes S, Iacobaeus E, Kroksveen AC, Kuhle J, Richert J, Tumani H, Villar LM, Drulovic J, Dujmovic I, Khalil M, Bartos A (2013) Consensus definitions and application guidelines for control groups in cerebrospinal fluid biomarker studies in multiple sclerosis. Mult Scler 19:1802–1809
32. Dufour-Rainfray D, Beaufils E, Vourc'h P, Vierron E, Mereghetti L, Gendrot C, Hommet C, Andres CR, Guilloteau D, Mondon K (2013) Total protein level in cerebrospinal fluid is stable in elderly adults. J Am Geriatr Soc 61:1819–1821
33. Kleine TO, Weber L, Zofel P (1988) Changes with age in leucocyte counts, protein and IgG content of lumbar cerebrospinal fluid (CSF) of patients with inflammatory, ischemic, and hemorrhagic diseases or tumors of the central nervous system (CNS). Zeitschrift fur Gerontologie 21:102–105
34. Seyfert S, Kunzmann V, Schwertfeger N, Koch HC, Faulstich A (2002) Determinants of lumbar CSF protein concentration. J Neurol 249:1021–1026
35. Blennow K, Fredman P, Wallin A, Gottfries CG, Karlsson I, Langstrom G, Skoog I, Svennerholm L, Wikkelso C (1993) Protein analysis in cerebrospinal fluid. II. Reference values derived from healthy individuals 18–88 years of age. Eur Neurol 33:129–133
36. Allen JA, Lewis RA (2015) CIDP diagnostic pitfalls and perception of treatment benefit. Neurology 85:498–504
37. Datar S, Singh TD, Fugate JE, Mandrekar J, Rabinstein AA, Hocker S (2015) Albuminocytologic dissociation in posterior reversible encephalopathy syndrome. Mayo Clin Proc 90:1366–1371
38. Jarius S, Hoffmann L, Clover L, Vincent A, Voltz R (2008) CSF findings in patients with voltage gated potassium channel antibody associated limbic encephalitis. J Neurol Sci 268:74–77
39. Lott JA, Mitchell LC, Moeschberger ML, Sutherland DE (1992) Estimation of reference ranges: how many subjects are needed? Clin Chem 38:648–650
40. Shah SS, Ebberson J, Kestenbaum LA, Hodinka RL, Zorc JJ (2011) Age-specific reference values for cerebrospinal fluid protein concentration in neonates and young infants. J Hosp Med 6:22–27