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Review article

Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs

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Abstract

Cerebrospinal fluid (CSF) analysis is a basic tool for diagnosis of neurological diseases. Knowledge regarding blood–CSF barrier function (molecular flux/CSF flow theory) and neuroimmunology is reviewed to aid understanding and evaluation of CSF data. Disease-related immunoglobulin patterns (IgG, IgA, IgM with reference to albumin) are described in CSF/serum quotient diagrams with the hyperbolic reference range for blood-derived protein fractions in CSF. Clinical relevance of complementary analyses (cytology, PCR, oligoclonal IgG, antibody detection and brain-derived proteins) is briefly discussed. Integrated CSF data reports are shown with numerical and graphical data representation, reference range-related interpretation and diagnosis-related comments. The principles and rationale of general CSF analysis reported in this review should enable the reader to accurately interpret CSF data profiles, and to plan a proper evaluation of new brain- or blood-derived analytes in CSF.

Keywords: Cerebrospinal fluid; Neurological diseases; Neuroimmunology; CSF immunoglobulins; Brain proteins in CSF; CSF evaluation programs

1. Introduction — Goals of CSF analysis

Are lumbar puncture and CSF analysis still the mainstays of neurologic diagnosis as stated in 1986 by the Health and Public Policy Committee of the American College of Physicians [1]? Have imaging techniques displaced conventional CSF analysis? Have there been any important advances in the analysis of CSF in the past decade?

In fact, new physiologically and biophysically based concepts of blood–CSF barrier function [2] as well as extended knowledge of neuroimmunology [3] have led to an increased awareness of the clinical reliability of albumin and immunoglobulin-based data patterns [4–6]. Complementary methods for sensitive detection of intrathecal synthesis of specific antibodies [7–9] and a reliable concept for evaluation of brain-derived proteins in CSF [10,11] and blood have yielded essential improvements in clinical relevance of CSF analysis. Advances include protocols for detection of oligoclonal IgG as set forth in a European consensus report [12] and the introduction of polymerase chain reaction (PCR) for sensitive detection of microorganisms [13,14] as well as analysis for brain-derived proteins in blood [15].

Other technological advances include on-line computer nephelometry programs for CSF analysis (CSF-COM [Dade–Behring] or CSF-Report [Beckman–Coulter]) and the development of knowledge-based interpretation software [16]. There is an increasing acceptance of integrated CSF reports (Fig. 1) with graphical representation of immunoglobulin response patterns. A new type of pattern-based quality assessment (CSF survey) was introduced in Germany and several European countries [6].

The description of patterns of CSF *data* cannot, of course, yield a definitive diagnosis in the absence of clinical information. Nevertheless, in contrast to single

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variable analysis, the analysis of patterns can yield the following:

- Sensitive controls for analysis (part of analytical quality assessment);
- Suggestions for further analysis (to specify the interpretation of basic CSF data);
- Answers to clinical questions that aid differential diagnosis and clinical outcome assessment by:
  - Identifying disease-related, typical data patterns (e.g. neurotuberculosis, neuroborreliosis or opportunistic infections);
  - Ruling out a suggested diagnosis by inconsistent findings;
  - Confirming the causative microorganism of an inflammatory process (antigen or antibody or nucleic acid detection);
  - Early characterization of chronic inflammatory processes (autoimmune type, e.g. multiple sclerosis);
  - Detection of meningeal carcinomatosis;
  - Specifying neuropathological origin of psychiatric symptoms (inflammation, degenerative process);
  - Early detection of post-surgical infection;
  - Determining extent of cerebral hypoxia or effects secondary to brain infarction;
  - Monitoring efficacy of therapy and course of disease.

This review explores these clinical applications, discusses the rationale for computer-based CSF evaluation programs and shows examples of representative disease-related patterns of CSF data.

It is beyond the scope of this contribution to review clinical relevance of specific CSF data patterns, as described in several textbook contributions [3,5,17,18] as well as in a variety of articles limited to a single disease [19–24]. For more general clinical, physiological, neuroanatomical or biochemical references, see Davson and Segal [25], Felgenhauer [17,26], Fishman [27], Herndon and Brumback [28], Thompson [29] and Reiber [3,18,30].

2. Analytical program

A systematic protocol of CSF analytical procedures might differentiate among an emergency program, a basic CSF program and an extended program with rarely analyzed but clinically significant analytes.

2.1. Emergency program

As part of point-of-care testing, this program includes total protein, cell count, visual inspection for hemorrhage, lactate analysis and differential cell count with characterization of bacteria [34].

