External Quality Assessment in Clinical Neurochemistry: Survey of Analysis for Cerebrospinal Fluid (CSF) Proteins Based on CSF/Serum Quotients

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Participants (230) from Germany and 20 laboratories in 11 European countries took part in a newly designed cerebrospinal fluid (CSF) survey distributed by INSTAND. Conventional proficiency testing for albumin, IgG, IgA, and IgM in CSF and serum, for total protein in CSF, and for oligoclonal IgG in CSF and serum was combined with evaluation and interpretation of CSF/serum quotients in quotient diagrams. The correct detection of a blood–CSF barrier dysfunction and the pattern of intrathecal immunoglobulin synthesis was judged. The accuracy of CSF/serum quotients and their clinically relevant interpretation was given first priority as a new concept in quality assessment. The main result of the surveys was to confirm that CSF/serum quotients of proteins represent method-independent values approaching the quality of reference values. This finding has consequences for internal quality control of CSF analysis and for accreditation bodies. The sensitivity of the methods for quantifying IgA and IgM in CSF and for detecting oligoclonal IgG fractions is discussed.

Indexing Terms: *albumin/immunoglobulins/oligoclonal IgG/internal quality assessment/proficiency testing*

This report is based on the Harmonized Proficiency Testing Protocol of the International Standardizing Organizations (*1*); relevant terms from this protocol are defined in the *Appendix*. Quality assurance in medical laboratories, as practiced in many countries (*2*), involves external quality assessment (EQA) and internal quality control.[1] External assessments usually involve interlaboratory surveys organized by an independent EQA organizer.

Here I present experience with a newly designed survey for cerebrospinal fluid (CSF) analytes distributed twice a year (1990–1994) by the Institut für Standardisierung und Dokumentation (INSTAND), Düsseldorf, Germany, to 280 participants in Germany and 20 laboratories in 11 other European countries (Austria, Belgium, Denmark, England, Italy, Netherlands, Norway, Portugal, Spain, Sweden, and Switzerland). I designed and evaluated the survey under the supervision of INSTAND as EQA organizer of the German Society of Laboratory Medicine.

After a 1-year pilot phase, the CSF survey was modified according to recommendations of the participants and two expert groups [a European group (*3*) and the German Society, Arbeitsgemeinschaft für Liquordiagnostik und Klinische Neurochemie]. It was not the aim of this European study to support or recommend a preference for an international survey. National institutions must develop in their own language and tradition a suitable CSF survey for competent handling by the technicians in individual laboratories.

Originally, participants' methods for CSF diagnosis varied from modern, highly sensitive, and specific protein analysis by a wide range of methods to such outdated methods as cellulose acetate foil electrophoresis. Many different evaluation and interpretation schemes for CSF data were in use. Moreover, routine analysis of CSF had long been hindered by insensitive methods, unsuitable standards, and inappropriate quality-control samples. It is still a problem to find automated methods with sufficient sensitivity for IgA and IgM in CSF, and no reference methods for CSF analysis are available.

Recently, a European expert group (*3*) reached consensus about which methods are the most relevant for CSF analysis to diagnose multiple sclerosis. In general, a basic analysis (*3–5*) should involve counting and differentiation of cells in CSF; determination of protein concentrations, including total protein in CSF, and albumin and immunoglobulins (G, A, M) in CSF and serum; and qualitative detection of oligoclonal bands in CSF by isoelectric focusing (IEF) as the most sensitive method (*3*) for detection of intrathecal synthesis of IgG.

Quantitative CSF protein analysis takes advantage of CSF/serum quotients (*6*) to reduce the influence of individual biological variations in blood concentration on the interpretation of CSF data (*7*). Moreover, method- and standard-dependent inaccuracy and interassay imprecision can be reduced if paired CSF and serum samples are analyzed in the same assay run (*4, 5*). Consequently, the CSF/serum quotients represent method-independent values, as shown below. The CSF/serum concentration quotient of albumin has been accepted as the best indicator for identification of the blood–CSF barrier dysfunction (*3*), or more precisely, for identification of an increased blood-derived protein concentration in CSF, due to a reduced CSF flow rate (*8*). However, discrimination between brain-derived and blood-derived protein fractions in CSF is possible by taking into account the individual blood–CSF barrier function/dysfunction, referring the CSF/serum quotients for IgG, IgA, and IgM to the albumin CSF/serum quotient of the individual patient (*3, 6–9*). This approach minimizes the influence of variations in non-

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[1] Nonstandard abbreviations: CSF, cerebrospinal fluid; INSTAND, Institut für Standardisierung und Dokumentation; EQA, external quality assessment; IEF, isoelectric focusing; and Q_{Alb}, Q_{IgG}, Q_{IgA}, Q_{IgM}, quotients for CSF/serum concentrations of albumin, IgG, IgA, and IgM, respectively.

