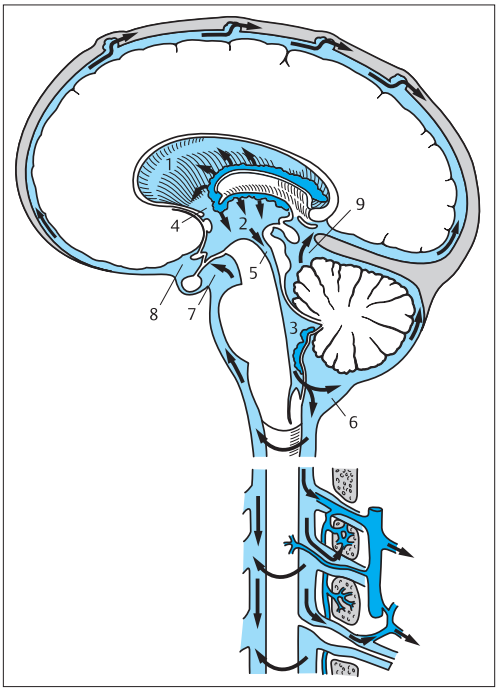
Cerebrospinal fluid

Laboratory Analysis, Evaluation and Interpretation



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Edition 2020

Preface

Dear Colleagues and Friends
This collection of practical helps in the daily laboratory practice of a CSF laboratory, collected over the last 40 years, would not have been possible without the basic support by some incredibly engaged colleagues and, meanwhile, dear friends.
Just to mention those which accompanied this work the longest time up to now. Peter Lange (Neurochemistry Laboratory Göttingen), Arno Wormek (CSF software, www.wormek.com), Werner Albaum (CSF software, www.albaum.it).
I have to appreciate the work of, a nearly distinct species in Medicine, the natural scientists Dr Dr Manfred Uhr (Göttingen, München), Dr Ernst Linke (Jena), Dr. Klaus Zimmermann (Dresden), Dr.Manfred Wick (München) and Dipl.-Chem. Helgard Möller (Detmold).
The international cooperation with Dr. Christian Sindic (Brussels, Belgium), Dr WW Tourtellotte (Los Angeles, USA), Dr Ed Thompson (London), Dr. Alberto Dorta (Havanna, Cuba), Dr. Jose Livramento (Sao Paulo, Brasil) helped to open the view for the different CSF analysis cultures and to develop and apply methods for the diagnosis of tropical diseases.
Thank you all for the obvious joy in a cooperative common work.
I think, for the sake of many neurological and psychiatric patients, the individual engagement was, and still is, worth it.

Hansotto Reiber
(Nov. 2019, Göttingen)

Content

Tables and Figures

CSF Software

The development of CSF software had/has four fields of applications.
Initially the creation of the Reibergrams and the on-line data interpretation in a cumulative CSF data report (A Wormek and W Albaum/Comed with Prospec /Dade-Behring).
The second phase was the creation of patient data systems either as a stand alone solution or as a solution with an interface to the general laboratory data systems, or for the data exchange with the hospital information system (www.wormek.com and www.albaum.it)
A.Wormek made for 30 years the CSF survey of INSTAND possible. W. Albaum created a statistics and graphics program for IgG, IgA, IgM and recently for free light chains. The recent development of a CSF App of Albaum/Reiber with INSTAND is a tutorial and calculator for the interpretation of patient data in the Android system of smartphones and Tablets. These programs are for free downloads from www.albaum.it.

Cerebrospinal fluid Analysis

Knowledge Base

1. Physiology and pathophysiology of CSF

The biophysical approach to describe the blood brain and blood CSF barriers as a molecular diffusion/ CSF flow interface got new support. In particular from considerations about the concentration gradients and disease-related dynamics of blood-derived, brain-derived and leptomeningeal proteins in CSF.
There are no leaks in the barriers it is just the reduced CSF flow rate which leads to increased concentrations of blood derived proteins in CSF:
1. Blood derived proteins form a nonlinearly increasing rostro-caudal concentration gradient, brain-derived proteins are decreasing linearly and leptomeningeal proteins increase linearly between normal ventricular and lumbar CSF.
2.In barrier dysfunctions the molecular selectivity and the constant interindividual variation for blood protein concentrations in CSF contradict a morphological change. Lumbar concentrations of brain proteins are invariant but leptomeningeal protein concentrations are increasing with increasing CSF flow rate. These facts are explained as reduced CSF flow rate with rostro-caudal direction.
3. In fetal development of humans early CSF production and mature barriers create 40 fold higher albumin concentrations in CSF, decreasing only with the later maturating arachnoid villi. Together with the rostro caudal flow direction this contradicts outflow into the lymph sytem in humans.
4.Fast respiration- and heart beat- related oscillations neither perturb the nonlinear rostro-caudal concentration gradient nor the net flow direction.
5. The pathophysiology of neurological inflammations, meningeosis, mechanical spinal stenosis or outflow restriction in Guillain-Barré radiculitis gives evidence for a common cause of barrier dysfunction: restricted CSF turnover rate.

Biophysics of the barrier functions

Protein dynamics follow the laws of diffusion with nonlinear concentration distribution along the diffusion way. The characterizing mean square displacement is modified by changing CSF concentrations, due to rostrocaudal gradients or reduced CSF flow rate. With Fick's 2nd law of diffusion barriers appear as molecular diffusion /bulk flow interface for CSF or interstitial fluid. The derived hyperbolic reference range is relevant in diagnostic quotient diagrams and barrier research.

Consequences

The focus on the pathologically reduced CSF flow rate instead on barrier structure leakages may change disease reseach in neurology fundamentally.

As an important consequence for a qualified analysis and data interpretation the clinical chemist must be aware of

- Particular connections regarding molecular transfer between blood and CSF
- Relevance of CSF flow rate for protein concentrations in CSF
- Concentration gradients between ventricular and lumbar CSF: increasing concentrations for blood-derived molecules and decreasing concentrations for brain-derived molecules. This has two consequences:
    - Different reference ranges for ventricular, cisternal and lumbar puncture
    - Concentrations vary with increasing volume of extraction

2. Analytical Methods

The low concentrations of molecules in CSF and the necessity to compare CSF and serum samples in the same analytical run are the reasons why new techniques replaced several of the classic methods used in blood analysis:

- Isoelectric focussing replaced serum electrophoresis
- Latest developments consider Free light chain-Kappa analysis in CSF and serum with reference to a hyperbolic reference range, as a possible complementary method to oligoclonal IgG
- Antibody Index with relative antibody concentrations replaced Antibody Titers with Cut off values.
- For Quality assessment the pure numerical analysis is replaced by a more general QA with combined data interpretation.

3. Interpretations

The sensitive discrimination between blood-derived and brain-derived changes of protein concentrations in CSF must refer to nonlinear reference ranges which take into account the dynamics of proteins in CSF

- The linear IgG-, IgA- or IgM –Index must be replaced by the hyperbolic function based, nonlinear reference range.

Actual main References

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It is a particular goal to learn that CSF analysis is never reliable without a concomittant blood analysis of the patient.
A second goal could be to learn about the diagnostik relevance of a complete analysis of all immunoglobulins and a cummulative data report which allows to recognize disease-related data patterns.

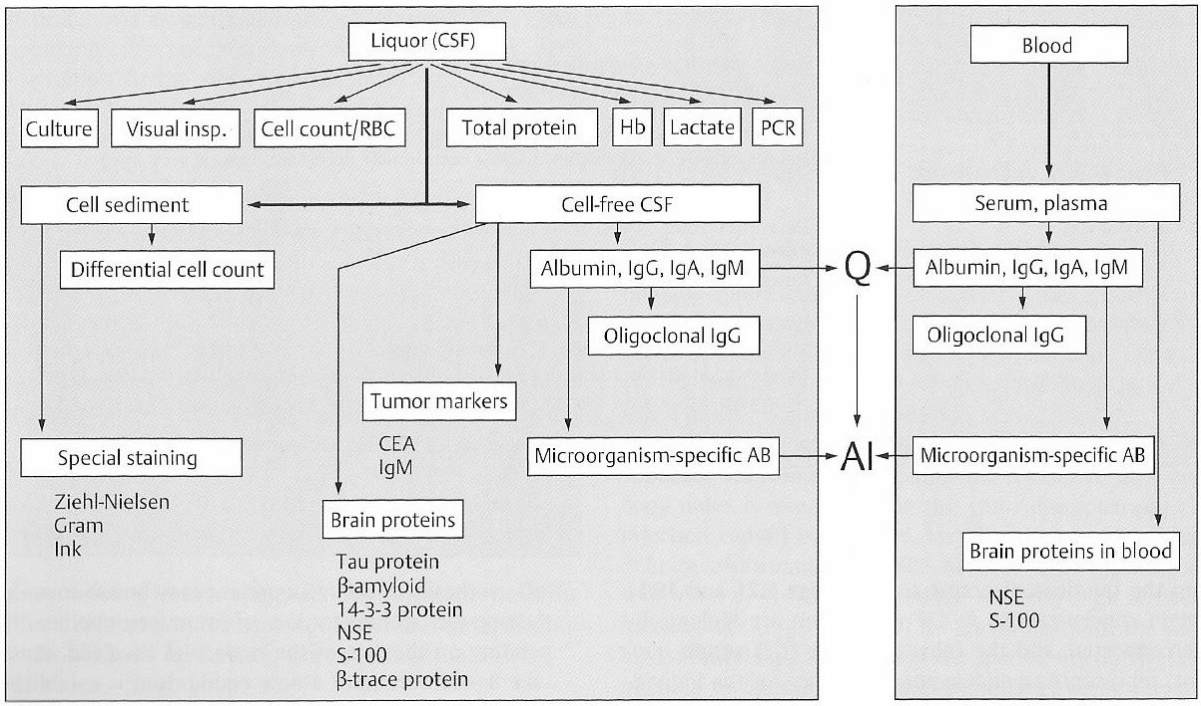
Laboratory organization – Program Levels of CSF analysis.

The physician's specifications regarding the diagnostic question or suspected diagnosis enable the clinical chemist to design a cost-effective flow chart for the analysis (Fig. 1, Table 1). When successively worked through, the flow chart helps make decisions about termination or continuation with a more detailed analysis. Because of the laboratory methods and the costs involved, it is recommended that decisions on further special analysis are only made once the basic program has been carried out. There are essentially three levels, ranging from emergency analysis to a maximum set of particular parameters.

Table 1 Program Levels of CSF Analysis

A. Emergency program	● Cell count ● Total protein (also semiquantitative determination) ● Lactate ● Quick screening test for bacterial pathogens
B. Basic program and more detailed analysis (depending on clinical problem and protein data)	● Cytology (cell count, differential cell count, detection of bacteria); if possible, always make a cell preparation (even when cell count is normal) ● Total protein (quantitative determination) (as reference for dilutions used in subsequent automated protein nephelometric analysis of single proteins) ● Albumin, IgG, IgA, and IgM in CSF and serum ● Qualitative, sensitive determination of oligoclonal IgG in CSF and Serum (if not QIgG >> QAlb) ● Detection of microorganism-specific antibodies ● Lactate in CSF (detect in all barrier dysfunctions with moderately increased cell counts).
C. Special parameters with confirmed clinical relevance	● Specific Antigen detection by PCR (acute inflammation) ● MRZ antibody response (chronic inflammation, MS) ● Tau protein, β-amyloid$_{1-42}$, protein 14-3-3, NSE, or S-100B (dementia, degeneration) ● CEA, IgM (tumors) ● β-Trace protein (CSF fistula, posttraumatic leak) ● Tumor cytology (differentiation of tumors) ● Antineuronal antibodies (neurological symptoms of systemic tumors) ● Ferritin in case of hemorrhage (including HSV-E)

Fig.1 Flow Chart of CSF Analysis



CSF Cytology

The total cell count in CSF and a differential cell count provide still the most relevant information about acute neurological diseases.
CSF Cytology needs long experience and we point to textbooks with brilliant pictures. (Kölmel, Kluge et al).

Cell Counts in CSF

Cell counts in the CSF are normal up to 4 cells/µL. Counts of > 20 cells/µL are interpreted as definite inflammation. The differential cell count is normal when 70–100% lymphocytes and up to 30% monocytes are present. Occasionally, the following cells are also found in normal CSF without having pathognomonic significance:

- Ependymal cells, choroid plexus cells, or cartilage cells
- Very rarely also a mitotic monocyte.

The following cell types in the CSF should always be characterized as an abnormal cytological finding:

- Granulocytes
- Plasma cells
- Macrophages
- Erythrocytes (as long as not artificially introduced by puncture)
- Cells in mitosis, such as tumor cells.
- In bacterial meningitis, pathogenic microorganisms are often directly visible in the CSF (Kölmel, 2005).

Differential cell count

In case of a corresponding differential diagnostic question, like a suggested tumor from all CSF samples still with a cell count < 5 cells/µL a preparation should be performed.

Hemorrhage

The treatment of CSF data from samples with hemorrhage is decribed in the attachment II.

Protein-Analysis

Total protein in CSF

The total protein value is not used for differential diagnosis (except for emergency analysis) The CSF/serum albumin concentration quotient is a much better indicator for blood-CSF barrier dysfunction; it is more sensitive and more specific.

Quantification of total proteins in CSF has mainly two purposes:

Rapid emergency information about the barrier function
Plausibility control of individual protein concentrations (Alb/TP)
Predilutions for subsequent analysis on nephelometer automates.

Detection Methods

A comparison of the quality of different methods is steadily reported in the CSF survey report, download from www.INSTAND-ev.de.

Reference Range

The absolute CSF reference values for proteins are given for orientation in laboratory analysis, Table . The connection between total protein and Q_{Alb} in the CSF is shown in Table

Pitfalls with Total Protein in CSF

The upper limit of the normal protein concentration in the CSF (about 400-500 mg/L) depends on the serum concentration and the intrathecal synthesis. This is the primary reason why it is necessary to use the CSF/serum albumin quotient for the sensitive and specific characterization of a blood-CSF barrier dysfunction. Elderly people or patients with a trypanosmiasis may have very low protein concentrations in blood, but normal quotients for sensitive detection of intrathecal protein fractions.

CSF/serum concentration quotients

With increasing concentration of a serum protein in blood its concentration in CSF is increasing correspondingly. By calculating the CSF/serum concentration quotient of a serum protein, the modulating influence of individual blood concentrations of the protein on its CSF concentration is eliminated. In mathematical terms, this means that a CSF/Serum Quotient represents a normalized CSF concentration. The dimensionless quotient has values between 0 and 1. From practical reasons a normal QAlb = 0,005 is written as QAlb = 5×10^{-3}

Albumin CSF/Serum Quotient, QAlb

Relevance

Albumin is synthesized exclusively outside the brain (namely, in the liver) and is therefore an ideal parameter for characterizing all influences and limitations on the passage of a protein from the blood into the lumbar CSF. The increase of the individual QAlb value is a direct expression of the decreasing individual CSF flow rate.

