Quality Assurance for Cerebrospinal Fluid Protein Analysis: International Consensus by an Internet-Based Group Discussion

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A group of neurologists and clinical neurochemists representing twelve countries worked towards a consensus on laboratory techniques to improve the quality of analysis and interpretation of cerebrospinal fluid (CSF) proteins. Consensus was approached *via* a virtual Lotus Notes-based TeamRoom. This new approach respecting multicultural differences, common views, and minority opinions, is available in http://www.teamspace.net/ CSF, presenting the implicit, complementary version of this explicit, printed consensus. Three key recommendations were made: CSF and (appropriately diluted) serum samples should be analyzed together in one analytical run, *i.e.,* with reference to the same calibration curve. Results are evaluated as CSF/serum quotients, taking into account the non-linear, hyperbolic relation between immunoglobulin (Ig)- and albumin-quotients rather than using the linear IgG index or IgG synthesis rate. Controls should include materials with values within the reference ranges (IgM: 0.5–1.5 mg/l; IgA: 1–3 mg/l; IgG: 10–30 mg/l and albumin: 100–300 mg/l).

The physiological, methodological and clinical significance of CSF/serum quotients is reviewed. We confirmed the previous consensus on oligoclonal IgG, in particular the usefulness of the five typical interpretation patterns. The group compared current external and internal quality assurance schemes and encouraged all members to maintain national or local traditions. Values for acceptable imprecision in the CSF quality assurance are proposed. Clin Chem Lab Med 2003; 41(41):331–337

Introduction

Use of virtual web technology in reaching a consensus on best practice

Consensus documents used to be produced by a selection of international experts who attended a series of meetings from which a document would emerge after a number of iterations (1). We used the current technology of internet-based discussions designated as TeamRoom (2), which is a Lotus Notes-based group-ware tool (Teamspace Solutions, London). The tool helps groups to move beyond simple information-sharing and co-ordination of work into genuinely collaborative activity, which facilitates the self-organizing, non-hierarchical process of interaction. As the work builds up, members can link their current contribution to both archived and recent work using document links (2), so that many complex paths of thinking can emerge. As a result, the picture forming in the virtual TeamRoom space represents a new kind of consensus, differing from a conventional written report by allowing a more complex and potentially richer representation of work,

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so that common views and minority perspectives are both accommodated (2). Direct access to our work is made available *via* the web (www.teamspace.net/CSF). This approach to improving and evolving diverse methodologies is more suitable for a global, multicultural environment than the singular view of "best" practice produced by the more traditional process of group discussions or formal workshops (2). This printed paper, therefore, as an explicit form of the consensus should be used in combination with the more complex, implicit consensus discussion on the website.

Quality assurance

National diversity of quality assurance in clinical chemistry

Depending on the health care systems in individual countries, different rules for quality assurance are required to fulfil legal preconditions for running a clinical chemistry laboratory or to get analytical costs refunded by the health insurance companies. The rules for internal and external quality assurance are intensively discussed by organizations for international standardization whose aim is to harmonize different approaches to quality control (QC) in clinical chemistry (for the various ISO rules see nomenclature in the TeamRoom website, and reference (3)).

In this paper, we use the term "quality assurance" in its general sense, implementing evaluation concepts together with QC for accuracy and precision of a single analyte (3).

Quality assurance in the cerebrospinal fluid (CSF) laboratory

This contribution reports on the practice of quality assurance in CSF analysis in different countries in order to develop guidelines for external and internal quality assurance in CSF analysis. An earlier publication (4) on external quality assurance for CSF analysis (CSF survey) showed how to take advantage of a methodological and clinical plausibility control for general quality assurance in CSF analysis. In particular, a CSF data report (5, 6) including quantitative CSF/serum quotients for albumin, immunoglobulin (Ig)G, IgA, and IgM, can help identify patterns typical for some neurological diseases (7).

The previously published International Consensus on CSF analysis in multiple sclerosis, including agreement on the five isoelectric focusing patterns (1), is now the basis for quality assurance used in the UK (NEQAS) and Germany (Ringversuch (4)), as well as other countries, *e.g.*, The Netherlands (8).