2.2. Basic CSF program

This program includes the parts shown in the integrated CSF report in Fig. 1:

1. Total cell count; differential cell count; total protein in CSF;
2. Albumin, IgG, IgA, IgM in CSF and serum;
3. Oligoclonal IgG;
4. Specific antibody indices (IgG class) when appropriate for measles, rubella, varicella-zoster virus, herpes simplex, HIV, CMV, toxoplasma, and *Borrelia burgdorferi* (IgM and IgG classes) as well as for *Treponema pallidum*;
5. Lactate in CSF or glucose in CSF and serum can be useful in some diseases;
6. The red blood cell count and Hgb in CSF, as well as the gross characteristics of CSF: clear, turbid, blood-contaminated or xanthochromic;
7. The laboratory team is helped by important clinical information including age of the patient and, in particular, the differential diagnostic question.

2.3. Extended program

Among the less common analyses of clinical relevance are:

1. PCR, in particular for HSV encephalitis [13], tuberculosis [31] and opportunistic infections (CMV, Toxoplasmosis) [14];
2. Tumor markers such as carcinoembryonic antigen (CEA) in CSF and serum [23];
3. Brain-derived proteins like neuron-specific enolase in blood [15] to characterize damage after hypoxia, or tau protein together with β-amyloid 1-42 in CSF [33,75] are important analytes in dementive processes as well as protein 14.3.3 [35], among others [3,17,34,68,69];
4. Beta-trace protein [32] to detect CSF in nasal secretions.

3. Physiology and pathophysiology of CSF

3.1. Blood–CSF barrier function and CSF flow

The ‘blood–CSF barrier’ for proteins represents a functional term that includes all processes that influence the final protein concentration in lumbar CSF, including blood–brain barrier, protein diffusion into CSF along its flow path and, in particular, the CSF flow rate [2,10,11]. The ‘blood–brain barrier’ refers to the morphological basis for restriction of protein diffusion from blood into the brain tissue, in particular by the brain capillary walls.

CSF Report

Client / Sender	Patient
Tel:	XXXXXXX, YYYY * 5 / 7 / 63 Sample receipt: 6 / 5 / 99

Differential diagnostic question	Punct. date: 6 / 5 / 99
Inflammatory process?	Physician:

Puncture			Visual inspection				Hgb				Vol.	TP (Pandy)		
(LP)	CP	VP	turbid	xanth.	bloody	artif. bl.	(0)	+	++	+++	6 mL	(0)	+	++

Cells

Cellcount	3	/mm³	RBC		/mm³
Lymphoc.	80	%	Mononucl.c. 20	%	PMNR (n.) %	Plasmac. few

Other Cells		
Activ. B-Cells		% of Lymphocytes

Proteins

	CSF		Serum		Q(CSF/Ser) · 10³		Intrathec. fraction
Tot. Prot.	270	mg/L					
Albumin	111	mg/L	41.4	g/L	Q_{Alb} =	2.7	
IgG	56	mg/L	8.8	g/L	Q_{IgG} =	6.3	73 %
IgA	1.9	mg/L	2.2	g/L	Q_{IgA} =	0.9	%
IgM	2.1	mg/L	2.0	g/L	Q_{IgM} =	1.0	56 %

Oligoclonal IgG – Bands in CSF (Type 2)

Specific Antibodies

Measles-AI	= 9.2	HIV-AI	=	Borrel.-AI(IgG)	=
Rubella-AI	= 12.3	CMV-AI	=	Borrel.-AI (IgM)	=
VZV-AI	= 8.1	Toxopl.-AI	=	.....-AI	=
HSV-AI	= 1.0	.....-AI	=	.....-AI	=

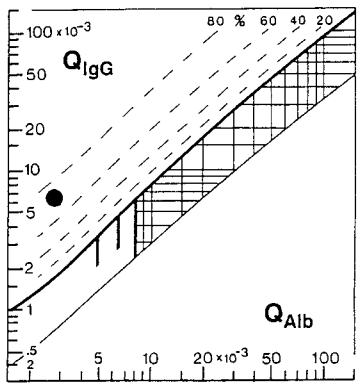
Lactate 1.6 mmol/L CSF

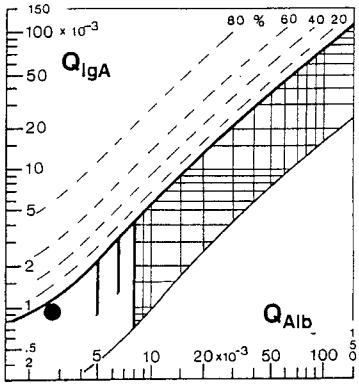
Interpretation

Normal CSF	☐	Normal CSF proteins	☐
B-CSF Barrier dysfunction	☐	Cellcount increased	☐
Inflammatory proc. in CNS	X	Spec. Ab-Synthesis in CNS	X