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specific individual variables such as CSF flow rate (*8, 10*), age of the patient (*11, 12*), and volume of CSF extracted.

The graphical presentation of the CSF and serum data in quotient diagrams is designed to benefit the clinician (*3*). The best discrimination function or formula for the identification of an intrathecal synthesis of immunoglobulin is still subject to discussion (*13–16*), but there is at least consensus (*3*) that a nonlinear approach is essential for discrimination between blood-derived and brain-derived concentrations of IgG, IgA, or IgM in CSF. In the surveys the hyperbolic function (*8, 9*) illustrated in Fig. 1, meanwhile shown to be the physiologically and physically correct form (*8, 16*), has been introduced. This diagram represents an improved evaluation graph (*8*) routinely used for CSF data reports in >80 German or other European neurological clinics for a clinically relevant pattern recognition (*4, 17–22*).

Materials and Methods

The CSF samples distributed for proficiency testing were taken from patients punctured for routine CSF diagnosis in the Neurologic Clinic, University of Göttingen. After the diagnostic procedures, residual CSF volumes were pooled and kept at −30°C. Pools of CSF samples and serum samples from patients were cleared by filtration and stabilized by adding 0.1 g/L thimerosal (sodium salt; Sigma, Deisenhofen, Germany).

Two pairs of CSF and serum samples (0.7 mL each) for quantitative analysis for total protein, albumin, IgG, IgA, and IgM were distributed, with a form for each CSF/serum pair. This data form contained the age of a fictitious patient and blank diagrams (see Fig. 1). Pooled CSF, which contains a polyclonal mixture of IgG, cannot be used for qualitative detection of oligoclonal bands. In most surveys the CSF samples for detection of oligoclonal bands originated from a single patient (e.g., ventricular CSF from a therapeutic CSF drainage, performed in the intensive care unit, which is usually discarded). In one survey single CSF samples from five different patients, each representing a similar band pattern, were distributed to participants. In another survey, for IEF, diluted serum instead of CSF was used to simulate a corresponding monoclonal pattern in CSF and serum, typical for a paraproteinemia (monoclonal pattern). In any case, for detection of oligoclonal IgG the participants were sent one pair of CSF and serum samples (>100 μL) and an evaluation form that included the method-dependent IgG concentrations in CSF and serum to avoid preanalytical faults.

Survey Procedure

Samples plus forms were distributed by regular mail by INSTAND, a nonprofit agency for EQA of the Society of Laboratory Medicine. This EQA organizer is controlled by the Bundesärztekammer (Federal Medical Society).

Three forms, two for quantitative and one for qualitative results, were returned to INSTAND by participants. Besides the individual data for CSF and serum values, CSF/serum quotients had to be reported, numerically and as points in the quotient diagrams on the supplied forms (Fig. 1). For interpretation of the results, the following comments were proposed: normal CSF; blood–CSF barrier dysfunction; inflammatory process; and intrathecal synthesis of IgG, IgA, and (or) IgM. The basis for this evaluation of immunoglobulin synthesis is given below. Age-related reference ranges for blood–CSF barrier function are listed in Table 1.

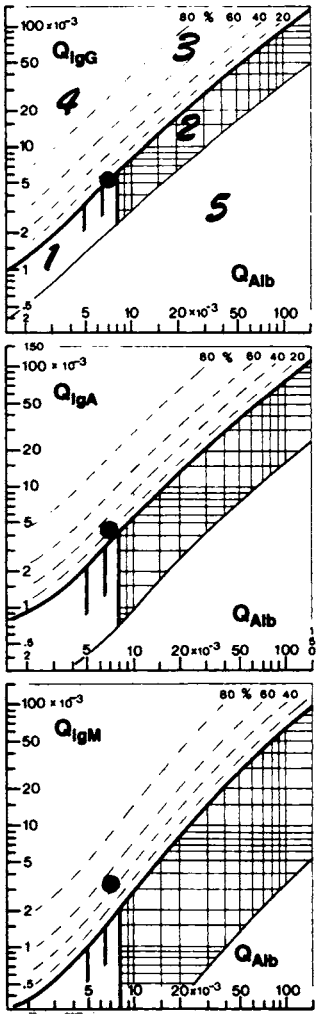

Fig. 1. CSF/serum quotient diagrams with hyperbolic functions for the ratio between Q_{IgG}, Q_{IgA}, Q_{IgM}, and Q_{Alb}.