In the virtual TeamRoom we present information about analytical methods and interpretation programs used in the 12 countries and discuss the individual experiences of several laboratories with regard to both internal and external quality assurance.

The CSF Consensus Group

As a voluntary group of biochemists, clinical chemists and neurologists involved in clinical neurochemistry, we have worked together for 4 years. The authors of this contribution originate from Belgium, Brazil, Czech Republic, France, Germany, Hungary, Italy, Portugal, Sweden, The Netherlands, the United Kingdom, and the USA. Most of the participants are members of the CSF research group of the World Federation of Neurology. The aim of this CSF consensus group is to help ensure that patients all over the world will ultimately benefit from improved CSF analysis to aid the diagnosis of neurological diseases. However, we do not advocate a uniform methodology to replace approved local traditions. In this publication, we summarize our discussions in TeamRoom about quality assurance and point to the more implicit consensus including minority opinions, which is accessible on the website of the group (http://www.teamspace.net/CSF), in particular on the Consensus Topics page, "Topic summary for QC" and "Analytical programs and methods from participating laboratories". Further topics in the discussion, relevant to this paper, are "Topic 2: proteins" and "Topic 4: focusing".

Results

Spectrum of analytes in CSF

The analytes used and the corresponding methods performed in 12 representative CSF laboratories in different countries were collected as a reference for analytical requests (website: Consensus Topics page, "Analytical programs and methods from participating laboratories"). Most frequently investigated are total cell count, differential cell count, total protein, albumin, and IgG together with oligoclonal bands. IgA and IgM are analyzed to complete the immunoglobulin response pattern. In this part of the website, three additional topics were considered: brain-derived proteins, specific antibodies, and the use of polymerase chain reaction (PCR) to identify infectious agents.

Analytical methods

With respect to quality assurance we will only refer to the basic analytes: total protein in CSF, albumin, IgG, IgA, IgM, and oligoclonal IgG in CSF and serum.

Analysis of albumin, IgG, IgA, and IgM in CSF and serum

The group's consensus is that CSF and appropriately diluted serum samples should be analyzed in the same analytical run, *i.e.*, with reference to the same standard curve, to obtain method-independent high sensitivity and specificity in the CSF/serum quotients.

If CSF and blood samples of the same patient are analyzed in this way, *i.e.*, with reference to the same standard curve, the error in the quotient of both values is smaller than in a quotient obtained from values determined independently in different assays with separate calibrations. The additional dilution of the serum sample (in the nephelometer) has less of an impact on the

Table 1 Method-dependent imprecision of absolute concentrations *vs.* CSF/serum quotients.

	CSF (mg/l)	Deviation*** (%)	Serum (g/l)	Deviation (%)	Q (× 10³)	Deviation (%)
Target value	2.1	---	0.70	---	3.0	---
Laboratory 1*	2.5	+19	0.56	−20	4.5	+50
Laboratory 2**	2.4	+14	0.82	+17	2.9	−3

IgM data from a CSF survey with target values and results from two different laboratories. * Laboratory 1 analyzed CSF in the particle-amplified nephelometric assay and serum in a usual immunochemical assay, *i.e.*, with reference to two independent calibrations. ** Laboratory 2 analyzed CSF and (appropriately diluted) serum paired in the same analytical run in the particle-amplified nephelometric assay. *** % deviation from target value.

quality than has the reference to two different standard curves. Examples from a CSF survey with given target values are shown in Table 1. The performance of two different laboratories was compared. Laboratory 1 analyzed CSF IgM in a particle-amplified nephelometric assay and serum IgM in a standard immunochemical nephelometric assay: the CSF value was 19% above the target values for CSF, and the serum value was 20% below the target value for serum. The corresponding quotient therefore shows 50% error. This is a classical example of error propagation. The deviation of each single value in CSF and serum would have been acceptable in the CSF survey, but the quotient would not reach the accuracy required for certification and could have lead to a false-positive interpretation of intrathecal IgM synthesis.