Comment:

Chronic inflammatory process. DD: Multiple sclerosis or autoimmune disease with involvement of CNS





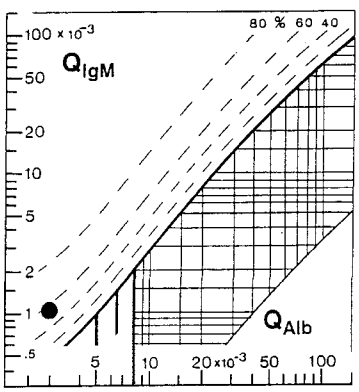


Fig. 1. CSF Report of the Neurochemistry Laboratory, University Göttingen. The data pattern originates from a patient with definite multiple sclerosis. At time of first diagnostic puncture, the clinician suspected an inflammatory process. By CSF analysis, a normal cell count, few plasma cells, lack of barrier dysfunction, together with an intense intrathecal humoral immune response (IgG_{IF} = 73% and IgM_{IF} = 56%) and oligoclonal IgG (interpretation type 2) were observed in CSF. These basic data suggested testing further for MRZ antibodies. The observed polyspecific intrathecal immune response (intrathecal synthesis of measles, rubella and varicella-zoster antibodies, with AI values >1.5) support the interpretation as a chronic inflammatory process of autoimmune type, already at the time of first clinical symptoms or in monosymptomatic cases. This statement extends significantly the information possible to obtain from oligoclonal IgG in any acute and chronic inflammatory processes. As this polyspecific MRZ reaction is not specific for MS, the differential diagnostic discrimination between multiple sclerosis and an autoimmune disease with involvement of the CNS needs further analysis, e.g. for antinuclear antibodies in blood. This type of integrated CSF data report is implemented in the on-line evaluation program of Dade–Behring (DSS-COM) or in the CSF-Report of Beckman–Coulter (Germany).

All blood proteins traverse capillary walls by passive diffusion (molecular flux) into brain, extracellular fluid and cerebrospinal fluid. According to the laws of diffusion, larger molecules, like IgM, are slower in exchange and subsequently form a steeper blood-to-CSF concentration gradient (3000:1) than do smaller molecules like IgG (500:1) or albumin (200:1).

Increased blood concentrations of a serum protein generally result in higher CSF concentrations, but the gradient remains constant at equilibrium; thus, variations in CSF values that are a direct result of individual variation in serum concentrations can be appropriately expressed as CSF/serum concentration quotients (e.g. albumin with $Q_{Alb}=CSF_{Alb}/serum_{Alb}$). Such quotients can be understood, biologically, as an overall concentration gradient or, mathematically, as a normalized, dimensionless CSF protein concentration, which is independent of blood variations.

The CSF flow rate (CSF turnover) modulates the concentration of molecules, e.g. decreasing CSF flow rate in certain neurological diseases results in an increasing serum protein concentration in CSF. The albumin CSF/serum concentration quotient, Q_{Alb}, is a widely accepted indicator of blood–CSF barrier function [12], including CSF flow rate. Increased albumin concentrations in CSF must always be due to blood–CSF barrier dysfunction, because albumin originates exclusively from blood.

A reduced CSF flow rate can originate from: (1) reduced production rate, (2) restriction of flow in the subarachnoid space, or (3) blocked flow through arachnoid villi into venous blood (examples with references in [2]). In general, a pathological increase of proteins (e.g. IgG) in CSF can occur either due to blood–CSF barrier dysfunction (notably, decreased CSF flow rate) and/or intrathecal synthesis. To discriminate between these causes, the IgG quotient, Q_{IgG}, is compared to the albumin quotient, Q_{Alb} [45,47,48].

Due to a steady diffusion of serum proteins into CSF along its flow inside the subarachnoid space, the concentration of blood-derived proteins steadily increases between ventricular and lumbar CSF [2]. This is the rostro-caudal concentration gradient, e.g. 1:2.5 for albumin.

To confirm intrathecal synthesis, the comparison of CSF/serum quotient levels of immunoglobulins has been done in linear approaches such as IgG Index [46] or by Tourtellotte's IgG synthesis rate [44]. However, the relation between Q_{IgG} and Q_{Alb} is non-linear [48–50] and, therefore, better described by the hyperbolic discrimination lines (numerical or graphical). The diagrams (Figs. 1 and 2) known as Reibergrams [30,37], include:

- vertical lines to indicate the age-related reference range for the albumin quotient (increased values indicating blood–CSF barrier dysfunction);
- a hyperbolic discrimination line to separate the reference range for the fraction of blood-derived IgG (below the line) from the intrathecally synthesized IgG fraction (above the line).