The upper discrimination line (*heaviest curve*) differentiates between a blood-derived and an additionally brain-derived CSF IgG, IgA, or IgM fraction with the following areas (marked on the IgG diagram): (*1*) normal range; (*2*) pure blood–CSF barrier dysfunction without local intrathecal IgG synthesis; (*3*) blood–CSF barrier dysfunction plus an intrathecal IgG synthesis in the central nervous system; (*4*) intrathecal IgG synthesis in the central nervous system without blood–CSF barrier dysfunction. Values in *area 5* are indicative of methodological error (unpaired CSF/serum samples, measurement in antigen excess range, etc.). The age-dependent evaluation of the blood–CSF barrier function is facilitated by the *vertical bars*, indicating (*left to right*): Q_{Alb} at ages 4 months to 15 years, to 40 years, and to 60 years; for newborn children, see Table 1. *Dashed lines* mark where 20%, 40%, 60%, and 80% of the measured immunoglobulin concentration in CSF originate from intrathecal synthesis (Ig_{IF}, intrathecal fraction; for calculation see *Materials and Methods*), referring to the discrimination line as 0% synthesis. Given the imprecision of the methods, any brain-derived fractions >10% are regarded as pathological. ●, points representing the report of a participant in the CSF survey indicating intrathecal synthesis of IgA and IgM with a blood–CSF barrier dysfunction (fictitious age of patient, 5 years). This pattern with a dominance of intrathecal IgM synthesis is a frequent observation in neuroborreliosis (*19, 21*).

Table 1. Age-related reference range of CSF/serum albumin quotients (Q_{Alb}, blood–CSF barrier function) in lumbar CSF[a].

Age	$Q_{Alb} \times 10^3$
Newborn	8–28
1 month	5–15
2 months	3–10
3 months	2–5
4 months–6 years	0.5–3.5
<15 years	<5.0
<40 years	<6.5
<60 years	<8.0

[a] These age-dependent limits of the protein reference range depend greatly on the volume of lumbar CSF extracted, given the concentration gradient in CSF (20% decrease of concentration between 1 and 12 mL extracted). For ventricular CSF of mature patients, divide the Q_{Alb} reference values by 2.3; for cisternal CSF, by 1.6 (27).

The results report form for oligoclonal IgG discriminates five types of CSF as recommended by the European expert group (*3*): (*a*) normal CSF; (*b*) CSF-restricted oligoclonal bands; (*c*) CSF-restricted oligoclonal bands with additional identical bands in CSF and serum; (*d*) identical bands in CSF and serum; and (*e*) monoclonal bands in CSF and serum.

Quantitation of Intrathecal Immunoglobulin Synthesis

The hyperbolic functions in Fig. 1 have the general form: $Q_{Ig} = a/b \sqrt{Q^2_{Alb} + b^2} - c$, where Q_{Alb} is the CSF/serum albumin quotient.

This equation represents the statistically defined upper limit [QLim(Ig)] of the reference range for the following respective values of a/b, b^2, and c (*8*): Q_{Lim}(IgG): 0.93, 6×10^{-6}, 1.7×10^{-3}; Q_{Lim}(IgA): 0.77, 23×10^{-6}, 3.1×10^{-3}; and Q_{Lim}(IgM): 0.67, 120×10^{-6}, 7.1×10^{-3}.

An intrathecal synthesis, for which Ig quotients are above the hyperbolic discrimination line, can be quantitated in two ways: (*a*) by the locally (Loc; intrathecally) synthesized fraction of the CSF concentration in mg/L (e.g., for IgG with the actual IgG quotient value Q_{IgG}), such that $IgG_{Loc} = [Q_{IgG} - Q_{Lim}(IgG)] \times$ IgG(serum); or (*b*) preferably, as a percentage of the total CSF IgG concentration, i.e., the intrathecal fraction $IgG_{IF} = [IgG_{Loc}/IgG(CSF)] \times 100$ or $IgG_{IF} = [Q_{IgG} - Q_{Lim}(IgG)]/[Q_{IgG}] \times 100$. Values of Ig_{IF} with 20%, 40%, 60%, and 80% intrathecal synthesis are indicated as dashed lines in Fig. 1.

Assigned Values/Consensus Values/Target Values (see *Appendix*)

Target values for the evaluation of the survey were determined in the pilot phase as the mean of 10 interassay values obtained by a single laboratory (method-dependent, assigned values). Alternatively, assigned values were obtained as the mean of data (interassay, 10 determinations per laboratory) from three different selected laboratories (trial). On the basis of the assigned values, the outliers (deviation >±30%) among the participants of the survey were detected and eliminated to calculate the consensus value from participants (i.e., all results except outliers). Consensus values were reported and used for evaluation only if >30 participants remained after exclusion of <10% outliers.