Albumin quotient is a measure of blood-CSF barrier function.

The CSF/serum albumin quotient (Q_{Alb}), i.e., the quotient of albumin in the CSF, Alb(CSF), to albumin in the serum, Alb(Ser), is generally accepted as a quantitative measure for the blood-CSF barrier function (Andersson et al., 1994; Reiber et al., 2003).

Albumin quotient is a reference for serum proteins in CSF.

By referring the CSF/serum concentration quotients of other serum proteins (Q_{IgG}, Q_{IgA}, Q_{IgM}) to the albumin quotient as a parameter of barrier function (Reiber, 1994), an excellent possibility emerges for determining, independent of the individual barrier function (CSF flow rate), the portion of a brain-derived (intrathecal) protein fraction in addition to a blood-derived protein fraction in the CSF (Fig. 2.**3**).

Reference Range of QAlb

The age-dependent reference ranges of the CSF/serum albumin quotient are presented with a formula for adults and in tabular form for young children (Table 2). The dynamics of albumin quotients in newborn children is also illustrated in Fig.2 The conversion factors for the different reference ranges of the albumin quotient in ventricular and cisternal CSF are reported as well. As the albumin concentration in CSF depends also on the—mostly unrecorded—extraction volume (rostrocaudal concentration gradient), the reference range limits should be interpreted generous (± 10% for average volumes of 6 ± 5 mL).

Age-dependent Reference Ranges of the Albumin Quotient

Because of the physiological conditions (Reiber 1994, Wildemann 2010) the albumin concentration in the CSF is age-dependent. The ranges and limits of upper border values are provided below.

The following equation is valid for adults (including children over 5 years) (Trendelenburg, 1994):

$$\mathbf{Q_{Alb}\ (Ref) < (4 + age\ (y) / 15) \times 10^{-3}}$$

Examples
Up to 15 years of age: $Q_{Alb} = 5 \times 10^{-3}$
Up to 40 years of age: $Q_{Alb} = 6.5 \times 10^{-3}$
Up to 60 years of age: $Q_{Alb} = 8 \times 10^{-3}$.

Tab.2 Reference ranges of the albumin quotient of newborn and young children

Age	birth	1. Mon	2. Mon	3. Mon	4.Mon/5 y
$Q_{Alb} \bullet 10^3$	8 to 28	5 to 15	3 to 10	2 to 5	0,5 to 3,5

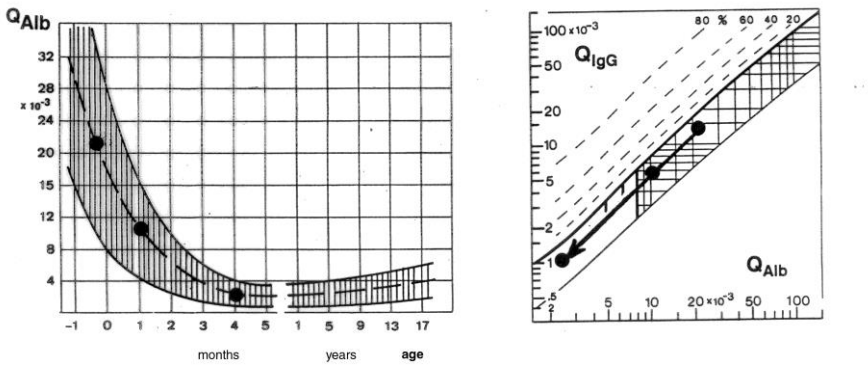


Fig 2 Age-related changes of the albumin quotient (a) and corresponding change of the relation QIgG/QAlb in a hyperbolic function in the IgG quotient diagram.

Albumin quotient in ventricular and cisternal CSF.

Corresponding to the rostro-caudal concentration gradient, the CSF concentration of serum proteins increases from the ventricles (V) to the cisterns (C) and lumbar spaces (L); accordingly, the age-related reference ranges for the albumin quotient change as well. The reference ranges for ventricular and cisternal CSF are thus calculated as follows from those valid for lumbar CSF:

V-QAlb = 0.4 × QAlb (ref)

C-QAlb = 0.65 × QAlb (ref)

The Albumin quotient, QAlb, is a reciprocal function of the CSF flow rate.

A blood-CSF barrier dysfunction, i.e. increased QAlb, is exclusively due to reduced CSF flow rate.

Immunoglobulins

Nomenclature of Immunoglobulins, Antibodies, and Isotypes

- The term immunoglobulin (Ig) refers to all antigen-binding molecules; they are either bound to the cell surface (receptors on B lymphocytes) or present as soluble antibodies.
- The term antibody usually refers to soluble immunoglobulins with known specificity that are secreted by plasma cells into body fluids.
- The term isotype refers to the various classes of immunoglobulins (IgG, IgA, IgM, IgD, IgE), which differ in their heavy chains.

Isotype switch.

In the course of an infection, a change from the primary IgM class reaction to the later longer lasting IgG class reaction is induced in the germinal centers of the lymph nodes.

Intrathecal antibodies derive from B cells which immigrate into brain already affinity maturated and isotype specific. (Reiber et al 2015)

The isotype switch is not observed in the brain (or in the CSF)

This is the base of disease-related immunoglobulin pattern in CSF

Quotient Diagrams (Reibergrams)

Hyperbolic Discrimination Functions.

The CSF/serum quotient diagrams for IgG, IgA, und IgM in double-logarithmic presentation with hyperbolic discrimination lines (Fig. 5) -also called Reibergrams- permit discrimination between blood-derived and brain-derived immunoglobulin fractions in the CSF. The diagrams are well suited for lumbar, cisternal, and ventricular CSF of patients of all ages. What is different, however, is the age-dependent reference range for the albumin quotient (above).

Hyperbolic function

The relation between two serumproteins in CSF follow a hyperbolic function derived from the laws of diffusion and the modulation by CSF flow rate: The molecular diffusion/CSF flow model (Reiber 1994). The mathematical function is also shown as diagram (Fig 3). The parameters a,b,c for the different molecules and their reference ranges are given in Table 3

$$Q_{IgG} = a / b \sqrt{(Q_{Alb})^2 + b^2} - c$$

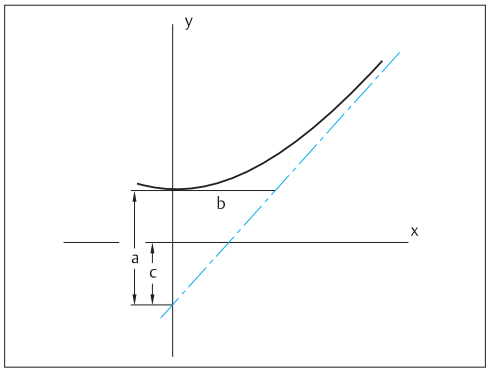


Fig 3 Relevant part of a hyperbolic function with the determinants a,b,c .

Tab. 3 Parameter of the Hyperbolic functions for IgG, IgA, IgM in Quotient diagrams according to Reiber 1994. The hyperbolic curves of the upper limit (Q_{Lim}), the mean value (Q_{Mean}), and the lower limit (Q_{Low}) (Fig.) have been characterized using the values shown for a/b, b^2, and c

IgX		**a/b**	**b^2** (x 10^6)	**c** (x 10^3)
	Lim	0,93	6	1,7
IgG	mean	0,65	8	1,4
	Low	0,33	2,0	0,3
	Lim	0,77	23	3,1
IgA	mean	0,47	27	2,1
	Low	0,17	74	1,3
	Lim	0,67	120	7,1
IgM	mean	0,33	306	5,7
	Low	0,04	442	0,82

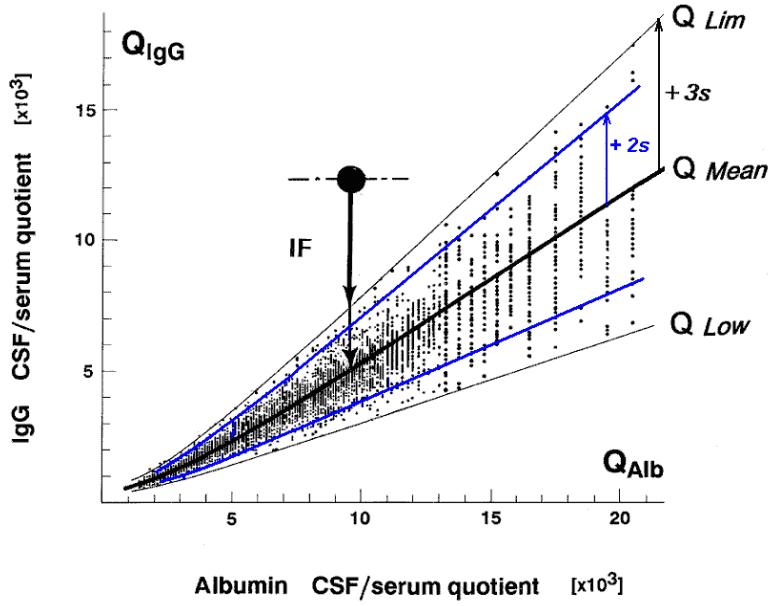
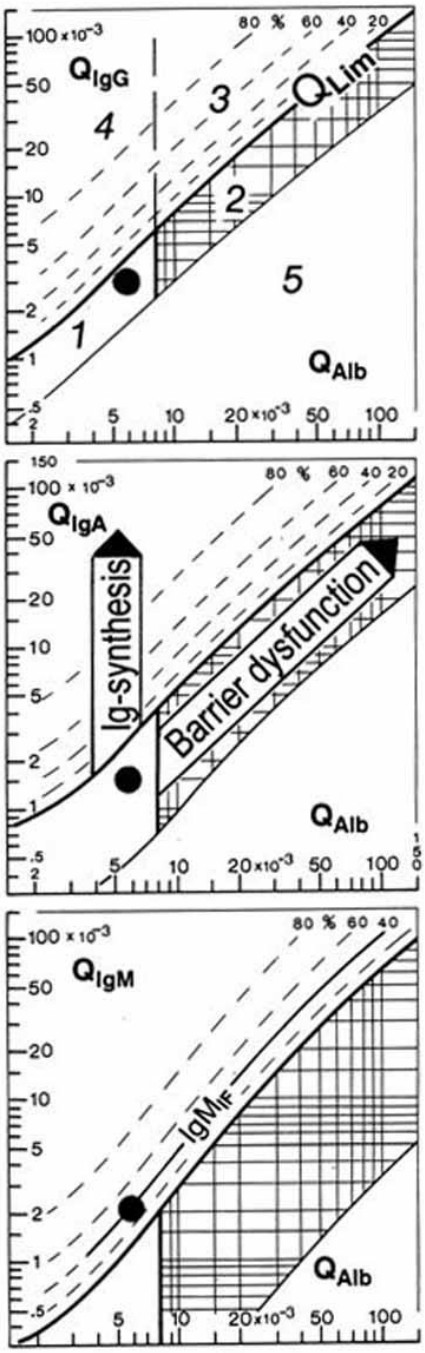


Fig. 4 Characterization of the reference range for blood-derived proteins in CSF. The upper discrimination line QLim = Qmean +3 SD includes 99% of the patients with noninflammatory diseases (oligoclonal IgG negative). This is regarded as the most specific discrimination line for diagnosis. For statistical purpose , comparing groups in diagrams , it is more appropriate to refer to the discrimination line Qmean +2 SD including 96% of the controls, but approaching the number of MS patients positive for oligoclonal IgG.

Construction of quotient diagrams

The so called Reibergrams for IgG, IgA and IgM were fitted from grouped data of 4300 patients with a mean,the upper and the lower border lines of the reference range. The range which involves 99% of all patients without an intrathecal Ig Synthesis, corresponding to Qmean ± 3 SD is used for the diagnostic relevant, most specific reference range in the Reibergrams (Fig 5). The range with 96% or Qmean ± 2 SD is used in statistics for the comparison of groups in the Reibergrams (free software of www.albaum.it).

Fig 5



The reference ranges of blood-derived IgG, A, M fractions in CSF (range 1 and 2) include between upper (QLim) and low- er hyperbolic discrimination lines 99% (+/-3 SD) of the 4300 patients investigated. The upper hyperbolic curves (thick lines) of the reference range represent the discrimination lines QLim (Lim from limit) between brain-derived and blood-derived immunoglobulin fractions in CSF. Values above QLim represent intrathecal fractions (IF) in percent of total CSF concentration as IgGIF, IgAIF, or IgMIF.

These intrathecal fractions can be conveniently and directly read from the quotient diagrams with lines for 20, 40, 60 and 80% in- trathecal synthesis with reference to the upper discrimination line (QLim) as 0% synthesis. The example shows IgMIF = 40%.

The limit of the reference range for QAlb between normal and increased CSF protein concentration (blood-CSF barrier dysfunc- tion) is age-dependent with a general for the age groups above 5 years: QAlb = (4 + age(y.)/15) $x10^{-3}$ (e.g. vertical line at QAlb = 8 x 10^{-3} for patients up to 60 years).

The diagrams depict five ranges: 1 = normal; 2 = pure blood-CSF barrier dysfunction (i.e., reduced CSF turnover); 3 = intrathecal Ig synthesis with a reduced CSF turnover and 4 = intrathecal Ig synthesis without change in CSF turnover. Values below the lower hyperbolic line, in range 5, indicate a methodological fault. The characterization of the hyperbolic functions has taken into account the analytical imprecision with coefficients of variation between 3 - 8% for the quotients of albumin, IgG, IgA and IgM. Due to larger variations between laboratories, intrathecal Ig synthesis should be considered elevated if the intrathecal fraction IgIF is larger than 10%.

The diagrams for IgG, IgA amnd IgM should be arranged above each other referring to the common albumin quotient to facilitate the recognition of the disease-related immunoglobulin patterns. The data in the diagram are from a patient with a facial nerve palsy. An intrathecal IgM fraction of IgMIF = 40% and oligoclo- nal IgG (with IgGIF = 0) led to further analysis of Borrelia-specific Antibody Index. The Borrelia (IgG) AI = 4.3 and Borrelia (IgM) AI = 3.2 indicates Borrelia as the cause of the disease.

General relevance of the hyperbolic reference line for blood proteins in CSF

With the recent demonstration for the small molecules like Free light chain Kappa (Reiber, Zeman et al 2019, Appendix VI) the general relevance of the hyperbolic function was shown. In this paper the general principles of the constructions ar demonstrated.
In General:

1. The concentration quotients (Q) of blood-derived proteins in CSF are characterized in quotient diagrams by the general hyperbolic function,

$Q = a/b\ [QAlb^2+b^2]^{0.5} - c$. Eq (1)

The meaning of the parameters a, b, c for this mathematical function are shown in Fig. 3. These parameters depend on the size of the molecule.