The hyperbolic function was introduced first as empirical observation [36] and is now buttressed by the molecular flux/CSF flow theory [2]. According to this theory, a reduced CSF flow rate is recognized as a sufficient, quantitative explanation of the dynamics of CSF proteins, including both blood-derived [2] and brain-derived [10,11] proteins.

The reduced CSF volume turnover has two related consequences [2]: the steady transfer of proteins from blood into CSF initially leads to a linear increase in protein concentration in CSF. As CSF protein concentrations continue to increase, the molecular flux increases, i.e. more molecules per time unit diffuse from blood into CSF (this cycle of increasing CSF concentration and increasing molecular flux is like a positive feedback loop in chemical reactions). The biophysical aspect of the increasing local concentration gradient at the border between meninges and subarachnoid space might seem to be paradox to a reduced overall blood/CSF concentration gradient [3,30]. But the increase of molecular flux into CSF [2] is actually the cause of a non-linear function of CSF flow rate on the blood–CSF barrier function. This function does not presume a morphological change of 'barrier' structures. In particular, the still widespread idea of a 'leakage' [17] with increased fluid flow from blood into CSF is contradicted by the empirically observed dynamics [2,30]. Imaging techniques like nuclear magnetic resonance spectroscopy provide further evidence that CSF flow rate changes occur in neurological diseases. The change in CSF flow rate can be regarded as the main modulator of the concentration of CSF proteins in pathological conditions characterized by 'blood–CSF barrier dysfunction'.

3.2. Dynamics of brain proteins in CSF

About 20% of the proteins in CSF are predominantly brain-derived, but only rarely are brain-specific [29]. Commonly, both brain-derived and blood-derived fractions contribute to the CSF concentration. The basic feature of predominantly brain-derived proteins is their higher concentration in CSF compared to serum, which induces a net flux out of CSF compared with blood proteins which exhibit a net flux into CSF [11].

The dynamics of brain-derived proteins have been investigated [11] with reference to the CSF flow rate as reflected by CSF/serum albumin concentration quotients. Three different groups can be discriminated: proteins from neurons or glial cells (e.g. tau protein, neuron-specific enolase and S-100 protein) which enter CSF primarily in the ventricular and cisternal space. Their concentration decreases between normal ventricular and lumbar CSF in

contrast to blood proteins, and in the case of pathologically decreased CSF flow rate, the concentration in lumbar CSF remains constant. Brain proteins with primarily leptomeningeal origin (e.g. β-trace protein and cystatin C) show an increasing concentration (11-fold for beta trace protein) between normal ventricular and lumbar CSF and, in the case of pathologically decreased CSF flow rate, a linearly increasing concentration in lumbar CSF [11]. The characterization of the ventricular-to-lumbar concentration gradient in addition to the CSF-to-blood gradient can yield important information about the source of the protein and the influence of pathophysiological processes.

These empirical results for brain proteins [11] buttress the general reliability of the molecular flux/CSF flow theory as a physiologically correct, biophysically derived concept for the evaluation of brain-derived proteins, blood-derived proteins or proteins with mixed sources in brain, leptomeninges or blood. In particular, the reference to the albumin quotient as a measure of CSF flow rate is important for evaluating and understanding protein concentrations and their change in CSF in health and disease. For example, failure to compare the concentrations to Q_{Alb} in the evaluation of angiotensin converting enzyme (ACE) [10], soluble intercellular cell adhesion molecule (sICAM) [63], prothromblin [62] or β-trace protein concentrations [11] could lead to erroneous interpretations of increased concentrations of these proteins in lumbar CSF. As another example, a non-linear saturation-like occurrence of sICAM