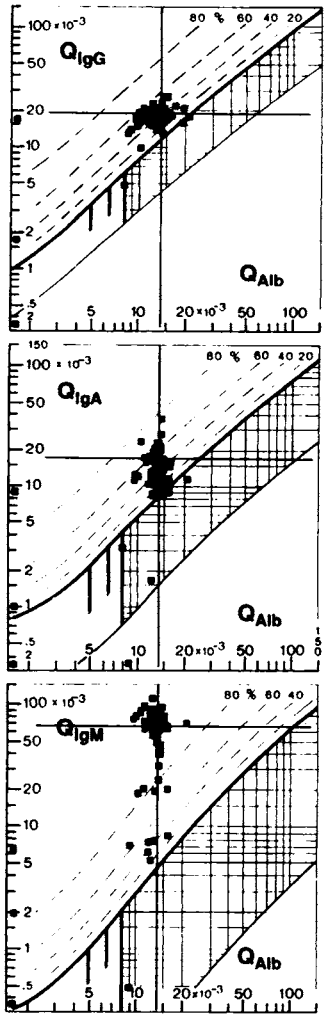

Fig. 2. Report of results of CSF/serum quotients (Q_{Alb}, Q_{IgG}, Q_{IgA}, Q_{IgM}) in CSF survey 12/92 (INSTAND), showing the data of all participants (including the outliers).

The *thin cross-hair lines* indicate the set of data of a single participant (reported in Table 2 together with a score for accuracy and the target values). The second pair of CSF samples in the same CSF survey had the following target values: $Q_{Alb} = 7.3 \times 10^{-3}$, $Q_{IgG} = 4.8 \times 10^{-3}$, $Q_{IgA} = 2.6 \times 10^{-3}$, $Q_{IgM} = 0.6 \times 10^{-3}$. Given the low IgA and IgM concentrations in CSF (IgA = 5.7 mg/L, IgM = 0.9 mg/L), the overall performance for analysis of the second pair was worse than for the sample pair shown here.

Assessment of Performance

The reports issued to participants included graphical representation of CSF/serum quotients in the diagram for the whole group of participants plotted with the individual results of each respective participant (marked by crossing thin lines in Fig. 2), to check for correct entry of their data. This evaluation was computer-aided with a program developed by COMED (Computerorganisation in der Medizin, Soest, Germany).

The accuracy of a participant's data was analyzed numerically as the percentage deviation from the tar-

get value: $[(x_p - \bar{x})/\bar{x}] \times 100$, where $\bar{x}$ is the target value and x_p the participant's value (Table 2). In addition to the graphical and numerical evaluation, the overall performance of the laboratory was summarized in the certificate of participation (Table 3) with a primary emphasis placed on clinically relevant interpretation and the accuracy of quotients (method-independent values). Second, in case of a large deviation in the quotients, the participant received a notation about the origin of the fault, whether in the absolute CSF value, the absolute serum value, or incorrect calculation.

Results

Albumin, IgG, IgA, and IgM

The typical report sent to a single participant in the CSF survey looks like Fig. 2 and Table 2 together with a certificate of participation (Table 3). The data in Fig. 2 represent the CSF/serum quotients of all participants and mark the individual data of the participant (thin lines) to verify correct entry of the data and to provide an individual comparison with the other participants. Table 2 gives the relevant target values, the consensus values, and the participants' performance as the percentage deviation from the target value. If the number of successful participants was too small (e.g., IgM in Table 2), or if the number of outliers was too large (>10%), no consensus value was reported.

The improvement of accuracy afforded by quotient evaluation compared with the absolute values in CSF and serum is shown with data from the single participant in Table 2. Despite poor accuracy for absolute IgM values in CSF and serum (−23% and −21%, respectively), the participant obtained excellent performance in the CSF/serum quotient, Q_{IgM} (+2%).

Table 2. Numerical data report for assessing individual performance in the CSF survey.