2. The asymptote $y = a/b\ x - c$ is determined preliminary as an approximation to the slope of the empirical curve in the range of largest QAlb values. In the range of low concentrations of x ($x \rightarrow 0$) we can estimate the value of (a-c) (Fig. 3) and calculate the value of a with c from the asymptote. With these preliminary parameter values we get a preliminary hyperbolic curve with the function Q_{kappa}(mean) corresponding to Eq (1). By iteration of this process for different values of the parameters a, b, c the best fitting function for the Qmean curve of FLC-K, can be found with the method of least squares, but using relative concentration differences, [(Qmeasured - Qtheor)/ Qmeasured]. This is needed to consider the theoretically founded, increasing standard deviation with larger QAlb.

3. With the mean CV value and the definition of the reference range as Qmean ± 3CV we can calculate the complete curves for the upper limit (Qlim) and the lower border (Qlow) of the reference range.

The general validity of the hyperbolic reference function is shown in the Fig 10 of the attachment VI. Large molecules as well as molecules smaller than albumin are shown. The slope of the asymptote of the hyperbolic curve correlates with the molecular size of the molecules passing from blood to brain and CSF. The insertion shows the complete curves for FLCK with an inverted hyperbolic function for $Q_{FLC\text{-}K} > 0.5$.

Graphical and numerical data evaluation

The graphical evaluation in Reibergrams is shown with explanation in Fig5.

The complementary numerical evaluation of intrathecal synthesis of an IgG, IgA,or IgM uses the two parameters, IgGloc the absolute amount of intrathecally synthesized Ig in mg/l or the IgGIF, the relative amount for IgGloc /total CSF IgG in %. The intrathecal fractions are directly read from the diagrams and allow the comparison of the different immunoglobulin class responses (Dominance). For statistical purposes the absulute amount has to be preferred**.**

All values can be easily gained from the software (CSF Statistics tool from www.albaum.it).

Calculation of Q_{Lim} (necessary for the AI)

Into the general hyperbolic function the values from Table 3 are inserted to get the following equations which describe the upper discrimination line

Q_{Lim} (Ig) for the reference range in the CSF/serum quotient diagrams:

$$Q_{Lim}(IgG) = 0{,}93\sqrt{(Q_{Alb})^2 + 6 \cdot 10^{-6}} - 1{,}7 \bullet 10^{-3}$$

$$Q_{Lim}(IgA) = 0{,}77\sqrt{(Q_{Alb})^2 + 23 \cdot 10^{-6}} - 3{,}1 \bullet 10^{-3}$$

$$Q_{Lim}(IgM) = 0{,}67\sqrt{(Q_{Alb})^2 + 120 \cdot 10^{-6}} - 7{,}1 \bullet 10^{-3}$$

Any values for Q_{IgG}, Q_{IgA}, and Q_{IgM} above these hyperbolic discrimination lines indicate intrathecal synthesis.

Example for Calculation of Q_{Lim} (IgG) with $Q_{Alb} = 5 \times 10^{-3}$

Q_{Lim} (IgG) = $0.93\ ((5.0 \times 10^{-3})^2 + 6 \times 10^{-6})^{0,5} - 1.7 \times 10^{-3}$

Q_{Lim} (IgG) = $[0.93 \times ((5)^2 + 6)^{0,5} - 1.7] \times 10^{-3}$

Q_{Lim} (IgG) = $[0.93 \times \sqrt{31} - 1.7] \times 10^{-3}$

Q_{Lim} (IgG) = 3.48×10^{-3}

Quantitation of Intrathecal Synthesis

Ig_{Loc} – Intrathecal Immunoglobulin Concentration in CSF

The increased amount of locally synthesized immunoglobulins secreted into the CSF, measured in mg/L, is calculated as the change in concentration in the CSF (Ig_{Loc}), according to the following equation:

IgLoc = [QIg – QLim (Ig)] × IgSerum (mg/L)

The value for Ig_{Loc} does not change with the CSF flow (Q_{Alb}). IgLoc is the best measure for demonstrating the dynamics of intrathecal synthesis of a specific immunoglobulin.

Ig_{IF} – Intrathecal Immunoglobulin Fraction

When determining the extent of intrathecal synthesis of different immunoglobulin classes (dominance, pattern) in a particular patient, it is of advantage to present Ig_{Loc} as the relative intrathecal fraction (Ig_{IF}). With Ig_{Loc} relative to the total immunoglobulin concentration in the CSF (Ig_{IF} = Ig_{Loc}/Ig_{CSF}) and with Q_{Ig} = Ig_{CSF}/Ig_{Serum}, the intrathecal fraction is calculated as follows:

IgIF = [1 – QLim (Ig) / QIg] × 100 (%)

The intrathecal fractions in % can be directly read from the percentage lines of the quotient diagrams (Fig. 5). The percentage line of the quotient diagrams in Fig. 5 for 20%, as an example, is calculated according to the formula for Ig_{IF} with $(1 - Q_{Lim}/Q_{IgG}) = 0.2$, or $Q_{IgG} = 1.25 \times Q_{Lim}$.

Dominance of an Intrathecally Synthesized Immunoglobulin Class.

Using the relative value for intrathecal synthesis (Ig_{IF}) takes into account that the amounts of IgG, IgA, and IgM synthesized in blood (and in the

brain) are fundamentally different: under normal conditions there is always more IgG synthesiszed then IgA or IgM . Thus, it is finally only possible to compare the data of different immunoglobulin classes by calculating the relative values of intrathecal synthesis.

Example: With a dominance pattern of IgMIF > IgGIF > IgAIF (three-class reaction), the dominant intrathecal fraction is IgM. The clinical relevance of these patterns is shown in Table 4.

Table 4 Intrathecal Immunoglobulin response types and the diseases they indicate.

Response type	***Diseases***
No IgG, IgA, IgM	● Early bacterial meningitis and viral encephalitis ● Guillain-Barré syndrome (polyradiculitis)
IgG dominance	● Multiple sclerosis (rare occurrence of IgM_{IF} in 2050%, and IgA_{IF} in 920% of cases) ● Neurosyphilis (2-class response, occasionally increased IgM_{IF}, IgA_{IF} very rarely) ● HIV encephalitis (1-class response)
IgA dominance	● Neurotuberculosis (isolated IgA_{IF} or combined with weak IgG response) ● Brain abscess ● Adrenoleukodystrophy !
IgM dominance	● Lyme neuroborreliosis (IgM_{IF} > IgA_{IF} > IgG_{IF}) ● Mumps meningoencephalitis (3-class response) ● Non-Hodgkin lymphoma with CNS involvement (1-class response, e.g., isolated IgM_{IF} > 0) ● Neurotrypanosomiasis (3-class response, frequency of IgM_{IF} > 0 in 95% of cases)
IgG + IgA + IgM without dominance	Opportunistic infections in immunodeficiency (CMV, toxoplasmosis)

Particular complementary parameters of the basic CSF Program

- Oligoclonal IgG in CSF and Serum
- Microorganism- specific antibodies in CSF and serum –Antibody Index
- Lactate in CSF or Glucose in CSF and serum
- Destruction marker analysis
- Tumor marker analysis

Oligoclonal IgG

Every immune reaction is polyspecific and also oligoclonal. The term “oligoclonal IgG” stems from a time when these interconnections were not yet clearly understood (for references, see Andersson et al., 1994). The different specificities of individual bands have been demonstrated by Western blot (Sindic et al., 1994). The following applies to the detection of oligoclonal IgG: Methods that use normal electrophoresis (i.e., without isoelectric focusing) for the separation of immunoglobulin fractions in CSF and serum are, per definition, unsuited to demonstrate “oligoclonal IgG” .

The interpretations of five classic types of band patterns according to international consensus are presented below.

Basic rules

- According to international consensus (Andersson et al., 1994), detection of oligoclonal IgG with isoelectric focusing and subsequent immunodetection is recommended (Figs. 4.**13** and 4.**14**).
- A precondition for comparing CSF and serum is the use of identical amounts of IgG in both samples.
- For the immunoblot demonstration two bands in the CSF are already sufficient for identifying type 2, whereas for the direct silver stain, 3-4 bands may be necessary for identifying type 2, depending on the homogeneity of the ampholine pattern. Only in 16% of cases examined in the immunoblot (n = 100), an isolated individual band in the CSF was associated with an inflammatory CNS reaction.
- Isoelectric focusing can detect an intrathecal IgG portion of just 0.5% in the (predominantly blood-derived) total IgG in CSF.

Fig 6 Interpretation of oligoclonal IgG bands in CSF and serum

A. Isoelectric focussing on agarose gels with immunoblot

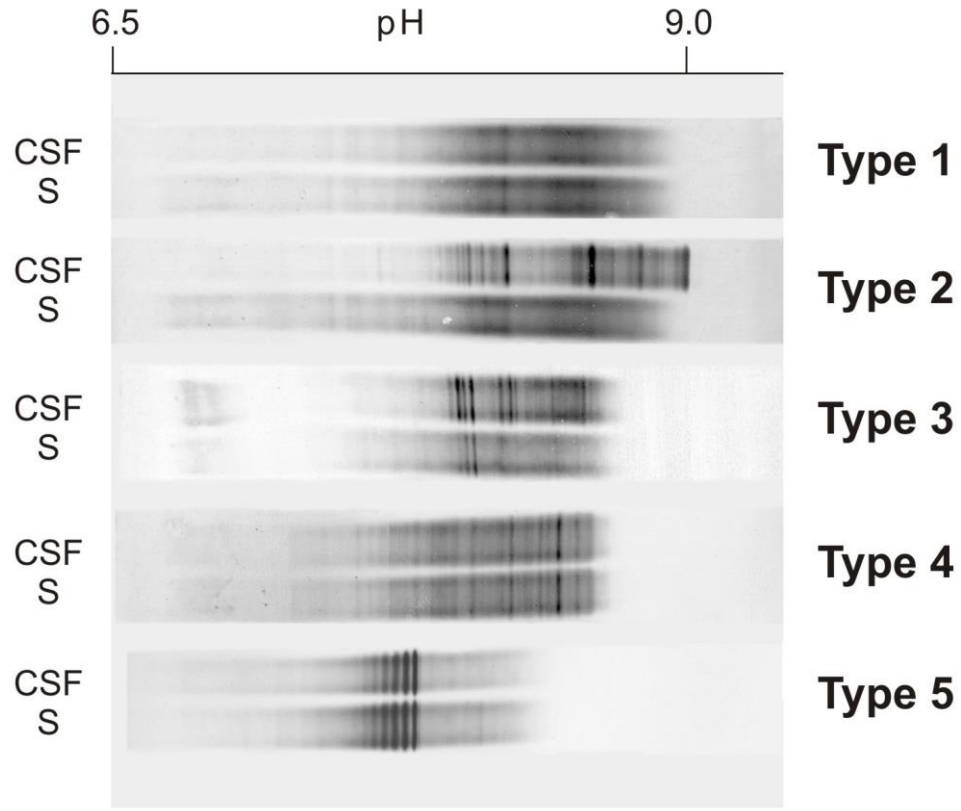


Fig. A: The figure represents the classical types 1 – 5 (Andersson et al. 1994):

- **Type 1:** No bands in CSF and serum.
- **Type 2:** Oligoclonal IgG-bands in CSF, not in serum.
  Interpretation: Intrathecal IgG-synthesis.
- **Type 3:** Oligoclonal bands in CSF (like type 2) and additional identical oligoclonal bands in CSF and serum (like type 4).
  Interpretation: Intrathecal IgG-synthesis
- **Type 4:** Identical oligoclonal bands in CSF and serum.
  Interpretation: No intrathecal IgG-synthesis but systemic immune reaction.
- **Type 5:** Monoclonal bands in CSF and serum.
  Interpretation: Systemic paraproteinaemia.

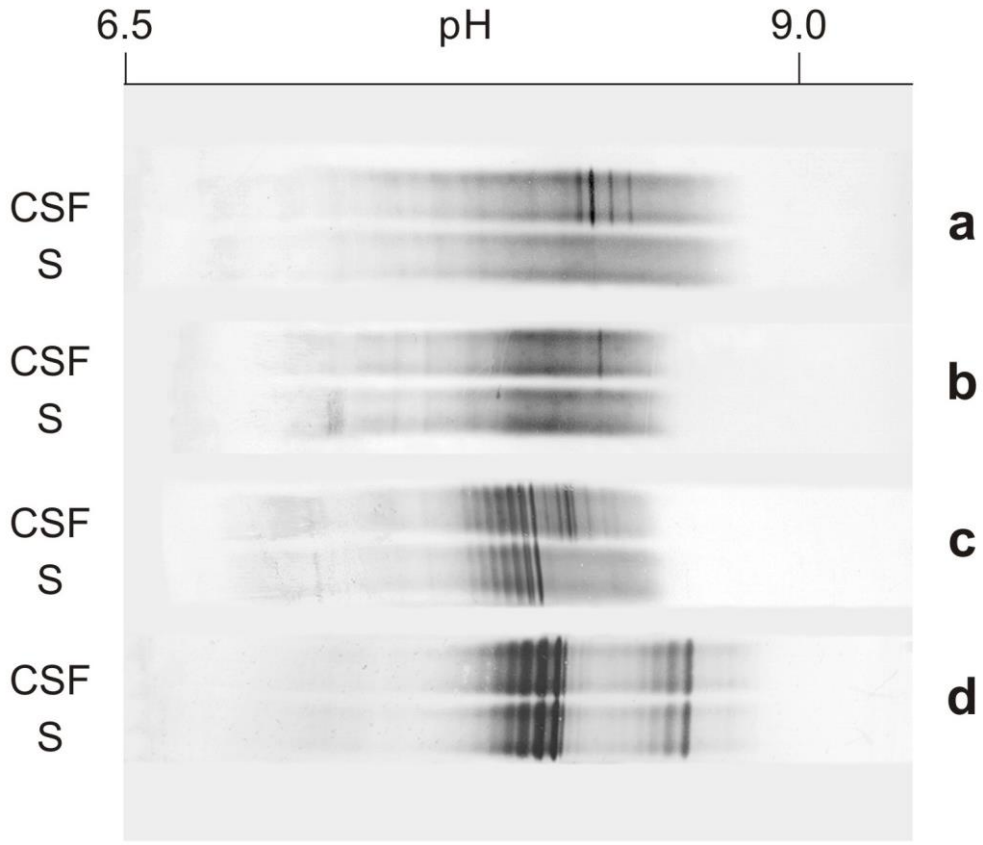


Fig. B: The figure represents four cases which are relevant for practical interpretation:

a) Few oligoclonal band in CSF, not in serum.
Interpretation: Type 2, intrathecal IgG-synthesis.
Comment: The number of bands and the position of the bands in the pH-range have no particular relevance for the interpretation.

b) One single IgG-band in CSF which is not regarded as oligoclonal IgG.
Interpretation: Type 1, no intrathecal IgG-synthesis
Comment: Only 16% of 100 cases with a single IgG-band in CSF have been associated with an intrathecal inflammatory process (D. Mehwald), identified by an increased cell count or intrathecal antibody synthesis (antibody index). 50% of the cases have been associated with a blood contamination (artificial) or subarachnoid haemorrhage.

c) Rare case of an oligoclonal IgG-pattern in CSF (like type 2) combined with additional identical monoclonal bands in CSF and serum (like type 5).
Interpretation: Type 3(b), intrathecal IgG-synthesis with systemic paraproteinaemia.

d) Identical monoclonal bands in CSF and serum which originate from two different monoclonal paraproteins.
Interpretation: Type 5, systemic, biclonal paraproteinaemia.