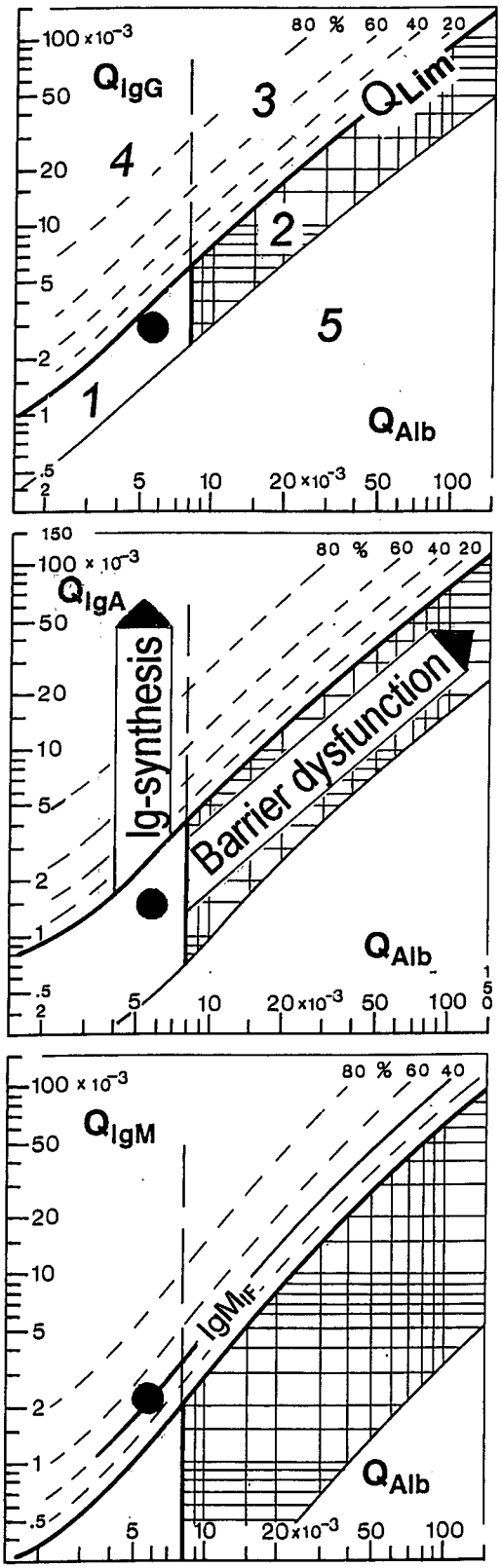


Fig. 2. CSF/serum quotient diagrams for IgG, IgA, IgM with *hyperbolic* graphs according to Reiber [2,6,30,37,38]. The reference ranges of blood-derived IgG, A, M fractions in CSF (range 1 and 2) include between upper (Q_{Lim}) and lower hyperbolic discrimination lines 99% (±3 S.D.) of the 4300 patients investigated. The upper hyperbolic curves (thick lines) of the reference range represent the discrimination lines between brain-derived and blood-derived immunoglobulin fractions in CSF, called Q_{Lim} (Lim from limit). Values above Q_{Lim} represent intrathecal fractions (IF) as percent of total CSF concentration as IgG_{IF}, IgA_{IF}, or IgM_{IF}. These intrathecal fractions can be conveniently and directly read from the quotient diagrams with lines for 20, 40, 60 and 80% intrathecal synthesis with the upper discrimination line (Q_{Lim}) as 0% synthesis. The example shows $IgM_{IF}=40\%$. The limit of the reference range for Q_{Alb} between normal and increased CSF protein concentration (blood–CSF barrier dysfunction) is indicated by the age-dependent vertical line, which in this case is for a patient aged 60 years. A general function describing the upper limit of the age groups above 5 years is $Q_{Alb}=(4+\text{age}/15)\times10^{-3}$. In the diagrams (Figs. 1 or 6–9), three vertical lines are implemented at $Q_{Alb}=5\times10^{-3}$ (up to 15 years); at $Q_{Alb}=6.5\times10^{-3}$ (up to 40 years); at $Q_{Alb}=8\times10^{-3}$ (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 and 8% for the quotients of albumin, IgG, IgA and IgM [38]. Due to larger variations between laboratories, intrathecal Ig synthesis should be considered elevated if the intrathecal fraction Ig_{IF} is larger than 10%. For construction of diagrams, see Appendix A. Patient example: the clinical information supplied (facial nerve palsy) together with the data in the diagrams (intrathecal IgM fraction of $IgM_{IF}=40\%$ and oligoclonal IgG [with $IgG_{IF}=0$]) 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. An alternative cause of facial nerve palsy, VZV-ganglionitis, is described in Fig. 8a.

in CSF [63] points to possible metabolic interference. In the case of β-trace protein in bacterial meningitis [32], the Q_{Alb}-independent reduction of β-trace protein concentration in lumbar CSF [32] points to a particular influence of the meningeal reaction on the release of β-trace protein from leptomeningeal cells [11].

This basic information about CSF dynamics including the ventricular/lumbar and CSF/blood gradients also helps to determine whether the absolute concentration (e.g. NSE, S-100, tau protein) or the CSF/serum concentration quotient of a brain protein (e.g. sICAM) should be used for most sensitive evaluation.