	Target values		Consensus values			Participant's results, %[a]
	$\bar{x}$	CV, %	n	$\bar{x}$	CV, %	
Method-independent values						
$Q_{Alb} \times 10^3$	13.6	2.4	72	13.6	10.5	+4
$Q_{IgG} \times 10^3$	18.1	3.9	71	18.3	9.0	+1
$Q_{IgA} \times 10^3$	11.3	7.3	44	10.2	20.1	+9
$Q_{IgM} \times 10^3$	56.7	7.3	23[b]	—	—	+2
Method-dependent values						
Albumin						
CSF, mg/L	323	2.6	72	371	7.5	0
Serum, g/L	23.7	2.1	65	26.6	7.4	−4
IgG						
CSF, mg/L	103	3.7	67	117	7.8	+5
Serum, g/L	5.7	2.3	69	6.5	6.8	+4
IgA						
CSF, mg/L	14.7	2.7	45	12.5	9.4	+1
Serum, g/L	1.4	7.3	54	1.2	9.7	−8
IgM						
CSF, mg/L	60.3	8.1	40	53.6	7.4	−23
Serum, g/L	1.1	8.3	25[b]	—	—	−21

[a] % deviation from target value: $[(x_p - \bar{x})/\bar{x}] \times 100$.
[b] Participants too few (n <30) for calculation of consensus value.

Table 3. Certificate of participation: EQA CSF Survey (I–III).

Participant _____ Specimen: 51

Qualitative evaluation		right	wrong
Result: Blood–CSF barrier dysfunction and inflammatory process with intrathecal synthesis of IgG, IgA, and IgM (dominant IgM fraction): Inflammatory process in the central nervous system.	Q_{IgG}/Q_{Alb}	○	○
	Q_{IgA}/Q_{Alb}	○	○
	Q_{IgM}/Q_{Alb}	○	○

Deviation of CSF/serum quotients from method-independent target value

	Q_{Alb}	Q_{IgG}	Q_{IgA}	Q_{IgM}
<10%	○	○	○	○
10–20%	○	○	○	○
>20%	○	○	○	○
Source of deviation in method-dependent values:				
CSF	○	○	○	○
Serum	○	○	○	○

Total protein

Deviation from consensus value ○ <10% ○ 10–20% ○ >20%

Oligoclonal IgG Specimen F

Result: CSF-restricted oligoclonal IgG ○ right ○ wrong

This method-independent performance of CSF/serum quotients is documented with consensus values from two groups of participants in Table 4, which used different standards (Beckman or Behring): Despite different absolute values for albumin and IgG in CSF and serum, both subgroups of participants showed an excellent compatibility of the consensus values for CSF/serum quotients.

From this empirical observation, which has been reproduced in the 10 CSF surveys, we conclude that:

1) CSF/serum quotients can be treated as method-independent values.

2) Target values (assigned values or consensus values) for CSF/serum quotients can be determined for the total group of all participants independently of the methods used.

Table 4. Comparison of the consensus values from CSF survey for CSF/serum quotients and absolute CSF or serum values analyzed with two different methods.

	Mean (CV, %)	
	Beckman[a]	Behring[b]
No. of participants	43	85
$Q_{Alb} \times 10^3$	10.0 (7.8)	10.2 (9.6)
$Q_{IgG} \times 10^3$	3.3 (5.0)	3.3 (6.9)
Albumin		
CSF, mg/L	259	285
Serum, g/L	25.9	28.3
IgG		
CSF, mg/L	37.5	44.3
Serum, g/L	11.2	13.4

[a] Beckman standards with Array analyzer.
[b] Behring standards with BN-100, BNA, or Turbitime apparatus.

3) The evaluation of CSF/serum quotients is more useful than the evaluation of absolute values in scoring the quality assessment of a single laboratory.

Several important restrictions must be considered: The application of the consensus values of all participants as target values instead of the assigned values is valid only if the number of participants is large enough and if the number of outliers is small compared with the total number of participants. For Q_{Alb} and Q_{IgG} we observed an acceptable 3 to 9 outliers among 128 participants. In contrast, Q_{IgM} (with the IgM in CSF = 1.1 mg/L) had 26 outliers among 37 participants, which does not allow the calculation of a consensus value. The problem of detecting outliers in a nongaussian distribution is obvious for IgM and partly obvious for IgA, whereas the distribution of the albumin and IgG quotients allows the data to be treated as a gaussian-like distribution. In general, the worse performance for IgM and occasionally for IgA analysis was due to an unqualified application of automated methods that were not sensitive enough for CSF analysis. The discrepancies between the Q_{IgA} consensus value and the target value in Table 2 originate from unpaired analysis of CSF and serum samples by some participants, as indicated by the larger CV for Q_{IgA} (20.1%) than for the absolute IgA values in CSF and serum (9.4% and 9.7%, respectively).

Total Protein in CSF

Total protein data were evaluated regardless of the methods used—dye binding; trichloroacetic acid precipitation with nephelometric or turbidimetric detection; or biuret method after preconcentration. The number of outliers decreased from 35% in survey 1990 to 7% in survey 1994. The consensus values for total protein were in excellent concordance (median difference 1.8%, n = 10 surveys) with the assigned values obtained with a nephelometric method for protein analysis in CSF (*23*).