B. Isoelectric focussing on polyacrylamidgel with silverstain

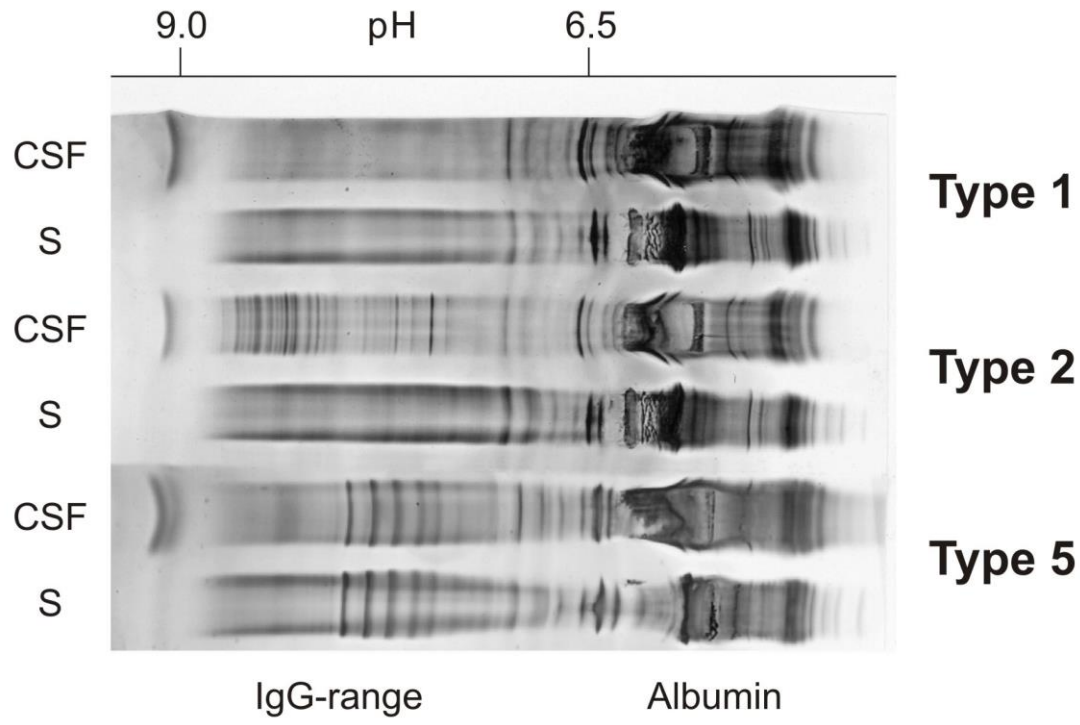


Fig. C: In contrast to the immune detection (Fig. 1 and Fig. 2) we find in the protein stain the albumin range (also place for application of the samples) at about pH < 5.0. In the alcaline range (at pH 9.3) we find cystatin C (gamma-trace-protein) as a single band in CSF (CSF marker).
The pH-range of the gradient starts on the right side with pH 3.5 (anode (+)) and reaches pH 10.5 (catode (-)).
In case of immunoblot the pattern is inversed. The nitro-cellulose-acetate foil is attached at pH 6.5.

Antibody Index (AI)

The detection of intrathecally synthesized antibodies is at its most sensitive when the corrected antibody index is used (Reiber and Lange, 1991). In principle, two cases should be distinguished when evaluating specific antibody quotients (Fig. 6):

AI = Q_{spec} /Q_{IgG} ($Q_{IgG} < Q_{Lim}$)

AI = Q_{spec} /Q_{Lim} ($Q_{IgG} > Q_{Lim}$)

Q_{spec} = AB(CSF) / AB(ser), specific antibody-CSF/serum quotient
Q_{Ig} = IgG(CSF) / IgG(ser), empirical immunoglobulin CSF/serum quotient for IgG, IgA or IgM
Q_{Lim} = upper hyperbolic discrimination line of the reference range for blood-derived immunoglobulins (IgG, IgA or IgM)

Case 1: The total IgG or IgM quotient is within the normal range, i.e., there is no detectable local IgG or IgM synthesis ($Q_{Total} < Q_{Lim}$, $IgG_{IF} \leq 0\%$)
Case 2:There is intensive antibody synthesis, and the resulting total IgG quotient lies above the discrimination line in the quotient diagram. ($Q_{Total} > Q_{Lim}$, or $IgG_{IF} \geq 0$).
Correction of the AI calculation relative to QLim instead of QIgG leads to a higher sensitivity, i.e., without this correction, 40% of the results of the MRZ reaction (against measles, rubella, and varicella zoster viruses) in MS patients would be false-negative (Quentin and Reiber, 2004).

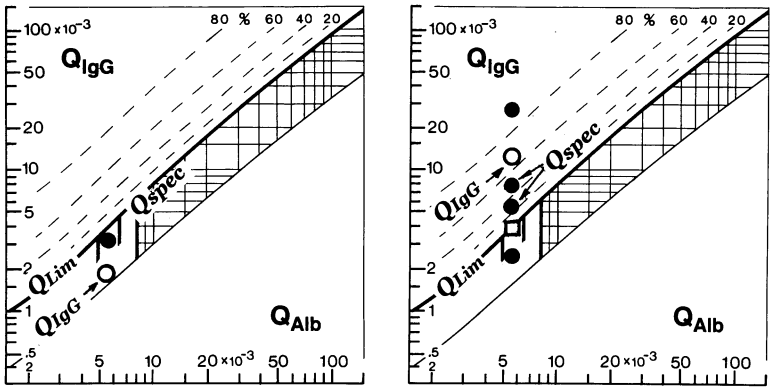


Fig. 7 Examples for corrected AI value: A case of zoster ganglionitis (left) with $Q_{IgG} < Q_{Lim}$ (no correction required) and a case of multiple sclerosis (right) with pronounced polyspecific immune reaction ($Q_{IgG} > Q_{Lim}$, correction for Q_{Lim} is required).

Correction of the specific antibody Index for polyspecific immune response (Fig 6 left side)
When calculating the VZV-AI in the MS patient with intensive intrathecal IgG synthesis, the result relative to Q_{IgG} would look as follows: VZV-AI = $Q_{Spec}/Q_{IgG} = 5.9 \times 10^{-3}/11.6 \times 10^{-3} = 0.51$. However, this value leads to the wrong interpretation (no synthesis). However, when the calculation for $Q_{IgG} > Q_{Lim}$ is based on AI = Q_{Spec}/Q_{Lim}, the following result is obtained: VZV-AI = $5.9 \times 10^{-3}/3.5 \times 10^{-3} = 1.8$. This value indicates intrathecal synthesis of zoster antibodies.

Improvements of sensitivity

The interpretation of Antibody-Index values reaches a higher sensitivity by combined evaluation of several Antibody-Index values as shown in the three cases in the table, where in case I a rubella-AI = 1.4 is the clear indication of an intrathecal antibody synthesis with reference to the other normal Antibody-Index values. Less reliability is found in the case II with a rubella-AI = 1.5 compared to the three other high Antibody-Index values. Case III represents a typical combination of non-matched CSF/serum samples (in spite of repetition the measles-AI remains < 0.5).

Table 5 Interpretation of borderline AI values

	Case I	Case II	Case III
Measles-AI	**0.8**	**1.2**	**0.2**
Rubella-AI	**1.4**	**1.5**	**1.1**
VZV-AI	**0.8**	**1.2**	**2.1**
HSV-AI	**0.7**	**1.1**	**0.7**

Analysis of small sample volumes.

If not enough lumbar or ventricular CSF is available for analyzing IgG and albumin, comparison of the Q_{Spec} values of different antibody species may suffice. When one of the Q_{Spec} values is 50% higher than one (or better, several) of the other values, there is evidence for specific intrathecal synthesis.

Reference range and interpretation

1. Method-related range of precision (mean± 2SD) :

AI = 1.0 ± 0,3

2. Clinically relevant reference range

Normal	**AI = 0.7 – 1.3**
Intrathecal synthesis	**AI ≥ 1.5**

Values of AI < 0.5 are an indication of non-matched CSF/serum samples or of analytical faults.

Lactate and Glucose

General Remarks

It is advantages to analyse lactate in CSF. Its CSF concentration does not correlate with the changes in blood. In Glucose analysis always CSF and serum have to be analysed to detect a decrease in CSF unbiased from serum variations (starving, diabetes).
The lactate analysis must consider age-related reference ranges (Table)

Determination of L-lactate has been introduced to differentiate between bacterial and nonbacterial meningitis. A moderate increase in lactate is observed with many inflammatory, vascular, metabolic, and neoplastic diseases of the brain and meninges, but it is less relevant for differential diagnosis (Thomas, 2005). When analyzed serially, it may help establishing a prognosis of the clinical course.

In severe bacterial meningitis, increased lactate levels in the CSF are caused largely by the mechanisms described above, and only to a minor degree by bacterial production of D- and L-lactates and granulocytic production of L-lactate in the CSF (Wellmer et al., 2001; Prange, 2004). The diseases shown in Table 5.**7** showed the highest mean granulocyte counts when the mean lactate values were also high (Felgenhauer and Beuche, 1999); nevertheless, it would be wrong to conclude from this that there is a direct connection. Cell counts may be high (12 700/µL) (*Pseudomonas*) with low lactate concentration (10 mmol/L) or may be low (1194/µL) (pneumococci) with high lactate concentration (29 mmol/L). This is supported by test results from a separate case of pneumococcal meningitis, with a cell count of 17/µL and 3.4 mmol/L lactate (Prange 2004).

Table Age-dependent reference ranges for L-lactate in CSF. Conversion factor: mg/dL × 0.11 = mmol/L

0-15 years	1.1-1.8 mmol/L (9.9-16.2 mg/dL)
16-50 years	1.5-2.1 mmol/L (13.5-18.9 mg/dL)
> 51 years	1.7-2.6 mmol/L (15.3-23.4 mg/dL)

Table 6 Reference ranges for lactate and glucose in CSF and serum. Under normal conditions CSF and serum values are similar. In the usual methods there are no matrix effects either. (Ref: L. Thomas (ed.) Labor und Diagnose. 7.Ed.,2008, p.193ff and p. 298ff).

	CSF			Serum	
	Lactate	Glucose abs	Glucose L/S	Lactat	Glucose
	mmol/l	mmol/l		mmol/l	mmol/l
Reference v.	1,2-2,1	2,7-4,2	> 0.5- 0,7	0,5- 2,2	3,3-5,5
Bact./Tub. M.	>3,4	<2,2	<0,5	0,5- 2,2	3,3-5,5
Diabetes					> 7 />11*)
*) jejunely / independent of food uptake					

Table 7 CSF lactate in patients with inflammation or tumor development (Felgenhauer and Beuche, 1999)

Disease	**Mean value (mmol/l)**	**Range (5-95%iles)**
Pneumococcal meningitis	14.2	3.5-19.5
Meningococcal meningitis	13.8	6.0-22.1
Listerial meningitis	7.1	2.9-11.1
Tuberculous meningitis	6.4	3.8-26.7
Metastases	5.5	3.5-12.5
Lymphoma, leukemia	4.9	1.5-19.5
Neurosarcoidosis	4.0	1.5-6.0
Guillain-Barré syndrome	2.7	2.2-3.3
Glioblastoma	2.3	1.9-5.7
Neuroborreliosis	2.5	1.4-3.6
Herpes simplex encephalitis	2.3	1.3-3.4
Viral meningitis	2.2	1.5-3.2
Herpes zoster ganglionitis	2.0	1.4-3.0
Multiple sclerosis	1.7	1.4-2.5

Beta-trace protein

Beta-trace protein is a sensitive marker for CSF Rhinorhea and CSF Otorhea

The mean beta trace concentration of normal lumbar CSF (18.4 mg/L) and normal serum (0.59 mg/L) from n =132 control patients, were 10% higher than reported earlier for smaller control groups.

The reference range of beta-trace protein in nasal secretions is very low (median: 0.016 mg/L, range < 0.003 mg/L to 0.12 mg/L, for total n= 29 controls).
Clinically confirmed cases of CSF rhinorhea (n=20) showed beta-trace concentrations between 0.36 mg/L and 53.6 mg/L, with a median of 2.4 mg/L .

A cut-off value of 0.35 mg/L is proposed above which a CSF contamination in the secretion is plausible. A clinically confirmed CSF otorhea had a value of 1.75 mg/L

Table 8 Reference ranges of beta-trace protein concentrations in normal lumbar CSF, serum and nasal secretions of control patients.

	Normal Beta-Trace Protein Concentrations			CSFRhinorhea
	Lumbar CSF	Serum	Nasal secretion	
Number of patients	132	132	29	20
Mean (mg/L)	18.4	0.59	0.016 [1)]	2.4 [2)]
CV (%)	22.6	16.5	---	---
Range (mg/L)	9.4 – 29.2	0.38 – 0.86	< 0.003 – 0.12	0.36 – 53.6

Markers of Dementia and Neuronal Destruction

Tau Protein

Tau proteins are microtubule-associated proteins of low molecular weight, found primarily in the axons of CNS neurons with different tau isoforms. In the human brain, only six isoforms are found; they are between 352 and 441 amino acids long.

The main function of tau is the stabilization of microtubules; it also promotes polymerization of microtubules. Tau binding to microtubules is influenced by phosphorylation, particularly near the microtubule-binding region of the isoforms. Up to 30 out of 79 possible phosphorylation sites of the amino acids serine and threonine have been described (Buee et al., 2000). The idea of determining tau protein in the CSF was based on the fact that the intracellular neurofibrillary tangles (NFT) found in the brains of patients with Alzheimer's disease consist predominantly of paired helical filaments (PHF) composed of hyperphosphorylated tau isoforms. The degree and localization of phosphorylation seem to vary with different diseases.

Alzheimer's disease. Elevated levels of total tau protein were described but not only in these patients (Andreasen et al., 1999 b), but also in patients with other forms of dementia (see below) and in particular in most inflammatory processes in brain. The predictive value of tau protein determination is therefore low.