3.3. Neuroimmunology — the particular immune response in CNS

The intrathecal synthesis of antibodies in CNS originates from perivascular infiltrates of B-lymphocytes that proliferate and mature locally. In contrast to the well known switch of IgM synthesis to IgG synthesis in blood of patients with an infection (Fig. 3), the immune response in the CNS is commonly characterized by lack of an intrathecal switch from IgM class response to IgG class response (Fig. 3). The initial pattern of the IgG/IgA/IgM class response in the CNS [3–5,17,38] is apparently related to particular diseases with particular causes and to the corresponding particular pathological processes (Table 1). As an example, neuroborreliosis [19] can exhibit a relatively constant relation between intrathecal IgG/IgA/IgM synthesis over many months (Fig. 3) in spite of a typical switch from an IgM class to an IgG class response in blood of the same patient (lower diagram in Fig. 3). The CSF/serum quotients do not exhibit the concentration changes of IgM and IgG in blood (a higher IgM concentration in blood induces a higher IgM concentration in CSF, but the quotient Q_{IgM} remains constant). This absence of classical immune regulation in brain might reflect the low level of regulatory cells and total antibody concentration in the brain.

As a consequence of the low transfer of blood-derived antibodies to brain and CSF, the brain-derived CSF antibodies in a CNS inflammation contribute a relatively large proportion of the total CSF antibodies. Indeed, the intrathecally produced IgG fraction can account for more than 90% of total IgG in CSF (e.g. IgM_{IF}=85% in Fig. 7b). In the blood, in contrast, an acute inflammation might increase total IgG by only a few percentage points. This is of tremendous analytical relevance for sensitive detection of oligoclonal IgG bands in the CSF. These CSF-restricted bands [12] when first observed in CSF by isoelectric focusing (IEF) were called oligoclonal IgG, long before a network theory of the immune system [40,41] was considered. The term ‘oligoclonal IgG’ has persisted despite the fact that the many bands in IEF reflect primarily the polyspecific and only secondarily the oligoclonal nature of immune response [9,12,21].

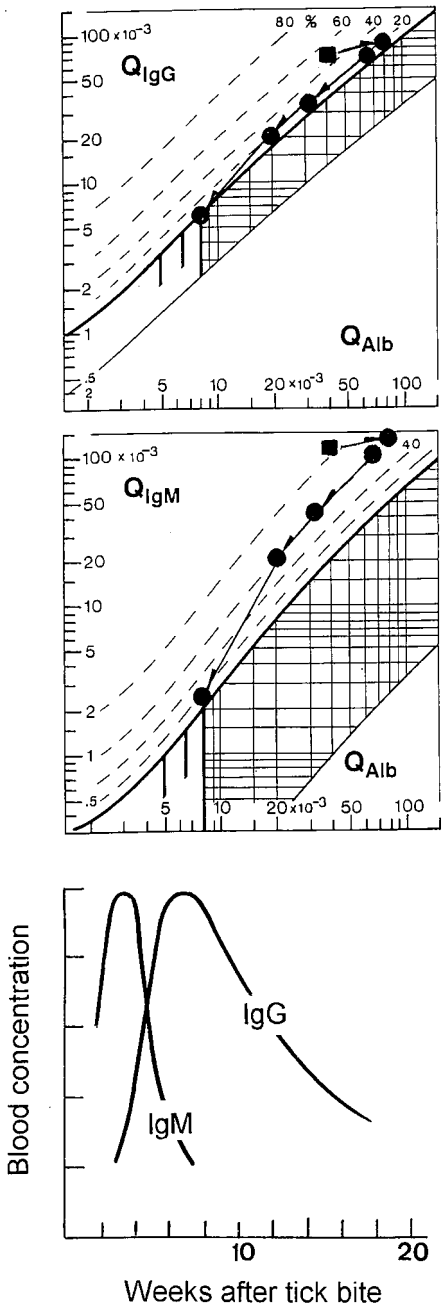


Fig. 3. CSF protein changes in a patient with neuroborreliosis. CSF samples were obtained at 3 (■), 4, 6, 10, 16 and 83 weeks after tick bite with the sequence of data indicated by arrows; cell counts were 132, 100, 39, 90, 15 and 3/μl, respectively. *Borrelia* AI (IgM)=31 and *Borrelia* (IgG) AI=42 were found at time of first puncture. The intrathecal fraction of IgM (IgM_{IF}) is constant between the 4th and 16th week after tick bite (2nd to 5th puncture) and is independent of serum variations of IgM (lower diagram). In contrast to relative intrathecal fraction IgM_{IF}, the absolute CSF concentration, IgM_{Loc} in mg/l, varies with the albumin quotient (CSF flow rate) according to Table 3 for the subsequent punctures (2nd to 5th). The lower diagram shows the relative serum concentrations of IgM and IgG depending on time after infection. In spite of decreasing IgM and increasing IgG in serum at the time of first diagnostic CSF puncture, this is not reflected in CSF/serum quotients, i.e. the intrathecal synthesis does not show this IgM/IgG switch which is present in blood.