Oligoclonal IgG Fractions

The performance of two surveys with similar CSF samples (four or five weak oligoclonal IgG bands in CSF from a single patient) paired with polyclonal serum (pooled from many different patients to avoid an oligoclonal pattern in serum) is summarized in Table 5. CSF-restricted oligoclonal bands (type 2) should have been reported. The reported results (Table 5) support the recommendations of the consensus paper (*3*): Oligoclonal banding must be detected by IEF; agarose electrophoresis is not sensitive enough. Moreover, detection with IEF on macrogels appears to be more sensitive than that on microgels (with direct silver stain of proteins). The good performance of the few participants who used immune detection after IEF on macrogels is in concordance with these recommendations (*3*). Some of the participants reported type 3 (oligoclonal bands in CSF with additional identical bands in CSF and serum) instead of type 2 (CSF-restricted oligoclonal bands). This partly wrong interpretation ("oligoclonal" bands in definitely polyclonal serum) was a consequence of a rough ampholine pattern in some methods and could have been avoided by comparison with results for other normal serum samples on the same gel.

Table 5. Detection of oligoclonal bands in CSF and serum.

	Reported results[a]		
Method	Type 2	Type 3	Types 1 or 4
IEF Macro PAG[b]	57/73	4/73	12/73 (16%)
IEF Micro (PHAST)[c]	20/40	5/40	15/40 (38%)
IEF immunodetection	13/14	—	1/14 (7%)
Agarose EPS[d]	1/14	—	13/14 (93%)
All others	5/20	3/20	12/20 (60%)

[a] Summarized from two CSF surveys with CSF specimens containing four or five weak oligoclonal bands and polyclonal (pooled) serum. Evaluation according to a European consensus (3): type 1 = normal CSF; type 2 = CSF-restricted oligoclonal bands; type 3 = CSF-restricted oligoclonal bands with additional, identical bands in CSF and serum; type 4 = identical oligoclonal bands in CSF and serum; type 5 = monoclonal bands in CSF and serum. For the samples reported here type 2 = right answer, type 3 = partly right, and types 1 and 4 are wrong.

[b] IEF on polyacrylamide gel, with direct protein stain (mainly silver stain).

[c] Various types of gel preparation, analyzed on Phast (Pharmacia), with direct protein stain.

[d] Agarose electrophoresis on Paragon (Beckman).

Performance of Surveys

The overall performance in the surveys improved successively from 1990 through 1994. An increasing number of participants were involved in the interpretation of their data reports, and an increasing number of participants learned to assess the restrictions of their methods with respect to CSF analysis, or learned to analyze CSF and serum paired in the same run by the same method. Consensus values from three expert laboratories (trial; 3 × 10 determinations, interassay) had CVs between 4% and 6% for Q_{Alb}, Q_{IgG}, Q_{IgA}, and Q_{IgM}. In the corresponding CSF survey the CVs from consensus of all participants were 7–15%.

Overall performance of survey 11/1993 (231 participants) was as follows: 60% of all participants reported accurate analytical data; 30% gave a correct clinical interpretation in addition to the accurate analytical data. Only 10% of the participants had a perfect and complete report: i.e., only 9 laboratories had a completely correct analysis and interpretation for all variables (Q_{Alb}, Q_{IgG}, Q_{IgA}, Q_{IgM}, and oligoclonal IgG) and for all pairs of samples (high and low CSF concentration). A somewhat larger number (n = 13) of participants in a group participating in a restricted program (no IgA and IgM) had good performance.

Consequently, one can conclude that sufficient quality in clinical neurochemistry is still restricted to a small but increasing number of expert laboratories.

Discussion and Recommendations

Accuracy in CSF Diagnosis

The main analytical problems observed in the CSF surveys were: (*a*) inappropriate performance due to unpaired analysis of CSF and serum, and (*b*) applica-

tion of automated methods used below their detection limits, particularly for IgA and IgM analysis in CSF. Only three analytical systems in use are sensitive enough to detect 0.5 mg/L IgM and 1 mg/L IgA: enzyme immunoassay, immunodetection by endpoint nephelometry (ICS from Beckman Instruments, Los Angeles, CA, and Dosascat from Dosatec, Munich, Germany), and latex-particle-amplified nephelometry (BN from Behring, Marburg, Germany). Turbidimetric and rate nephelometric systems are not sensitive enough for normal IgA and IgM values in CSF: 63% of normal IgM values in CSF are <0.5 mg/L and 25% are <0.2 mg/L (unpublished data from my study of 220 patients with normal albumin quotients and no humoral immune response).