Laboratory 2 (see Table 1) analyzed paired CSF and appropriately diluted serum together in one analytical run in the particle-amplified assay. The deviation of +14% in CSF is similar to the accuracy of Laboratory 1. However, with a deviation of +17% in serum (*vs.* −20% in Laboratory 1) the CSF/serum quotient shows only 3% deviation from the target value. This synchronous deviation of CSF and serum values from the target value, resulting in a correct quotient, is a typical advantage of the reference to the same calibration curve. This method- (calibrator-) independent quality of quotients has also been documented in the earlier report (4). The accuracy of quotients, comparable between different laboratories, can be guaranteed if the reliability of standard curves is tested to give the same result over a range of high as well as low concentrations (this is easily done by a serial dilution of a serum sample down to the range of the suitable standard curve for CSF analysis). Only under these conditions can the CSF/serum protein quotients be method-independent values.

The spectrum of different methods for quantitative protein analysis (nephelometry, turbidimetry, ELISA, rocket electrophoresis, and radial immunodiffusion) has been discussed by the group with regard to analytical sensitivity, costs, and precision (see website "Topic 2: proteins").

Table 2 presents an evidence-based proposal for acceptable deviations from target values in CSF surveys. The single CSF survey reports a target value and the

Table 2 Average performance from 10 surveys of CSF protein analysis (International CSF survey, INSTAND, Germany (1999–2002)).

Analyte		Mean imprecision[1] (%)	Outliers[2] (%)	Max. deviation[3] (%)
Total protein		11.0 ± 1.1	11.0	30
Albumin	CSF	7.3 ± 0.5	3.6	24
	Ser	6.6 ± 0.5	2.1	24
IgG	CSF	8.6 ± 1.5	4.5	30
	Ser	6.7 ± 0.7	2.5	30
IgA	CSF	11.3 ± 1.6	2.6	45
	Ser	9.9 ± 0.6	1.8	45
IgM	CSF	13.4 ± 1.0	5.7	45
	Ser	10.1 ± 0.9	3.3	45
Q_{Alb}	CSF	8.0 ± 0.8	3.1	30
Q_{IgG}	CSF	8.2 ± 0.9	3.7	30
Q_{IgA}	CSF	12.1 ± 1.0	4.5	30
Q_{IgM}	CSF	11.3 ± 1.1	9.8	30

[1] Mean ± SD of the deviations (CV) from consensus values in the group of participants after elimination of outliers. Outliers are defined by values > ± 30% deviation from target values (> ± 45% for IgA and IgM, respectively). [2] Mean fraction of outliers in n = 10 surveys, as % of the total group of participants (approx. n = 200). [3] Maximal deviation characterizes the acceptable maximal deviation of the single participant's value from target value in the CSF survey. This is an experience-based proposal according to the rules of the German Bundesärztekammer by which maximal deviation of the single value is 3 times the CV of the whole group.

consensus value of the participants (after elimination of outliers) together with the variation of the participants' data around the consensus value (CV in %). On the basis of the performance of 10 CSF surveys from INSTAND (4) with about 200 participants each, we calculated the mean of these deviations (CV) in the surveys and the corresponding SD (Table 2). These data support the above statement that quotients have a higher precision (and sensitivity) than the absolute protein concentration: the mean CV of IgM quotients with 11.3% is smaller than the expected value (23.5%) derived from two independent absolute values with error propagation (13.4 + 10.1 = 23.5).

Control samples for total protein, albumin and immunoglobulins in CSF

QC samples for CSF from commercial suppliers often have serious drawbacks: the albumin concentration may be given only as its electrophoretic fraction (percentage of total protein); IgA and IgM samples are not commercially available, or the samples may contain unsuitably high amounts of IgG, IgA, and IgM. Moreover, control samples with oligoclonal IgG fractions are not commercially available. Disadvantages of artificial CSF control samples include the lack of CSF-specific proteins and a significant difference between total protein concentration and albumin concentration, with albumin in some cases accounting for 90% instead of 40–80% of total protein. A suitable normal CSF control should contain IgM (0.5–1.5 mg/l); IgA (1.0–3.0 mg/l); IgG (10–30 mg/l), and albumin (100–300 mg/l) (4).