Table 1
Humoral immune response patterns in CNS at time of first diagnostic CSF puncture

Reaction type	Disease
No IgG, IgA, IgM	Early bacterial meningitis and viral encephalitis, Guillain–Barré polyradiculitis
IgG dominance	Multiple sclerosis (lower frequency of IgM, 25%, and IgA, 9%)
	Neurosyphilis (low frequencies of increased IgM, no IgA)
	Chronic HIV encephalitis
IgA dominance	Neurotuberculosis (IgA dominant with weak IgG response)
	Brain abscess
	Adrenoleukodystrophy
IgM dominance	Lyme neuroborreliosis (IgM dominant: $IgM_{IF} > IgA_{IF} > IgG_{IF}$)
	Mumps meningoencephalitis (IgM dominant)
	Non-Hodgkin lymphoma involving CNS (isolated $IgM_{IF} > 0$)
IgG + IgA + IgM	Opportunistic infections (CMV, toxoplasmosis)

Together with specific antibodies against the causative antigen, there is a large fraction (>70%) of immunoglobulin with many different antigenic specificities not related to the cause of the disease. In HSV encephalitis [21], for example, HSV antibodies in CSF represent only up to 20–30% and in subacute sclerosing panencephalitis [39], measles antibodies represent only up to 30% of the intrathecally synthesized IgG fraction. In chronic diseases, like multiple sclerosis, in spite of an intense intrathecal immune response, the amount of intrathecally synthesized antibodies for a single species (e.g. measles antibodies) is less than 1% of intrathecally synthesized IgG [21].

This phenomenon of heterogeneity of the immune response is explained by modern immune network theory [40] which holds that each immune reaction induced by a single microorganism or antigen involves the whole immune network [40,41]. In addition to the specific antibody against the causative microorganism there is also increased production of many other antibodies and autoantibodies with different specificities — a phenomenon called polyspecific immune response. The polyspecific antibody response in CNS can persist even in the absence of a corresponding antigen [21]; the polyspecific autoantibodies and the antibody synthesis in blood of patients with Guillain–Barré polyradiculitis represent an important example [42]. The Desert Storm syndrome has been proposed to be a consequence of such a polyspecific concomitant immune response after immunization [41]. These reactions can be misleading for differential diagnosis, e.g. an increased Toxoplasma Antibody Index suggesting intrathecal toxoplasma antibody synthesis is actually ascribed to a polyspecific immune response in 10% of multiple sclerosis patients [21].

Another consequence of the larger contribution of local CNS immune reaction than blood-derived fraction in CSF is the possibility to observe the slow decay of the intrathecal response after the acute phase of a disease, e.g. intrathecal IgG *Treponema pallidum* antibodies (Fig. 4) are still detectable in some patients 20 years after recovery. This type of slowly diminishing intrathecal antibody synthesis is also described for HSV encephalitis [3,17] and in neuroborreliosis, albeit with shorter duration of observation [19] (Fig. 3).

From these examples, one can conclude that the intrathecal humoral immune response is not necessarily indicative of the current disease activity; rather, it can reflect three different processes: (1) Acute inflammatory disease of CNS manifest by an increased CSF cell count and an increased CSF/serum concentration of the Q_{Alb}. (2) Residual intrathecal antibody synthesis from an infection in the past, not relevant to the current clinical symptoms (characterized by absence of barrier dysfunction and low IgM titers in blood). (3) A chronic autoimmune type inflammatory process (suggested by polyspecific MRZ antibody reaction).