Amyloid Beta (Aβ) Peptides

Amyloid beta peptides are generated through enzymatic cleavage of amyloid precursor protein (APP) by α-, β-, and γ-secretases and constitute the main ingredient of amyloid plaques in Alzheimer's disease. These peptides form a heterogeneous group of 37-42 amino acids in length, with the Aβ peptide 1-40 clearly dominating. The most frequently investigated **Aβ peptide 1-42** differs from Aβ peptide 1-40 by two additional neutral amino acids at the carboxyterminal end; its proportion under physiological conditions is only 10% of the total.

Although the primary sequences are almost identical, the longer Aβ peptide$_{1-42}$ is less soluble than Aβ peptide1-40. It aggregates faster and forms neurotoxic β-amyloid depositions. These neuritic extracellular plaques consist predominantly of Aβ peptide1-42.

Pathological levels of Aβ peptide1-42 are usually below 450 pg/mL. Aβ peptide1-42 is significantly diminished in Alzheimer patients. In combination with the increase in tau protein, this finding is relatively characteristic for Alzheimer's disease

For diagnostic purposes the relation or Aß 40/Aß42 is calculated

14-3-3 Proteins

14-3-3 proteins are a family of regulatory proteins with a molecular weight of about 30 kDa. At least 7 isoforms are known; they exist as dimers and have a highly conserved amino acid sequence in almost all eukaryotic species. 14-3-3 proteins are thought to play a role in signal transduction, particularly in mediating the binding of kinases.

Several studies have confirmed the high diagnostic reliability of the SDS-PAGE immunoblot techniques currently used for 14-3-3 proteins in the CSF. Since then, patients who fulfill the clinical criteria of “possible” CJD and show positive CSF results are classified as “probable” CJD cases, independently of their EEG results. With vCJD, however, the SDS-PAGE immunoblot is not always positive. False-positive results may occasionally occur in Alzheimer patients, in CSF changed by inflammation, after ischemic incidences, and in glioblastoma patients. It should be kept in mind, however, that clinical differential diagnosis can distinguish these diseases in most cases. Determination of total tau protein permits a similarly clear-cut distinction. The 14-3-3 immunoblot usually becomes positive with tau protein levels above 1100-1300 pg/mL.

S-100 Proteins in CSF

S-100 protein is an acidic calcium-binding protein with a molecular weight of 21 kDa, and it occurs primarily in the nervous system of vertebrates. Native S-100 is found as homodimer or heterodimer with two isomeric subunits, α and β. According to the new nomenclature, the α-subunit is called S-100A, and the β-subunit is called S-100B All three possible combinations occur. The isoforms have a molecular weight of 10.5 kDa each. S-is found in glial cells with the exception of Schwann cells. In peripheral tissues, S-100 occurs in much lower concentrations than in the central nervous system. Experiments suggest that S-100 functions as a neurotrophic factor. Several studies have found elevated S-100 levels in the CSF of patients with sporadic CJD and vCJD. The sensitivity, however, is lower than that for 14-3-3 protein and tau protein in the CSF analysis

The diagnostic importance of S-100 protein is increasing not only as a tumor marker in malignant melanoma, but also in making a prognosis of ischemic cerebral infarcts and in evaluating neuropsychological deficits after minimal craniocerebral trauma.

Neuron-specific Enolase in CSF

Neuron-specific enolase (NSE) is a 78 kDa glycolytic enzyme that is localized as a γ,γ-dimer in neurons and neuroendocrine cells. In the CSF, 98% of the protein stems from the brain.

Pathological levels have so far been measured in the serum and CSF of patients with hypoxemic brain damage, brain tumors, cerebral hemorrhage, and cerebral trauma. In the differential diagnosis of dementia, NSE is gaining in importance as one of the first surrogate markers. The cut-off level for NSE in the CSF is 35 ng/mL; it permits to diagnose patients as CJD cases with a sensitivity of 78% and a specificity of 88%. With the assays used so far, no significantly different levels have been found in the serum of CJD patients.

The Cummulative CSF data report, Fig 8

The development of a cummunlative data report which contains all laboratory data of an individual patient was a great step forward in the relevance of CSF analysis for differential diagnosis in Neurologicl diseases. With the integration of the Reibergram which provides neuroimmunological response patterns this concept, developed already 40 years ago, gained further improvement.
To analyse the complete set of data typical for a basic program ( ) may seem to expensive for the routine diagnosis. The reality ids of course different in different countries with restricted resources or missing technical facilities. If in gana a HiV positive patient sees the doctor with neurological symptoms he gets without further investigation a treatment against toxoplasmosis without further investigation. This is the most probable opportunistic disease and the treatment is cheap. Or in India where a doctor sees a patient with a neurological disease and there is any anmnestic information of a tuberculosis in the family or in the patients biography he gets a tuberculosis treatment without preceding further analysis. This may be a cost reducing rational based on statistical evidence, but it is not possible in Germany where tuberculosis is so rare that it is easily overlooked. But a dominant intrathecal IgA synthesis with barrier dysfunction and increassed lactate value could clearly point to this rare diagnosis of a neurotuberculosis in this country. Csf analysis may in some diseases like a facial palsy the only diagnostic option to discriminate between a viral (VZV) and a bacterial (borrelia) cause avoiding fatal consequences for the patient by a wrong treatment.
But there is also another basic challenge in some diseases if the CSF is analysed but without serum. A sytemic African trypanosomiasis with a tremendous high concentration of IgM in blood and subsequently in CSF would easily misinterpreted as an involvement of the brain if the QIgM is not calculated. Or a solely restricted analyis of low total protein concentration in CSF would let miss an intrathecal synthesis of individual proteins (IgM) due to the extremely low concentrations of total protein in Blood of these paients.
It is one of the bad habits to request just a single parameter analysis from CSF like in dementia. But the results of the dementia maker analysis with 95% normal values teaches that a basic CSF analysis could exclude or point to another disease. As CSF punctures are not frequently repeated like blood extraction, it is advisable to analyse always a basic program (including serum). This is cost reducing and avoids wrong treatments of the patient. The bad performance of dementia marker analysis with many false positive and false negative interpretation documents this aspct (Reiber et al 2014). The publication attached (Reiber and Peter 2001) gives some ideas of the disease related typical patterns which might support, contradict the suggested diagnosis or point to an unsuggested different option. The tables and… help to find a diagnosis in many cases which might otherwise be missed.
The knowledge based interpetations of modern software can support the clinical chemist not to overlook options of interpretations in the daily routine analysis. (Wormek, Albaum, Comed, Siemens, Beckman).

CSF REPORT

CSF LABORATORY
INSTITUTE ,Adress,
Phone, Fax, Email

Sender/Client	Patient		sex
Hospital	Name	**Nunez, Roberto**	F / M
Address	Birth/ Age	xx/yy/19zz	**40 y**
	Puncture	xx/yy/2015	**LP**
Phone	Receipt	xx/yy/2015	CP
Email	Physician		VP

Differential diagnostic question /suggested Diagnosis	Add. Information
Inflammatory Process?	

Visual Inspection					Hemoglobin				Lactate	Tot. Protein
clear	turbid	xanth	blood	art. blood	0	**+**	++	+++	**6,0** mmol/l	**1563** mg/l

CELLS

Cell count **337 / µl** Ery 0 / µl

Lymphoc % | Monoc % | N. Granuloc. % | Plasma c. %

Other Cells

PROTEINS

	CSF		Serum		Q x 10^3	Intrath. Fract, (IF)
Albumin	**809**	mg/l	**35,2**	g/l	QAlb = **23**	
IgG	**189**	mg/l	**13,6**	g/l	QIgG = **13,9**	%
IgA	**55,7**	mg/l	**2,9**	g/l	QIgA = **19,2**	**22** %
IgM	**31,4**	mg/l	**5,9**	g/l	QIgM = **5,3**	%

Oligoclonal IgG CSF-spec. bands 0 o/+ Type **1**

Specific Antibodies

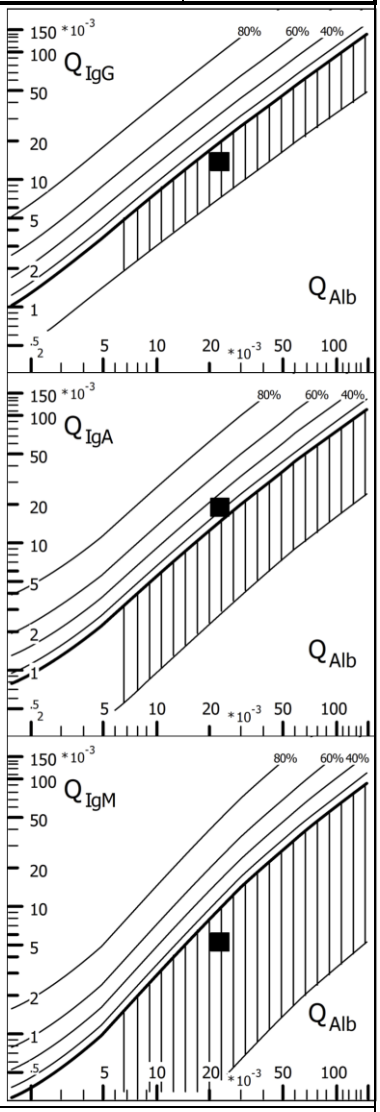
Synthesis in CNS : AI≥1.5 , n.d. = not detectable

Measles-AI =	Borr.-AI(IgG) =	TP-AI (IgG) =
Rubella-AI =	Borr.-AI(IgM)=	TP-AI (IgM) =
VZV-AI =	HIV-AI =	
HSV-AI =	Toxopl –AI =	
CMV-AI =	EBV-AI =	

Surrogate marker (Tumor, Dementia, Hemorrhage, Psychiatric diseases)

Interpretation

Normal CSF-Report	☐	Normal proteins	☐
B-CSF barrier dysfunction	☒	Cell count increased	☒
Inflammatory process	☒	Lactate increased	☒
AB-synthesis in CNS	☐	Path. Dementia markers	☐



Comments

Date : Sign.:

Interpretation and Comments

No diagnosis can be established only from individual pathological values, not even from a combination of several parameters. The diagnosis can only be established by the clinician on the basis of the patient's history, the clinical findings, and supplementary analytical tests (e.g., imaging procedures).

List of particular analytical and diagnostic Comments

Software programs (e.g., www.wormek.com) allow for the storage of frequently recurring comments that can be retrieved using a specific command. Analysis-related comments include all explanations and clues to carrying out the analysis and to problems that may occur during the analysis. Diagnosis-related comments are interpretations that may contribute to the differential diagnosis, but they must never be understood as a diagnosis.

The following examples are from the Neurochemical Laboratory in Göttingen, Germany. They are added individually to the respective findings in the CSF data report (Fig.), rather than being printed out automatically as part of the knowledge-based evaluation program:

Analysis-related comments:

- Analysis of "parameter X" was repeated.
- Further analysis was not possible due to small sample volume.
- Analysis of oligoclonal IgG was not performed because $Q_{IgG} > Q_{Alb}$.
- Intrathecal IgA synthesis was detected because $Q_{IgA} > Q_{IgG}$ (even if $IgA_{IF} = 0\%$).
- Total cell count was corrected for blood contamination (reduction of 1 leukocyte/µL per 1000 erythrocytes/µL in the CSF).
- Differential cell count could not be interpreted because of heavy blood contamination.
- Correction of CSF albumin and Ig concentrations for blood contamination (in the range of 1000–7000 erythrocytes/µL) resulted in $Ig_{IF} \leq 0\%$.
- Due to heavy blood contamination (> 7000 erythrocytes/µL), interpretation of CSF/serum quotients is unreliable.
- Despite heavy blood contamination, there is evidence that a humoral immune response took place in the central nervous system: $Q_{IgG} > Q_{Alb}$ (or $Q_{IgA} > Q_{IgG}$; or $Q_{IgM} > Q_{IgA}$). Absolute values of the quotients, however, are not reliable enough for calculating the intrathecal fraction.

Diagnosis-related comments:

- Positive MRZ response: chronic inflammation (autoimmune type). Differential diagnosis: multiple sclerosis or autoimmune disease with CNS involvement.
- Large albumin quotients ($> 20 \times 10^{-3}$) or high cell counts (> 90/µL), or absence of oligoclonal IgG do not support the suspected diagnosis of

multiple sclerosis.

- Markedly increased albumin quotient, dominant intrathecal IgA synthesis, increased CSF lactate level (> 3.4 mmol/L), and moderate pleocytosis indicate suggest tuberculous meningitis with a high probability (recommendation: PCR).
- Lactate levels of > 3.4 mmol/L with cell counts of > 500/µL indicate bacterial infection.
- Intrathecal three-class responses with HIV AI ≥ 1.5 indicate opportunistic infections (recommendation: analysis of the respective antibodies, or PCR for relevant microorganisms).
- Isolated intrathecal IgM synthesis without other inflammatory signs (normal cell count, no oligoclonal IgG) may indicate lymphoma.
- Intrathecal synthesis of carcinoembryonic antigen (CEA) with $Q_{CEA} > Q_{Lim}$ (IgA) indicates tumor metastases in the brain.
- The combined findings of decreased β-amyloid$_{1-42}$ (< 450 pg/mL) and increased tau protein (> 450 pg/mL) are compatible with the suspected diagnosis of Alzheimer's disease.
- Tau protein values of > 1300 pg/mL are observed in Creutzfeldt-Jakob disease (recommendation: determination of protein 14-3-3 and neuron-specific enolase in the CSF).

Tab. 9 Parameter combinations for differential diagnosis in Neurology

Table 19.6 CSF data patterns in neurological diseases and the frequency of pathological values (in %) at the time of diagnostic lumbar puncture. Bold numbers indicate typical data.

Diseases	*Cell count (cells/µL)*				*Lactate (> 3.5 mmol/L)*	*Barrier function $Q_{Alb} \times 10^3$*			*Intrathecal fractions*				*Specific antibody*	*Optional tests*
	5	*5–30*	*30–300*	*> 300*		*< 8*	*8–25*	*> 25*	*IgG > 0 %*	*(oligo)*	*IgA > 0 %*	*IgM/F > 0 %*		
Bacterial meningitis	< 5*	5	20	**70** (40 % > 2000)	**90**			**100**			20†			I, III, VI
Neuroborreliosis		10	**60**	30 (< 900)			**60**	40	**38**	(63)	**33**	**75**	1	I
Neurotuberculosis		10	**80**	10 (< 500)	**80**			**100**	15	(20)	**85**	~ 30		I, III, IV
Neurosyphilis	**50**	40	10			**70**	30 (< 15)	0–5	**85**	(90)	35	44	2	I, VI
HSV encephalitis	4		**96**				**100**						3	I, IV
VZV meningitis			**60**	40 (< 600)		10	**90**		15	(15)			4	I, IV
VZV ganglionitis	20	30	**50**			**90**	10 (< 10)		15	(30)			5	
HIV encephalitis, stages I, II	**60**	40				**85**	15		10	(30)			6	I, VII
HIV encephalitis, stage III	20	**80**				40	**60**		20	(45)			6	I
Opportunistic infection (toxoplasmosis, CMV, cryptococcosis)	**60**	30	10			25	**75**		**50**	(50)	**50**	**50**	7	I, IV
Multiple sclerosis	**40**	**55**	5‡			**90**	10		**72**	(98)		**40**	8	I
Guillain-Barré syndrome	**80**	20§					**100**							
Neurodegenerative diseases	**100**					**100**				(5)¶				VIII
Creutzfeldt-Jakob disease	**100**					**80**	20 (< 15)			(7)¶				V

* Rare cases (apurulent meningitis), e.g., early lumbar puncture in meningococcal meningitis with bacteremia, followed only a few hours later by a strong influx of neutrophilic granulocytes.
† Microorganism-dependent (e.g., meningococci, pneumococci); may indicate an abscess.
‡ Up to a maximum of 90/µL (2 % > 40/µL).
§ Mild pleocytosis may be present only in the early phase of disease.
¶ Few patients with degenerative disease have a humoral immune response, which may be interpreted either as a scar of an earlier disease or as a secondary immune response on tissue destruction.