Until CSF proteins are commercially available for internal QC, a pool of CSF samples stored as frozen aliquots remains a good and inexpensive approach. For internal QC of total protein in CSF, a 1:200 diluted certified serum control sample is sufficient. To ensure analytical accuracy for albumin, IgG, IgA and IgM, paired analysis of a CSF pool with a certified commercial serum control sample is preferred, provided both samples are analyzed in the same analytical run. The day-to-day precision of the CSF values should be controlled primarily by determination of the method-independent CSF/serum quotient, calculated from both CSF and serum control samples. Several group members indicate, *via* links on the website, the control samples which they use for internal QC (website: Consensus Topics page: "Topic summary for quality control").

Interpretation of quantitative protein data in CSF

Blood-CSF barrier dysfunction

The preferred method for the measurement of blood-CSF barrier dysfunction is the analysis of the albumin quotient (6) and its evaluation with regard to the age-related reference range (5, 7). The analysis of total protein is still frequently used for evaluation of barrier dysfunction because it does offer some clinical value, in spite of its broader reference range, *i.e.*, lower sensitivity, and specificity than the albumin quotient. A comparison between the biological variation of total protein and albumin quotients is available for the European population (7). Due to systemic inflammatory diseases with high total protein concentrations in blood, high total protein concentrations in CSF are subsequently observed. This could lead to a false-positive interpretation but could be avoided by using the albumin quotient.

Interpretation of intrathecal immunoglobulin synthesis

We regard the determination of CSF/serum quotients as a suitable evaluation and interpretation method for the detection of quantitative intrathecal IgG, IgA or IgM synthesis. As reviewed recently (5, 7), the frequently used IgG Index or IgG Synthesis Rate give up to 90% false-positive values for high albumin quotients (blood-CSF barrier dysfunction) compared to the "gold standard", which is the detection of oligoclonal bands on isoelectric focusing (IEF). Regarding quantitative analyses of intrathecal immunoglobulin synthesis, the group agreed to refer to the hyperbolic discrimination line, expressed in either a numerical or a graphical format (5, 7). This is often facilitated by the use of PC-based software for evaluation of CSF data profiles (5). Knowledge-based interpretation programs support routine CSF analysis (5). The case reports and discussions of neurological diseases in the CSF Consensus Group also refer to this method of interpretation.

Oligoclonal IgG – an update

The recommendations of the first consensus report on oligoclonal IgG as the "gold standard" for detection of intrathecal IgG synthesis (1) are confirmed by the practical experience of the group (website, Consensus Topics page, "Topic 4: focusing"). The recommended method is IEF followed by immunodetection of IgG. CSF and serum samples with similar amounts of IgG (as dictated by limits of the dynamic ranges for each method) should be run in parallel. The interpretation should be evaluated according to the five typical patterns (example in (6)) used to distinguish local from systemic synthesis of oligoclonal IgG: *type 1* = normal, *i.e.*, no oligoclonal band (OCB) in CSF and serum; *type 2* = intrathecal IgG, *i.e.*, OCB in CSF but not in serum; *type 3* = intrathecal IgG and systemic oligoclonal IgG, *i.e.*, some OCB in CSF only and some additional matched bands in CSF and serum; *type 4* = no intrathecal IgG synthesis but a systemic inflammatory process, *i.e.* identical OCB in CSF and serum; *type 5* = paraproteinemia, *i.e.* identical patterns of monoclonal bands in CSF and serum.

Internal QC for oligoclonal IgG

QC of oligoclonal IgG requires running of several samples (including positive and, especially, negative controls) on a single gel to discriminate between genuine OCB and the background pattern due to discontinuous pH gradients. Positive and negative controls for oligoclonal IgG are available from the laboratories' previous samples. By definition, a sample pool is not possible.