The accuracy requested by the accreditation bodies for albumin, IgG, IgA, and IgM in blood (*24*) is not relevant for the absolute values of these analytes in CSF, given the more sophisticated methods needed for quantifying the very low concentrations in CSF. It makes no sense to suggest a maximal allowable deviation from the target value in serum of 3% (*24*) for total protein in CSF, the concentration of which is only 1% of the serum reference values.

From the latest (1994) CSF survey an allowed deviation (CV) from the consensus value of 10% would still exclude 34% of the participants.

CSF/Serum Quotients as Method-Independent Values

One of the most important results of the CSF survey was the confirmation that CSF/serum quotients can be treated as method-independent values (Table 4). Quotient values approach the quality of reference values, usually obtained only with reference methods. For judging the accuracy of single serum proteins in CSF, referring to the accuracy of CSF/serum quotients appears to be the best approach in CSF surveys. However, this is valid only if paired CSF and serum samples from the same patient are analyzed with the same method in the same analytical run, and are referred to the same calibration curve.

As shown in the results, this is one of the most prominent faults in the surveys, leading to larger CVs for the quotients compared with the CVs of the absolute values in CSF or serum of the single protein.

Target Value, Assigned Value, Consensus Value

The discussions of the international standardizing organizations led to helpful definitions (see *Appendix*) but left some controversies unresolved. One concerns the hierarchy between the terms "assigned value" and "target value." In this survey the target value ("Zielwert") refers to the best available value for statistical evaluation of the survey. This value can be obtained as a reference value (rarely available) or from an assigned value ("Sollwert"), which is a method-dependent value. Because for CSF diagnosis no reference methods are available, the target value in this case has to refer to assigned values originating from a consensus of few invited expert laboratories (trial) or, less expensively, from a consensus from all participants of the survey, with some restrictions. Consensus values of all participants as target values may be acceptable for albumin and IgG, in particular for the method-independent quotients. For IgA and IgM, however, the situation was completely different: Occasionally, the low number of successful participants (<30), the large variation among results (and nongaussian distribution), and large number of outliers did not allow calculation of participants-based consensus values that were acceptable as target values. For example, in Table 2 the Q_{IgA} consensus value was adversely affected by results from a group of participants who measured CSF and serum in different runs or with different methods. In such cases the consensus value from the three expert laboratories is a better choice for the target value (*1*).

Clinically Oriented Accuracy

For general quality assessment, the certificate of participation (Table 3) documents the "patient-related" true results with greatest priority: reporting the albumin quotient with reference to its age-appropriate value (Table 1) and the detection of intrathecally synthesized immunoglobulin in diagrams (Fig. 1) as a goal of CSF diagnosis. The integration of CSF data into a pattern with disease-related, differential diagnostic relevance has proved to be of great benefit for the neurologist (*4*). The absolute concentrations of a single protein in CSF and serum were of secondary value, used only to determine the source of any analytical faults.

As a training program the survey has contributed to a more general quality assessment involving proficiency testing and plausibility control by comparison of data (e.g., the albumin in CSF must be less than total protein concentration, or a Q_{IgG}/Q_{Alb} ratio in range 5 of Fig. 1 is not allowed). The survey also allows the participants to check the clinical relevance of their interpretation scheme (quotient diagrams, formulas, index values, etc.): The discrepancies between linear approaches (*25, 26*) and nonlinear discrimination curves and formulas (*3, 8, 13–16*), for example, are obvious in the interpretation of IgA and IgM quotients, particularly in cases of blood–CSF barrier dysfunction (increased Q_{Alb}). Recently, the hyperbolic function (*8, 9*) in Fig. 1 has been shown to be the physiologically and physically correct form (*16*) of the discrimination line between a blood-derived and a brain-derived protein fraction in CSF.

These IgG, IgA, and IgM diagrams in Fig. 1 gained an increasing acceptance, being routinely used for CSF data reports in ~80 German or other European neurological clinics. Several firms (Behring, Beckman) developed software for on-line or off-line evaluation of CSF protein data in this quotient diagram, integrated in a form, including cytology and other information, relevant for CSF diagnosis.

External quality assessment systems cannot substitute for internal quality control for actual reliability of an analytical series.