Specific antibodies:
1 Borrelia-specific antibody index (IgG and IgM classes).
2 Treponema-specific antibody index.
3 HSV antibody index not increased earlier than 6–7 days after disease onset; antigen detection (by PCR) is successful before that time.
4 VZV antibody index increased in 100 % of cases, by definition.
5 VZV antibody index increased in 100 % of patients.
6 HIV antibody index.
7 Antibody index for Toxoplasma or CMV.
8 Measles, rubella, and/or VZV antibody indices are increased in 90 % of patients with definitive MS; differential diagnosis: autoimmune diseases with CNS involvement.

Optional tests: I, differential cell count; II, bacterial culture; IV, DNA or RNA detection by PCR; V, neuron-specific enolase, protein 14–3-3, and tau protein in CSF; VI, Gram staining; VII, β_2-microglobulin as an activation marker; VIII, tau protein (increased) and amyloid β_{1-42} (decreased).

Attachment I: Quality assessment in the CSF laboratory

H. Reiber in Wildemann et al 2010

Quality assessment in laboratory medicine

Accuracy and precision in laboratory medicine are kept on a high level controlled mainly by the following activities:

- Internal quality control (QC)
- External quality assurance (EQA)
- Knowledge based interpretation concepts. In CSF analysis this is the integrating CSF data report
- Laboratory protocols (laboratory accreditation)

Internal quality control (QC)

QC is part of the daily analysis protocol using certified commercial control samples for assurance of accuracy in a single analytical series and of precision calculated from a series of daily control values. The mean of these data measured between two calibrations of an analyzer may be used to control the calibration dependent variation of accuracy .

External quality assurance system (EQAS)

EQAS is a major attempt to improve the quality standards in laboratory medicine and to keep interlaboratory variation low. Many national and international institutions have defined rules and regulations and also organize meetings (links in INSTAND e.V.). In Germany INSTAND (s. INSTAND e.V.) is one of two institutions for quality assurance working from mandatory guidelines defined by the German Federal Medical Association (BÄK, 2008 and Table).The following description of EQA for CSF analysis is based on the development of INSTAND with the German society of CSF analysis and clinical Neurochemistry (www.dgln.de) as an implicit consensus of 400 participating laboratories from Germany and sixteen different European countries. (Reiber 1995, Reiber and Uhr 2003).

Interpretation concepts

As a generally accepted definition (ISO15189) of the EQAS we find:
“ External quality assessment programmes should , as far as possible, provide clinically relevant challenges that mimic patient samples and have the effect of checking the entire examination process, including pre- and post-analytical procedures.”
It is not only about finding the adequate samples for a survey, which might be demanding, EQA is also considering the postanalytical procedures, i.e. the data handling and interpretation.
General guidelines (BÄK 2008) regulate quality of results (mostly numbers for absolute concentrations in a sample), but have problems with very particular rules for medical interpretation or do not consider them at all. But this is the relevant issue for the patient.

Laboratory accreditation

The accreditation of a laboratory by an accreditation body is a big business driven by competing laboratories. This cost- and time-consuming activity leads to a tremendous consumption of time for documentation of everything and is, compared to the other activities, of questionable relevance for diagnostic quality provided to the medical doctor.

Special features for quality of CSF protein analysis

1.The CSF/serum quotient of serum proteins in CSF, a mathematically normalized CSF concentration, is based on a biological relation.
Most proteins in CSF are blood-derived. The CSF concentration of a serum protein is

modulated by its serum concentration (and the CSF flow rate). So the CSF/Serum quotients represents a value independent of the varying concentration in blood. This is the reason why CSF/serum concentration quotients of serum proteins, which represent a biologically founded entity, are different from any other calculated ratio in clinical chemistry, e.g. the percentage of albumin in total protein calculated from serum electrophoresis.
The CSF/serum quotient has a smaller *biological* variability then the absolute CSF concentration and offers therefore a more sensitive and specific reference value for the discrimination of a brain-derived pathological fraction from normal blood-derived fraction in CSF.

2. The CSF/serum quotient is a method-independent value if derived after paired protein analsis in CSF and (appropriately diluted) serum samples.
If CSF and serum samples are analysed in the same analytical run compared to analysis with reference to two different calibration curves (and by this way different accuracies) also the CV is smaller (Andersson et al., 1994).
But, a basic precondition the calibraton curve must give concentration independent accuracy. This is controlled by measuring a serially diluted serum sample with dilutions covering the complete analytical range of the corresponding calibration curve.

3**.** CSF flow-related, i.e. barrier function (QAlb)- related interpretations of Ig-quotients are most specific for detection of an intrathecal Ig fraction in CSF (referred to a hyperbolic discrimination function in quotient diagrams).
In contrast to the linear IgG Index or linear functions with many false positive interpretations (Reiber et al 2001) the hyperbolic discrimination line is an empirically and theoretically founded function (chapter 2).

4. The age-related evaluation of the QAlb to detect a barrier dysfunction must take into account different reference values for ventricular , cisternal or lumbar CSF.

5. The cummulative CSF data report is part of a quality assessment by plausibility controls for the accuracy of numerical results and of clinical interpretations.

Internal Quality Control for CSF proteins (TP, Albumin, IgG, IgA, IgM)
CSF assays must take into account the low protein concentrations in normal CSF and the large variation of concentrations in case of intrathecal synthesis, 10-to100-fold larger then in pathological serum. This is the basic demand to industrial development to allow a sufficiently high sensitivity and a reasonable protocol for change of the default dilution to avoid extreme positions on the calibration curve. The use of a cummulative CSF data report for reporting laboratory data to the medical doctor is a basic part of the internal quality assessment in the CSF laboratory.

Precision.
For interassay precision CSF and serum control samples are measured with each analytical run and documented together with the CSF/serum quotients. Variation and trends between two calibrations can be read directly from the charts or by statistics calculation.

Accuracy.
The accuracy control of absolute values is ensured by two certified reference serum samples (normal range and pathological range), and two CSF samples in the upper and lower range of the calibration curve. The control samples can be measured alternating in different analytical runs. A calibration dependent variability is recognized from mean

values of the precision controls.
With each new assay or a new batch of reagents the laboratory ensures the method-independent accuracy of the paired CSF and serum analysis: A serum control sample is serially diluted down to CSF concentrations with dilutions covering the complete analytical range of the corresponding calibration curve in the CSF assay.

Control material for CSF analysis
Certified control samples for proteins in serum are used as available from commercial sources.
But, up to now (2009) there is no certified commercial protein control available suited for normal CSF protein concentration (in particular for IgA and IgM) . The concentrations of analytes in commercial control samples are too high.
A suitable control sample for normal CSF should yield (Andersson et al, 1994):
IgM values between 0.5 and 1.5 mg/L
IgA values between 1.0 and 3.0 mg/L
IgG values between 10 and 30 mg/L
Albumin values between 100 und 300 mg/L.

EQAS - The CSF Survey of INSTAND
Concept
Already 20 yeas ago the CSF survey of INSTAND in Germany (www.INSTAND-eV.de) established an interpretation - oriented evaluation in addition to a single result control. This EQA concept (Reiber 1995) takes the post analytical part, the data interpretation as an aspect of a general quality assessment, serious. With this kind of training program for interpretation of CSF data, participants also improved their awareness for methodological discrepancies. But this concept is demanding fort he manufacturing and combining clinically relevant values in the samples. The evaluation of the survey data is based on two references the parameter related reference ranges and the differential diagnostic relevant result oft he combined data.

Detection of Target values
The evaluation of participants data in a survey refers to target values (defined for cases of analytes with a reference method , like for glucose or total protein in serum) or assigned values (defined for analytes without a reference method). The assigned values for nephelometric analysis in the CSF survey were former detected by reference laboratories. Now the use of the median of a group can be used as a target value. Under these aspects of group specific target values the survey is not testing the real accuracy of reported results with serious consequences for the patients: In particular in the decision range between normal and pathological values this concept supports false positive or false negative interpretations, which finally must lead to wrong information for the medical doctor (Reiber et al 2009). This discussion is actually also lead for the Antibody Index (AI) of microorganism- specific antibodies. For the viral antibody assays it is easier to find a method-independent common target value for the AI then for the bacterial antibodies with a much larger variation of coating antigens in the different ELISAs. (see www.instandev.de , English, EQAS, Reports, Category: Cerebrospinal fluid analysis).
It must become the target for the manufacturers to evaluate and adapt new assays sufficiently to allow a patient-related accuracy instead of a relative, method-related result. To allow the best accuracy is the responsibility of the manufacturers but the users in the laboratories are responsible for the decision of choosing an assay found reliable by the own testing.

I. CSF proteins (Albumin, IgG,, IgA, IgM)
The survey for these combined analytes contains three levels:

1. Interpretation for differential diagnosis

Based on the interpretation of the CSF/serum quotients in the quotient diagrams the participants in the CSF survey have to judge:

- Barrier function, normal or pathological
- Intrathecal IgG-,and/or IgA- and/or IgM- synthesis, detectable or absent
- Inflammatory process in CNS, detectable or absent

The certificate (Reiber 1995) documents, as primary result the correct patient-oriented interpretation of the data.

2. Evaluation of CSF/serum quotients

Regarding quantitative results we refer with priority to the CSF/serum quotients as the specific feature for quality of CSF analysis and judge .:

- Accuracy of CSF serum quotients and deviation from target value
- A possibly serious deviation in the accuracy of a quotient is commented by pointing to the source of deviation in the absolute value of the analyte (CSF or serum or both)

3. Evaluation as independent values in CSF and serum

- Absolute values in CSF and serum are individually certified to meet the RILIBÄK specifications (BÄK, 2008) with the new permissible borders in Table.

Target value, consensus value of the group after subtraction of the outliers, CV of the interlaboratory variation and the individual deviation of the participants value from target value are reported, including a graphical demonstration of the participants position in the group performance in quotient diagrams. (Reiber 1995).

Survey report:

Numerical statistics and method related performance are reported to the participants in a summarizing letter, recent examples of which can be optained from the website of INSTAND (www.instand-ev.de , English, EQAS, Reports, Category: Cerebrospinal fluid analysis). This evaluation protocol was enabled by the knowledge based software of A.Wormek (www.wormek.com).

II. Total Protein

Samples of one normal and one pathological total protein concentration in the CSF pool are tested. The method-related evaluation shows large differences of the medians between methods compared in Youdenplots (www.instandev.de , English, EQAS, Reports, Category: Cerebrospinal fluid analysis).

III. Specific antibodies in CSF and serum

Like with total immunoglobulins , the detection of specific antibodies in CSF and serum by evaluating the CSF/serum quotients is better than the evaluation of absolute values. Titers are not suitable for CSF analysis. It is also a basic fault in the field if predecissions on the base of cut offs are made for the further proceedings.

As a basic challenge for the standard microbiological laboratory we have to regard the low concentration of the antibodies in blood, frequently below the cut off for a systemic infection in blood. Accurate analysis of serum antibody concentrations, also below the cut off value, are the challenge for the usual methods used in serology: In this case of a CSF related serum analysis the usual report in serology for such a case with a comment like “blood-titer is negative” is obviously not helpful at all.

In the CSF survey where the calculated antibody indices are interpreted, only the Q_{Spec} has an impact on the result because the IgG and albumin values are passed on to the participants in advance to ensure that uniform IgG values serve as a basis for calculating the Antibody Index (Quality control of IgG analysis is carried out separately). For a correct result participants also have to calculate and choose the relevant reference quotient QIgG or Qlim (IgG).

The AI values are evaluated regarding deviation from target values and certified as right

/wrong. Additionally the participants interpretations are compared with the correct interpretation which contains the following spectrum of options:

- Normal AI values
- Intrathecal antibody synthesis with specification of the antibody species that is pathologically increased
- Chronic inflammation (when certain combinations of antibodies are increased, like MR MZ or RZ in the MRZ antibody response, Chapt. 19.3)
- Implausible data combination (a single value with AI < 0.5 among other different, normal values).
- The combination of only VZV- and HSV-AI increased may need further control for a Herpes- or Zoster- encephalitis.

IV. Oligoclonal IgG

The collection of samples which suit for the control of oligoclonal IgG in the CSF survey is a challenge, as these samples can not be obtained from pooled CSF, rather need to be from a single patient. To collect the volume necessary for a group of 200 participants in the INSTAND survey this is only possible from patients with a ventricle catheter in a neurosurgical intensive care unit. Multiple lumbar punctures are comparably rare, but with predilution of the samples occationally it is possible to recruit samples in this way.
The bands in paired CSF and serum sample are evaluated according to the 5 types of the international criteria together with the clinical interpretation.

V. CSF survey: Lactate, glucose

Glucose and lactate concentrations in CSF are of similar size and there are no different matrix effects in the assays. This allows the usual survey conditions for serum to be applied to CSF samples.

VI. Neurochemical dementia diagnosis

The analysis of Tau-Protein, Aß1-42 peptide and Phospho-Tau 181 protein has been invented by INSTAND a few years ago. The numeric result of the analysis is better then the interpretation of the combined data. This shows the deficits by missing controls and clinically founded, common reference values. (Reiber et al 2014).

VII. CSF Cytology – Combined Quality control and interpretation training

The internal and external quality controls for CSF cytology are very demanding. There are actually two successful approaches. The“*Ringversuch vor Ort*“ (On-site survey) founded by E. Linke (Linke et al., 2005) in Stadtroda, Germany; it offers regularly education and, at the same time, examination of each participant on four real cytological CSF preparations, tob e investigated with the microscope. The written exam is then certified by INSTAND e.V. The second system established by Controllab in Rio de Janeiro takes advantage of modern cloud technology by which the participants are able to download monthly new high performance digital cytology pictures for training and testing on computer screen.