External QC for oligoclonal IgG

Several techniques are in use for the detection of oligoclonal IgG bands in CSF and serum (1). The performance of 180 laboratories using these different experimental approaches has been systematically analyzed in six sequential CSF surveys, shown in Table 3. There was no significant difference between Silver staining and immunodetection in the assessment of sensitivity, shown in the case of three weak oligoclonal bands in CSF (survey 10/99 in Table 3). A somewhat different experience is reported in a recent publication (8) on the results of the Dutch QC study on OCB , which strongly

Table 3   Performance in the CSF surveys for detection of oligoclonal IgG (INSTAND, Germany).[1]

Survey	Expected result type	Correct interpretation			Main faults	
		Total	Silver stain[2]	Immuno-detection[2]	Type	Frequency
10/00	1 = Normal	120/141	73/84	39/47	4	9%
5/00	2 = OCB in CSF[3]	138/146	92/96	42/44		
10/99	2 = Weak OCB[4]	58/119	27/63	18/46	1	50%
5/99	5 = Paraprotein	48/130	28/76	18/47	1 and 4	50%
10/98	2 = OCB in CSF	95/114	64/77	28/35	3	8%
5/98	1 = Normal	70/97	53/67	16/23	4/2	14%/8%

[1] Only data from participants using IEF are evaluated in this Table. The small number of participants using electrophoresis or electrophoresis with immunodetection failed frequently, due to low sensitivity or false-positive reports. The evaluation refers to the reported interpretation, which in some cases (*e.g.*, survey 5/99) may be biased more by a lack of experience in interpretation than by technical performance (*e.g.*, in survey 10/99). [2] IEF on macrogels and on microgels is evaluated together. [3] OCB = oligoclonal bands. [4] 2–3 faint bands in CSF.

suggests that IEF in combination with immunoblotting is superior to any other combination of techniques However, both surveys (4, 8) strongly support the group's consensus that IEF is superior in performance to other electrophoretic techniques (including electrophoresis combined with immunodetection). The evaluation of surveys in Table 3 shows that the performance of many laboratories may be biased more by lack of experience than by technical performance, as demonstrated by many of the incorrect interpretations of a type 5 pattern. The type 1 pattern is frequently misinterpreted as type 4 due to rough ampholine gradients (surveys 10/00 or 5/98 in Table 3).

External quality assurance – national CSF surveys

TeamRoom facilitates the examination of the reports of different CSF surveys from various countries (website, Consensus Topics page, "Topic summary for quality control"). The group consensus favors national CSF surveys, which use the native language and thus take account of local differences.

Germany: The CSF survey distributed by INSTAND (Institut für Standardisierung und Dokumentation im Medizinischen Laboratorium e.V.) for about 300 German CSF laboratories and 20 European and American laboratories has been described (4). Meanwhile the extended survey program involves QC of specific antibody synthesis (Antibody Index, (5, 7)), lactate, and glucose. In this approach, CSF/serum quotients are given priority over a single absolute value in CSF and serum. The INSTAND survey asks for interpretation of the barrier function relevant to the age of a patient and indication of IgG, IgA, and IgM intrathecal synthesis by reference to the hyperbolic discrimination lines in quotient diagrams (4). Tables 1–3 are based on the performance of these CSF surveys. Wormek's program (www.wormek.de) facilitates the evaluation of CSF surveys. These concepts of general quality assurance are expressed in the proposal of the "German Society of CSF analysis and Clinical Neurochemistry" (www.dgln.de).

In the United Kingdom QC for oligoclonal IgG in CSF is organized by the NEQAS (National External Quality Assurance Scheme) which covers 200 UK and 30 European and other laboratories (www.ukneqas.org.uk). Paired normal and pathological CSF and sera are distributed (IgG concentrations are given for each), and the result is reported according to one of the five types of IEF patterns (1). A more recent scheme distributes only artificial CSF for the surveys of total protein, albumin, IgG, glucose, lactate, and heme pigments.

The CSF survey in The Netherlands (8) is distributed by the Stichting Kwaliteitsbewaking Ziekenhuis Laboratoria (section on multi-component analysis) to 54 laboratories. Twice a year two pairs of CSF and serum are analyzed for total protein, albumin, and IgG. Interpretation of CSF total protein and the CSF/serum albumin quotient is requested, referring to the age of the patient. Interpretation of intrathecal IgG synthesis according to an empirical discrimination line is also requested. Furthermore, two samples are analyzed for oligoclonal IgG and reported according to one of the five types of isoelectric focusing patterns.