The quality-control samples from commercial suppliers often have several deficiencies: Sometimes the albumin concentration is given only as its electrophoretic fraction (percentage of total protein); IgA and IgM concentrations <10 mg/L are not available; or the samples contain unsuitably high amounts of IgG, IgA, and IgM. A suitable normal CSF control should contain 0.5–1.5 mg/L IgM and 1.0–3.0 mg/L IgA. Representative sets of data for normal and pathological CSF protein data are reported in Table 2 and in the legend of Fig. 2. CSF collected postmortem is not suitable as a normal control, because the large protein concentrations accumulated after the stop of CSF flow exceed even usual pathological values. Disadvantages of contrived CSF control samples include the lack of CSF-specific proteins and a small concentration difference between total protein concentration and albumin concentration, with albumin in some cases accounting for 90% instead 30–60% of total protein. Moreover, samples with oligoclonal IgG fractions are not distributed commercially at all. Therefore, for internal quality control a pool of CSF samples stored as frozen aliquots remains a good and inexpensive approach.

For a complete internal quality-control system, one could use a commercially distributed control serum and a homemade CSF pool, analyzing both daily paired in the same run with the same method. The accuracy and precision of the (diluted) serum would be controlled by the assigned value of the commercial control sample. The accuracy and precision of the CSF values should be controlled by determination of the method-independent CSF/serum quotient, calculated from both the control samples.

In conclusion, accreditation bodies judging a single laboratory for CSF analyses should refer primarily to the accuracy and imprecision of these CSF/serum quotients. Standardization committees and accreditation bodies that require arguments for quality assessment in clinical neurochemistry can now consider quotient values as method-independent values and introduce priority to medical relevance and judgement of physiologically based patterns of data as part of the quality-assurance program.

As a consequence of this CSF survey as a training program, the performance of many nonspecialized laboratories improved dramatically but still has not reached that of the expert laboratories. Clinical chemists not trained in clinical neurochemistry became more aware of the specific methodological aspects in CSF analysis and changed their methods accordingly. Manufacturers as suppliers of reagents and apparatus responded by developing more suitable methods.

Appendix: Definitions from Harmonized Proficiency Testing Protocol (*1*)

Internal quality control. The set of procedures undertaken by the laboratory staff for continual monitoring of operations and results to decide whether the results are reliable enough to be released; internal quality control primarily monitors the batchwise accuracy of results for quality-control materials and the precision of replicate analysis of test materials.

External quality assessment by survey or trial (proficiency testing). The system for objectively checking laboratory results by an external agency. This includes comparison of a laboratory's results at intervals with those of other laboratories, the main object being the establishment of trueness. Proficiency testing is designed to assess accuracy.

Survey. Procedure in which aliquots of a specimen, as nearly identical as possible, are sent to participating laboratories where they are investigated or processed, and the results are returned to the EQA organizer. The survey is open to anyone but needs a sufficient number of participants for the conclusion to be statistically valid.

Trial. Interlaboratory test comparison by a limited number of invited participants who accept and apply the protocol of the organizer.

Quality assessment program/system. The sum total of a laboratory's activities aimed at achieving the required standard of analysis. Although internal quality control and proficiency testing are very important components, a quality-assurance program must also include staff training, administrative procedures, management structure, etc. Accreditation bodies judge laboratories on the basis of their quality-assurance program.

True value. The actual value of the analyte in the matrix.

Assigned value. The value to be used as the "true" value by the proficiency test coordinator in the statistical treatment of results; it is a practical estimate of the true value of the analyte in the matrix. [This definition is not generally accepted; some use "target value" as the term for the best value for evaluation of the survey, a definition used here too (see definition below and the text). In such cases "assigned value" is understood to be any method-dependent value.]

Target value. Result that is achieved to guide the assessment of the results in EQA. At least 30 stochastically independent results (after elimination of outliers) are necessary. [Alternatively—and the definition used in this paper—the value used for evaluation of the survey, obtained either by Reference Methods, by trial for method-dependent assigned values, or as a consensus of all participants.]

Consensus value from referee/expert laboratories. A value produced by a group of expert or referee laboratories using the best possible methods (trial). This is probably the best procedure for determining the true values for representative materials under practical circumstances. There are obvious reasons for using

such a value if it is available. There are also arguments against using it, namely: (*a*) it would be expensive to execute, and (*b*) there might be lingering doubts about the validity of the consensus value, especially among the participants.

Consensus value from all participants. Usually, estimated mean of the observations remaining after outliers have been detected and eliminated, but other possible estimators include the robust mean and the modal value. The consensus of participants is clearly the least expensive estimator to obtain. Objections against such a value include: (*a*) there may not be a real consensus among the participants, and (*b*) the consensus may be biased because of the general use of faulty methodology.

Samples. The samples to be distributed in the scheme must be generally similar in type (matrix) to the unknown samples that are routinely analyzed in the laboratory with respect to composition of the matrix and the concentration range or loading of the analyte. It is essential that the samples are of acceptable homogeneity and stability. [Note: For CSF surveys a small sample volume is another condition for providing the most realistic simulation of a patient's sample.]

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