Permissible maximum deviation from target value in CSF Survey, according to the German Federal Medical Association (BÄK) are updated regularly on their website.

Attachment II: Reference values of CSF - and related serum-solutes

Table 10 Reference values of osmolality, pH and low molecular weight solutes in lumbar cerebrospinal fluid and in plasma

Substance		CSF	Plasma
Osmolality -mOsm/L(37°C)		281.0	289.0
pH		7.31 ± 0.028	7.39 ± 0.017
pCO_2	mmHg	49.5 ± 2.37	39.1 + 1.87
HCO_3^-	mmol/L	22.7 ± 1.15	23.67 ± 0.98
Na	mmol/L	(138 – 150)	135 – 145
K	mmol/L	2.7 – 3.9	3.6 –4.8
Mg	mmol/L	0.38 – 1.4	1.8 – 2.6
Ca[1)]	mmol/L	1.05 – 1.35	1.15 – 1.35
Cl	mmol/L	116 - 127	92 – 105
P_i	mmol/L	1.1	0.84 – 1.45
Glucose	mmol/L	1.1 – 4.4 [2)]	3.9 – 5.5
Lactate	mmol/L	1.2 – 2.1 [3)]	0.5 – 2.2
Uric acid[4)]	µmol/L	25.5 ± 9.2	254.0 ± 79
Total amino acids [5)]		0.72	2.62
Total lipids [6)]	mg/L	19	7500

1) Ionic form (free Ca) ≈ 45% of total Ca.
2) Normal ratio CSF/serum > 0.7
3) CSF-lactate does not vary with serum variations!
4) Normal ratio CSF/serum = 0.1 ± 0.023 (strongly associated with Q_{Alb} , Ref. Reiber, Ruff, Uhr 1993)
5) See Table 8.4 for single amino acids.
6) See Table 8.5 for single lipids.

Table 11 Reference range of blood-derived proteins in CSF and serum of adults as a rough orientation [1)]

Protein	CSF [mg/L]	Serum [g/L]	Q(CSF/ser) [2)] x 10^3	MW kD
Total Protein	200 – 500 [3)]	70		
Albumin	110 – 350	35 – 55	4.5	69
IgG	10 – 40	7 - 18	2	
IgA	1.5 – 6.0 ?	0.9 – 4.5		
IgM	0.05 – 0.8	0.6 – 2.8		800
α_2-Macroglublin	2.0	2.22	0.9/1.1	798
Ceruloplasmin	(1.0/0.97 ± 0.37)	0.366 (0.18–0.45)	2.7/1.9	152
SICAM-1	0.0015		5.0 [4)]	90
Fibrinogen	0.65	2.96	0.22	340
ß-Lipoprotein	0.6	3.7	0.16	2.239
Transferrin	14.4	2.0	7.0/5.7	81

1) CSF concentrations of blood-derived proteins depend on their blood concentration and blood-CSF barrier function, CSF concentrations increase with age-related increase of Q_{Alb}. A most sensitive evaluation therefore needs quotient evaluations with reference to the albumin quotients with a hyperbolic function.
2) Mean values for the whole age group 5 – 60 years.
3) See Table x.x… for relation to Q_{Alb}.
4) 70% of sICAM-1 in normal CSF are blood-derived.

Table 12 Mean concentrations of primarily brain-derived proteins in CSF (Intrathecal fraction in CSF > 90%)

Protein	MW (kDa)	CSF	(% of TP)	Serum
Transthyretin (Präalbumin)	55	17 mg/L	(4.6)	250 mg/L
Prostaglandin-D-synthetase (ß-trace)	27	17 mg/L	(4.6)	0.3 mg/L
Neuron specific enolase	78	8 µg/L	(2,1)	5.8 µg/L
Apolipoprotein E [5]	34	6 mg/L	(1.6)	93.5 mg/L
Cystatin C (γ-trace)	13	3,1 mg/L??	(0.84)	0.75 mg/L
$ß_2$-microglobulin (CSF? < 1.8 mg/L)??	12	1,6 mg/L??	(0.4)	1.7 mg/L
Ferritin	473	6 µg/L	(0.0016)	120 µg/L
S100 protein	21	1,5 µg/L ??	(0.0004)	0.1 µg/L
Myelin basic protein	19	0.5 µg/L	(0.0001)	> 0.5 µg/L
Interleukin 6	26	10.5 ng/L		12 ng/L
Tumor necrosis factor α	17	5.5 ng/L		20 ng/L
Acidic gliafibrillar protein	49	0.12 µg/L		
Neuronal acetylcholine esterase	290	13 U/L		3 U/L
Tau protein	55-74	< 200 ng/L		20 ng/L
ß-Amyloid 1-42		> 600 ng/L		

Table 13 Reference ranges of free amino acid concentrations in cerebrospinal fluid (CSF) and serum of adults (Kruse et al. 1985).

Amino acid	CSF concentration		Serum concentration		CSF/serum ratio	
	Mean µmol/L	C.V. [%]	Mean µmol/L	C.V. [%]	Mean µmol/L	C.V. [%]
Isoleucine	8.1	25.9	80.1	25.8	0.107	33.4
Leucine	13.4	37.3	144.1	30.5	0.096	33.3
Phenylanine	9.7	26.8	70.0	40.4	0.155	31.0
Methionine	11.9	24.4	57.0	27.9	0.205	25.4
Proline	3.8	13.2	181.8	29.8	0.023	30.4
Valine	18.6	25.8	197.3	20.7	0.093	21.5
γ-Aminoburic acid	5.4	29.6	30.0	34.7	0.192	30.2
Alanine	33.3	25.8	382.5	33.5	0.098	48.0
Ethanolamine	9.1	23.1	11.7	29.1	0.804	27.4
Lysine	29.0	22.8	158.9	23.1	0.188	23.9
Ornithine5.5	5.5	40.0	69.9	43.1	0.082	41.5
Tryptophan	2.3	30.4	62.1	22.1	0.038	34.2
Tyrosine	13.8	19.6	83.2	25.1	0.173	20.2
Taurine	6.5	30.8	95.9	53.9	0.087	69.0
Cysteine/Cystine	3.0	- [1]	63.9	21.0	-	-
Glycine	11.1	46.8	310.3	34.9	0.039	41.0
Hydroxyproline	6.9	8.7	33.7	17.8	0.211	19.4
Glutamine	546.6	16.1	668.6	22.0	0.828	24.5
Theonine	25.5	36.9	156.9	43.3	0.179	46.4
Asparagine	37.7	13.0	168.0	19.5	0.238	14.7
Serine	29.8	29.5	136.6	28.8	0.227	37.9
Arginine	17.5	44.6	82.4	29.9	0.214	57.5

Part of the sample below the detection limit.

Table 14 Concentrations of lipids in CSF

Lipids	Concentration mg/L
Total cholesterin	5.4
Free cholesterin	1.9
Cholesterinester	3.5
Lecithin	2.0
Sphingomyelin	1.0
Cephalin	1.3
Cerebroside	0.4
Total lipids	19

References in Wildemann et al 2010:

Table 15 Mean concentrations (± SD) of Vitamin C and Vitamin E in CSF and serum [1]. Examples of active transport from blood into CSF (Vitamin C) and passive protein-associated transfer (Vitamin E)

	CSF [µmol/L]	Serum [µmol/L]	R(CSF/S)
Vitamin C	160 ± 34	42 ± 18 [2]	4.0
Vitamin E [3]			
?? α-tocopherol	(0.53 x C_{ser}) [4]	42 ± 15	0.0015 [5]
?? γ-tocopherol	(0.67 x C_{ser}) [4]	3.1 ± 1.8	

1) See Fishman and Thomas for further vitamins.
2) Serum (!) concentration of vitamin C (ascorbic acid) depends on Q_{Alb} (Ref. Reiber, Ruff, Uhr 1993).
3) Vitamin E is a mixture of 75-87% α-tocopherol and about 10% γ-tocopherol amongst others. In serum 70% of α- and γ-tocopherol are associated with HDL-LDL. α-tocopherol and γ-tocopherol pass the blood-CSF barrier associated to a protein with MW > 100 kD.
4) The tocopherol CSF/serum quotient correlates strongly with the albumin quotient: $Q_{\alpha\text{-}T} = 0.83 \times Q_{Alb}$ and $Q_{\gamma\text{-}T} = 0.76 \times Q_{Alb}$ (Ref, Uhr 1995).

Attachment III Relative Sensitivities of Immunodetection Methods

Antibody index vs. Western blot for detecting viral infections.

As has been convincingly shown (Felgenhauer and Reiber, 1992), the antibody index is more sensitive in cases of herpes encephalitis and zoster meningitis than a Western blot.

Antibody index vs. Western blot for detecting bacterial infections (borreliosis).

Over recent years, the detection of neuroborreliosis has triggered intensive discussions among different schools of thought. Because this disease is caused by very different species of *Borrelia* and the antibodies to be detected in different patients are directed against different antigens, it is very important that appropriate antigen coating is used in the ELISA. If the matching antigen is missing, specific intrathecal synthesis cannot be detected.

The inclusion of nonspecific antigens (e.g., p41 flagellin) in the coating for detecting Borrelia does increase the sensitivity, but it may also lead to misinterpretation (e.g., cross reaction with Treponema antibodies).

In this case, inclusion of a positive control in the Western blot (Fig. 4.**12**) may show whether antigens are present in the CSF which are not present in the serum and which have possibly escaped the testing with ELISA. The risk of cross reactivity between *Borrelia* species and *Treponema pallidum* is considered not so critical as sometimes claimed, as long as a complete CSF analysis is performed. When a positive *Borrelia* AI has been found, a Treponema AI can still be detected if this distinction was not already obvious from the patient's history, clinical picture, and basic CSF diagnosis.

Provided the ELISA for Borrelia has the appropriate antigen coating (Borrelia extract and vslE antigen), the Western blot may be abandoned. ELISA has a higher sensitivity.

Antibody index vs. oligoclonal IgG for detecting acute and chronic viral infections.

In case of varicella zoster meningitis, the antibody index is more sensitive than the detection of oligoclonal IgG (Felgenhauer and Reiber, 1992). This is also true for varicella zoster-induced facial paresis, where oligoclonal IgG can be detected in only 50% of cases while the antibody index is always increased. In general, the following rule applies:

- In chronic inflammation, the detection of oligoclonal IgG is more often successful (98% in patients with MS) than the detection of specific antibodies (MRZ reaction in 90% of patients with MS).
- In acute inflammation, the detection of intrathecal antibodies against the causative antigen is more sensitive than the detection of oligoclonal IgG.

This rule is also based on the fact that the intensity of intrathecal IgG synthesis is up to 60 times higher with a reaction against the causative antigen than with a polyspecific co-reaction (Quentin and Reiber, 2004).

Table 16. Relative sensitivities of qualitative vs quantitative methods

No. of patients	100
Oligoclonal + ve	98
AI-Index > 1.5	90
Plasma cells ↑	79
Q_{IgG} ↑	75
Pleocytosis (> 4/µL)	71
Q_{Alb} ↑	12

Felgenhauer K and Reiber H (1992).

Table 17.The diagnostic sensitivity of antibody indices (AI) as compared with the Western blot

Case	Diagnosis	ASI	Western blot
F.K.	Herpes encephalitis	2.1	Negative
R.S.	Zoster meningitis	3.2	Negative
W.S.	Herpes encephalitis	4.4	Positive
K.J.	Zoster meningitis	9.8	Positive
G.B.	Herpes encephalitis	37.9	Positive

Table 18. Five representative cerebrospinal fluid (CSF) findings in zoster meningitis. The blood-CSF barrier was not impaired except in case K.J., and the locally synthesized antibodies remained undetectable with isoelectric focussing or the differentiation diagram except in case S.K. There was a definite yet variable cross-reaction with HSV antigens in all cases

Day	Case	Cells/µL	Q_{Alb} x 10^{-3}	IgG_{Loc} %	Oligos.	VZV	HSV
3	M.A.	589	5.5	0	0	0.8	1.0
6	S.M.	211	7.8	0	0	2.2	1.9
9	K.J.	267	24.0	0	0	9.8	2.2
14	S.K.	239	4.6	38.0	+	28.6	2.8
24	R.P.	60	4.6	0	0	3.6	2.9
38	S.M.	9	4.7	0	0	2.5	2.0

Table 19. Five representative CSF findings in herpes simplex encephalitis. The blood-CSF barrier was disturbed in all cases, and the locally synthesized antibodies became visible as oligoclonal bands after isoelectric focussing. The humoral response is generally dominated by IgG and may persist for years. There is a cross-reaction with zoster virus antigens in two cases.

Day	Case	Cells/µL	Q_{Alb} x 10^{-3}	IgG_{Loc} %	Oligos.	VZV	HSV
6	B.K.	284	18.8	0	0	0.8	0.8
9	F.K.	227	22.7	13	+	2.1	0.8
17	G.B.	45	9.8	63	+	37.9	13.0
25	J.H.	44	14.0	69	+	46.7	-
90	G.P.	7	23.0	75	+	21.2	15.3

Table 20. Analytical sensitivity of FLC-K compared with Oligoclonal IgG. From the subsequent analysis of 320 patients in the routine laboratory with total 116 OCB positive CSF samples (OCB$\geq$2 Bds) we get the described frequencies of 2 to 24 bands. Of the CSF samples neg for OCB, 7/204 had a single band of which 3 had an intrathecal FLC-K synthesis. 26/204 cases without intrathecal IgG synthesis had ≥ 2 oligoclonal FLC-K bands in CSF.

	total	2 Bds	3 Bds	4-24 Bds
OCB +	116	7	8	101
FLC-K +	110	3	6	101

Attachment IV: Blood in CSF

A blood contamination in CSF can be due to a brain hemorrhage, but also by the CSF puncture, called arteficial hemorrhage. These cases are discriminated by the serial collection in three tubes.

In general it is not advisable to interprete data from a seriously bloody CSF sample in quotient diagramms as there is a frequent wrong interpretation as an IgM synthesis s. Example below).

In cases of emergency some few options are available. Independent of the most serious blood contamination we can detect an intrathecal synthesis of a immunoglobulin class if the quotient of the larger molecule is larger then that of a smaller molecule (QIgM > QIgA, QIgA > QIgG, QIgG> QAlb).

In a few cases it is also possible to correct for the artificial blood contamination.

Correction of Cell Count and Protein concentration in case of artificial blood contamination

As shown in Table 1 the error of the IgG value is < 20% even with 2000 erythrocytes/µL in CSF. When considering the large range of biological variation, minor artificial contaminations with blood (< 1000 erythrocytes/µL) are negligible regarding the protein values.

CSF with an artificial blood contamination of > 7000 erythrocytes/µL should not be used for interpretation in the quotient diagram because mathematical correction is no longer reliable.

Correction of leukocyte counts.

For every 1000 erythrocytes/µL, 1 leukocyte/µL should be subtracted from the leukocyte count.