Laboratories in Belgium do not yet have their own scheme but participate in programs organized in Germany or The Netherlands.

Portugal does not yet have a national CSF survey, so to obtain certification and accreditation for total protein, albumin, and IgG, and most public or private laboratories apply for QC surveys developed by other countries.

In Italy, quality control is performed in both public and private laboratories. Rules for performing QC are devolved by central government to individual regions, as is the organization of programs for external evaluation. Two Italian laboratories may therefore have totally different rules and programs. For example, in the Lombardia region many analytes are under the control of an external evaluation program but none is specifically designed for CSF analysis.

The United States currently has a rather restricted service for relevant external quality control for CSF

analysis. The CSF survey of the College of American Pathologists (www.cap.org) offers only CSF without paired sera and uses albumin values derived from CSF electrophoresis.

In Hungary, 15 laboratories participate in a national QC survey for CSF analysis. The procedure described by INSTAND in Germany has been translated into Hungarian.

Brazil does not yet have an external QC survey for CSF analysis.

We believe that accreditation bodies judging CSF analyses in a single laboratory should refer both to the accuracy and precision of the CSF/serum quotients and to the detection of oligoclonal IgG bands. The protein quotient can now be considered method-independent, thus introducing clinical relevance and recognition of physiologically based patterns of data as part of the quality-assurance program. In particular, accreditation bodies should consider that diluted serum is analyzed in the CSF assay, *i.e.*, if quality control of a serum sample is run in a second serum assay, this is not strictly an appropriate comparison.

Discussion

Preferential interpretation of CSF/serum quotients of proteins in CSF

There is still widespread hesitation by clinical chemists and, in particular, by the accreditation bodies, to give the priority for CSF/serum quotients over absolute values. But CSF analysis offers three particular advantages which are found as an almost unique precondition in CSF analysis.

Physiology of blood-CSF barrier function

The CSF concentration of a blood-derived protein depends, among other things, on that protein's concentration in blood, *i.e.*, an increasing concentration of IgM in blood would result in an increasing IgM concentration in CSF, but the IgM CSF/serum quotient would remain constant in the absence of blood-CSF barrier dysfunction and the absence of intrathecal IgM synthesis. There is therefore a good biological reason to refer these values to each other. In fact, these quotients are normalized (relative) CSF concentrations *i.e.*, dimension-less values between 0 and 1.0, unbiased by the variations in blood concentration of the protein involved.

Clinical significance of CSF/serum quotients

The detection of intrathecal synthesis of immunoglobulins (*e.g.*, IgM) has to take into account the dynamic changes of blood concentration and blood-CSF barrier dysfunction. In the case of neuroborreliosis (7), the extent of intrathecal IgG and IgM synthesis between the 1st and 13th week after admission to hospital with neurological symptoms remained constant. Meanwhile, in blood the classical initial increase in the IgM class synthesis was followed by a decrease (IgM = 3.8 g/l to 1.6 g/l), concomitant with a switch to an increasing concentration of the IgG class (Figure 3 in (7)). Only the use of quotients and their interpretation based on the hyperbolic reference range will allow such interpretation of CSF data on the neuroimmunological reaction, unbiased by the dynamics of the immune reaction in the blood and the changing blood CSF barrier function. It is the only methodology which guarantees the necessary accuracy of such an investigation over many months.

Perspectives for an improvement of general quality assurance in the CSF laboratory have been described in an integrated report (5–7), where all CSF data of a patient are reported together in one file (a corresponding discussion is also displayed on the website, Consensus Topics page, "Topic 1: reporting"). This type of disease-related data pattern represents a plausibility control on a methodological level (5, 7) as well as on the basis of medical evidence (7). A knowledge-based evaluation program (5) can also help to maintain standards in the CSF laboratory.

Acknowledgements

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