Correction of CSF protein values.

The empirical protein concentration in the CSF (y) is mathematically corrected (y') on the basis of the erythrocyte count (z) in the CSF (z) according the following equation (Reiber et al., 2001):

$$y' = y - x \cdot z/v$$

x = Protein concentration in the serum in mg/L (e.g., total protein, albumin, or IgG)
y = Measured protein concentration in the CSF in mg/L, matching x
v = Erythrocyte count in the blood
z = Erythrocyte count in the CSF

If serum values are not available, an approximation can be arrived at by using the following mean values from a healthy cohort: v = 4.5 × 10^6/µL; total protein = 69 200 mg/L; Alb = 40 600 mg/L; IgG = 11 400 mg/L.The corresponding correction values of the protein concentrations and the percentage of deviation are shown in Table 1.

Table 21 Correction for artificial blood contamination. The fault in % of the uncorrected value refers to the following mean values in CSF: TP = 350 mg/L, Alb = 240 mg/L and IgG = 23,4 mg/L. Faulty deviation: $(y - y' / y) \cdot 100$ (%).

	Z	500	1000	2000	4000	8000
GEW	correction mg/L fault	7,7 2 %	15,4 4 %	30,8 8%	61,6 15%	123 30%
Alb	correction mg/L fault	4,5 1,8%	9,0 3,6%	18,0 7%	36,0 13%	72,0 23%
IgG	correction mg/L fault	1,3 5,1%	2,5 9,7%	5,0 18%	10,0 30%	20,0 46%

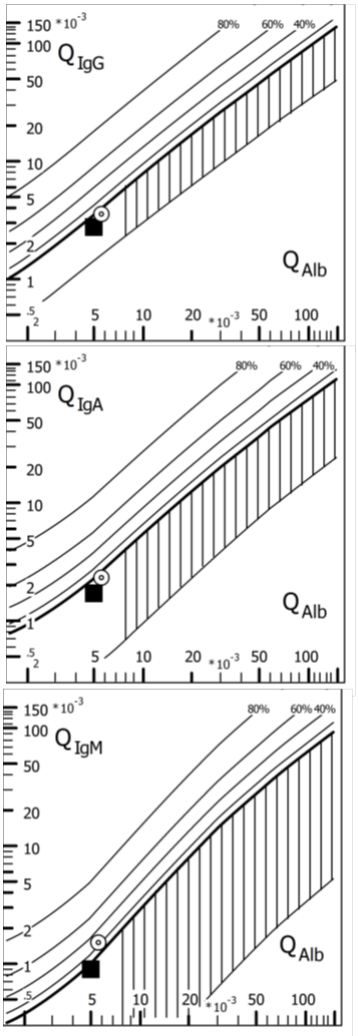
Calculation example with results in Reibergrams:

Table 21 : Correction of Blood contamination in Reibergrams. Calculations for patient example in Fig 8 with RBC Z = 2944/ µl CSF.

	X in Serum	X(Z/V)	Y in CSF	Y' corrected
Albumin	41800	26,8	239	212
IgG	11300	7,2	39,1	32
IgA	2100	1,3	4,9	3,6
IgM	800	0,5	**1,2**	**0,7**

The larger the molecular size of the protein the larger the relative correction. This is the reason why IgM is most frequent a risk for wrong interpretations, in particular at low QAlb values (flat Qlim).

Fig 8. CSF data correction in case of arteficial blood contamination 2944 RBC / µl CSF. Empirical data (circle with centerpoint) with slight intrathecal IgM synthesis . After Correction (s. Table 2) the intrathecal IgM synthesis disappears.



Attachment V: Statistical treatment of CSF Immunoglobulin data
Knowlegde base in the CSF Research tool of www.Albaum.it

Statistics for groups in quotient diagrams.

In contrast to the diagnostic approach with reference to the upper limit of the reference range QLim, the statistical approach for comparison of groups must refer to the mean of the control group, Qmean.

Diagnostic CSF Interpretation: **Single patient**

- Reference to Qlim
- IgG-IF for pattern recognition (IgG,IgA and/or IgM dominance)
- Detection of a pathological fraction with highest specificity > Qlim = Qmean +**3** SD (99% level)
- Statistical CSF interpretation: **Group analysis**
- Reference to Qmean
- IgG-loc (m) for quantitation of mean intrathecal synthesis in a group
- Frequency for pathological cases > Qmean +**2** SD (96% level)

Mean Intensities: The calculation of the mean amount synthesized uses the Igloc(mean) with reference to Qmean instead of Igloc with reference to Qlim.

Mean frequencies: The program uses as reference for statistically significant intrathecal Ig-synthesis the new borderline of Qmean+2SD instead of Qlim (=Qmean+3SD).

Attachment VI. Free light chain kappa in Reibergrams

(Reiber H, Zeman D, Kušnierová P, Mundwiler E, Bernasconi L. Diagnostic relevance of Free light chains in Cerebrospinal fluid – The hyperbolic reference range for reliable data interpretation in quotient diagrams. Clin Chim Acta 2019; 497: 153-162).

Background: Free light chains, type kappa (FLC-K), in cerebrospinal fluid (CSF) were compared to oligoclonal IgG in many studies for sensitive detection of immune reactions in brain. The missing consensus about CSF data interpretation prevents reliable conclusions. This can be overcome by a theory-based hyperbolic reference range in CSF/serum quotient diagrams.

Methods: Mean Quotients for FLC-K, Q_{Kappa}, and albumin, QAlb, of grouped, biochemically defined controls (N=433) were fitted with the hyperbolic function $Q_{Kappa}(mean) = a/b\ (QAlb^2 + b^2)^{0.5} - c$ by a generally applicable procedure excluding outliers.

Results: With $Q_{Kappa}(mean)$, the coefficient of variation CV (22.5%) and the reference range (QKappa(mean) ± 3 CV) we get the discrimination line $Q_{Kappa}(lim) = (3.27(QAlb^2+33)^{0.5} - 8.2)\ x10^{-3}$ in a FLC-K Reibergram. Intrathecal FLC-K was found in 8% of another control group without OCB (N=388) but is missed in 7% of patients with definite Multiple Sclerosis (N=95). In MS the mean intrathecal fraction is threefold larger for FLC-K (95%) compared to total IgG (36%). Similar mean quantities of intrathecal FLC-K contradict an immunological conversion between a Clinically isolated syndrome and MS.

Discussion: The hyperbolic reference range is superior to linear FLC-K Index (10 to 15 % false negatives) and exponential curves (30 % false positive interpretations for controls) in the analytical range of MS data, with excellent data fits for up to ten-fold larger QAlb values. Dynamics of the small molecule FLC-K contribute to the understanding of molecular size dependent barrier functions.

Normal laboratory data based Controls (oligoclonal IgG bands negative) of FLC-K are shown in a FLC-K Reibergram in Fig. 9.
The construction process of Reibergrams is described in details in this paper, as refered to also above.
The Table 22 shows the numerical data set for the upper, mean and lower reference lines with the restriction of the range of relevance typical for molecules smaller than albumin in the quotient diagrams.

Table 22. Parameters of hyperbolic functions, $Q_{Kappa} = a/b\ [QAlb^2 + b^2]^{0.5} - c$ (Fig.1). The functions are shown in FLC-K Reibergrams for the upper border line Qlim, the mean Qmean, and the lower border line Qlow in Fig 2. The theoretical limit of the valid range for the Q_{Kappa} functions in the QAlb quotient diagram is defined for $Q_{Kappa} << 0.5 = 500 \times 10^{-3}$. As example for QKappa(mean) = 500×10^{-3} we get for QAlb (0.5) = $[(504{,}85/1.95)^2 - 33]^{0.5} = 256 \times 10^{-3}$. $Q_{Kappa} = 1$ is reached at a QAlb(1) with values calculated as approximation from the asymptote. Corresponding calculations are performed for Qlim and Qlow.

	a-c	a/b	a [x10³]	b² [x10⁶]	c [x10³]	QAlb (0.5)	QAlb (1)
Q_{Kappa} (lim)	10,6	3.27	18.8	33	8.2	$< 155 \times 10^{-3}$	308×10^{-3}
Q_{Kappa}(mean)	6,35	1.95	11.2	33	4.85	$< 256 \times 10^{-3}$	515×10^{-3}
Q_{Kappa} (low)	2,54	0.78	4.48	33	2	$< 643 \times 10^{-3}$	$>1000 \times 10^{-3}$

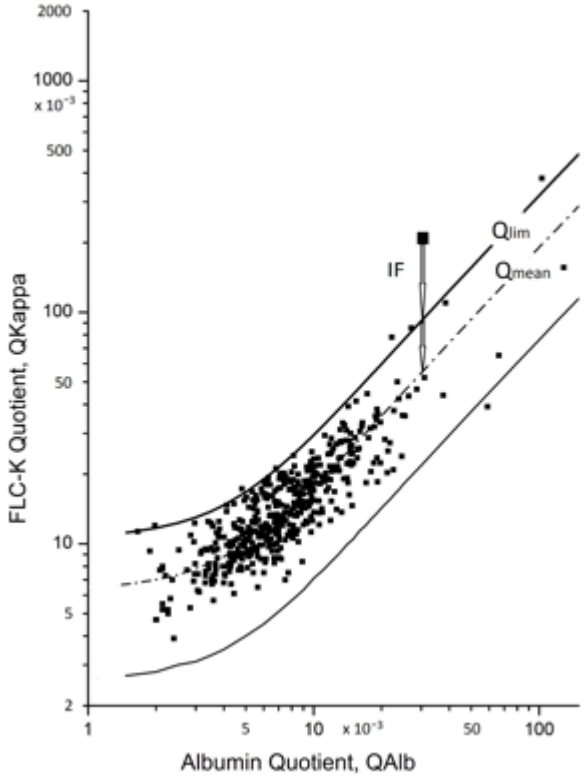


Fig 9. Reference range of CSF/ serum quotients, Q_{Kappa}, in a quotient diagram with linear axis. Qlim and Qlow are calculated from Qmean ± 3 CV (Table 22). Increasing standard deviations (SD) have nevertheless a constant coefficient of variation, CV = 22.5%. The variation of the grouped individual data support the theory-based constancy of the CV and symmetrical distribution around Qmean. The reference range includes 99% of the controls.

Universal validiy of the hyperbolic function is a consequence of the basic biophysical parameters: Diffusion und Fluid flow.

The evaluation of the FLC-K data contributed essentially to the flux /flow model (Reiber 1994) in the way to complete the curves up to the value of QFLC-K = 1 (Fig 10)

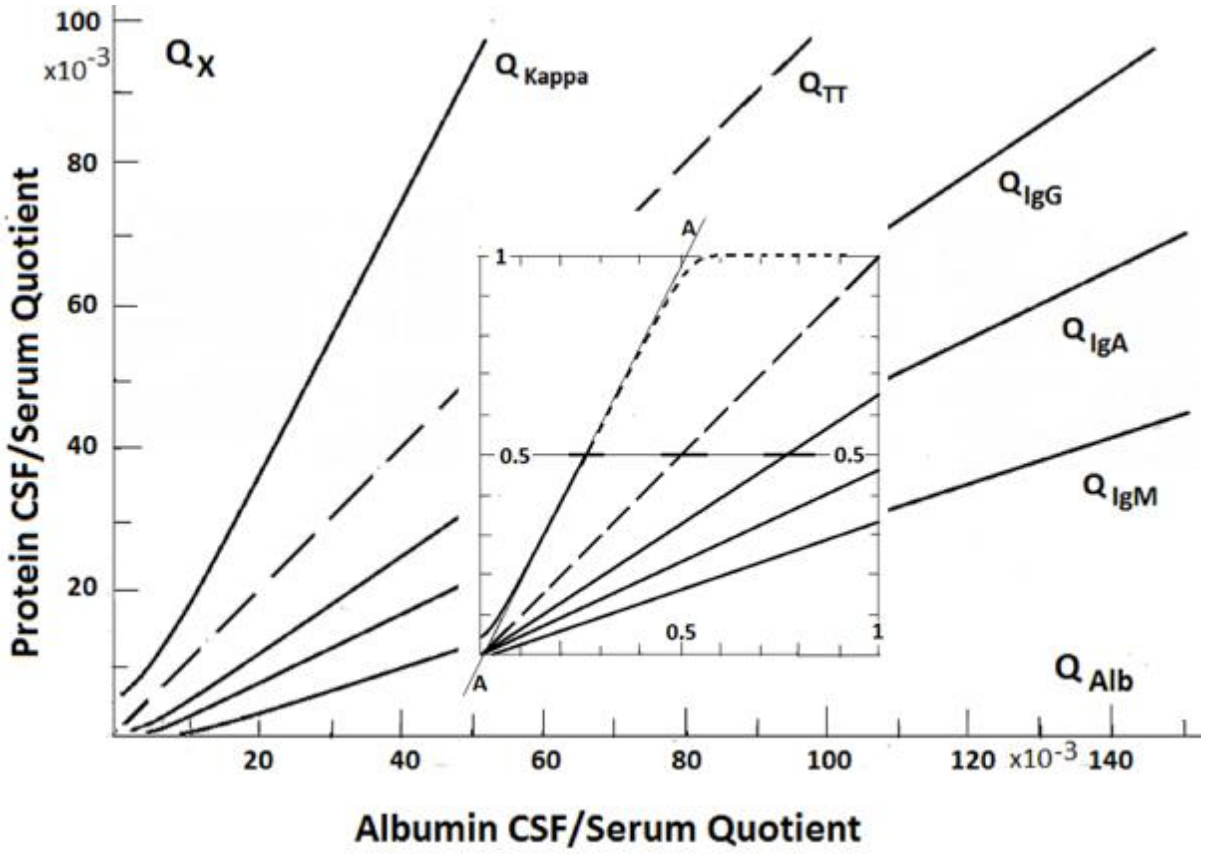


Fig 10 The general validity of the hyperbolic reference function.

Mean concentrations of blood-derived molecules as function of molecular size and increasing blood CSF barrier dysfunction. The CSF/serum Quotients of immunoglobulins (QIgM, QIgA, QIgG), Transthyretin associated with retinol binding protein, Q_{TT} and Free light chain kappa, Q_{Kappa}, are fitted with hyperbolic functions for the independently calculated Qmean values of the grouped QAlb intervals. Transthyretin (TT)/retinol binding protein complex (55 + 21 kDa) with similar molecular size as albumin represent the 45° line (dashed). The insertion shows the complete quotient diagram up to QAlb=1 and Q_{Kappa} =1 with the complete Q_{Kappa} curve. The curve for QKappa shows the application of error function, erfc, at low concentrations and error function, erf, at high concentrations. (Reiber H. Flow rate of cerebrospinal fluid (CSF)- a concept common to normal blood-CSF barrier function and to dysfunction in neurological diseases. J Neurol Sci 1994; 122: 189-203.)

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