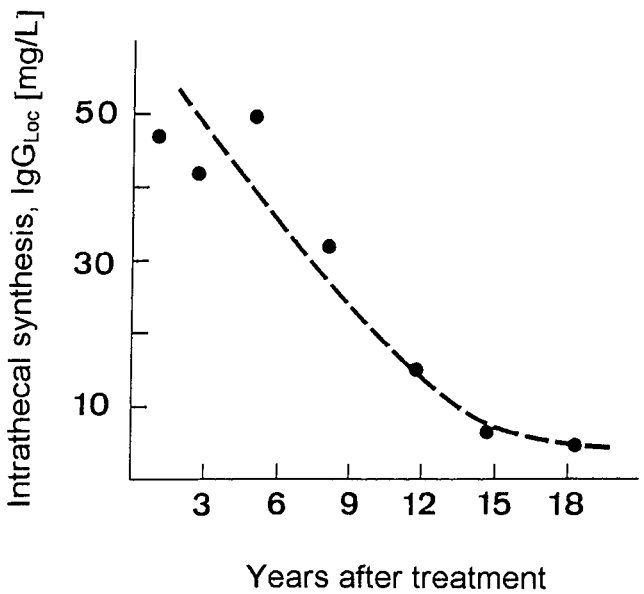


Fig. 4. Delayed normalization of intrathecal immune response in seven different patients with neurosyphilis. The CSF punctures were done at different times after sufficient treatment of acute disease. Data from two different patients in the acute phase are shown in Fig. 7b. Comparison of IgG_{Loc} values is possible, as changes of the blood–CSF barrier function are negligible in this disease group.

4. Evaluation and interpretation of CSF data

Total protein, cell counts, individual protein values or specific antibody titers in CSF are often reported at different times on separate forms to the clinician, making it difficult to visualize the complete picture. Compilation of all CSF data into a single data report allows the physician to recognize disease-related patterns and to identify areas for further analysis; it also allows a form of quality control.

Fig. 1 shows a CSF data report developed in the Neurochemistry Laboratory Göttingen that is used (with modifications) in hundreds of laboratories and neurological centers. Commercial PC on-line programs for protein analysis and evaluation (e.g. Dade–Behring or Beckman–Coulter) support use of this integrated report. Interpretation can be facilitated by a knowledge-based evaluation program [16].

The CSF report includes several sections, some of which are discussed in the following paragraphs:

- Clinical information provided by the attending physician;
- CSF gross characteristics;
- Cytology;
- Protein analysis including immunoglobulin class analysis in CSF and serum;
- Graphical evaluation in quotient diagrams;
- Immunoglobulin class response patterns;
- Oligoclonal IgG as the complementary and most sensitive, qualitative method;
- Specific, intrathecal antibody response (Antibody Index);
- Brain protein markers for tumors, brain destruction, dementia, cerebral hypoxia, CSF leaks, etc.;
- CSF lactate (or glucose in CSF and serum);
- Reference range-related interpretations;
- Interpretive comments regarding the analysis or differential diagnosis.

4.1. Quantitation of blood–CSF barrier dysfunction

The calculated CSF/serum concentration quotient, Q, e.g. for albumin: $CSF_{Alb}/serum_{Alb} = Q_{Alb}$ has a higher sensitivity for barrier dysfunction than the absolute CSF concentrations (Table 2). In particular, if CSF and serum are analysed in the same analytical run, the precision of quotients is higher and values are independent of method. Total protein concentrations in CSF show about threefold larger variations than the albumin quotient in almost all concentration ranges (Table 2). Table 2 also helps to convert ranges of total protein into ranges of Q_{Alb}.The ratio of albumin to total protein in CSF (35–80%, mean 57%) is sometimes useful as a plausibility control in case of discrepant CSF protein values.

To be taken into account, however, are the age-related variations of the reference range of Q_{Alb} [6,16] as well as the fact that the albumin concentration in lumbar CSF (like all blood-derived proteins) decreases with increasing volume of CSF extracted. Due to the ventricular–lumbar concentration gradient, the CSF albumin concentration as well as other blood-derived proteins decreases 20% between 1 and 12 ml of CSF extracted by lumbar puncture. The influence of volume extracted is usually ignored but makes clear how important the Q_{Alb}-related evaluation is for detection of intrathecal Ig synthesis. Three age-related reference ranges of Q_{Alb} are laid out in the quotient diagrams (Fig. 1 or Fig. 2). The extremely high protein concentrations in the newborn decrease in the first 4 months of life and, hence, require other reference ranges [6]. A continuous depiction of the reference range for Q_{Alb} (Appendix A) is valid in age groups above 5 years [16].

Table 2
Blood–CSF barrier dysfunction. Relationships among CSF/serum albumin quotients (Q_{Alb}), total protein (TP), and ratio of CSF albumin/CSF TP in CSF ($n = 20$ per group)

Q_{Alb} ($\times 10^3$)		TP (mg/l)		Albumin/TP (%)	
Range	Mean	Range	Mean	Range	Mean
2.0–3.5	2.7	174–323	232	34–61	51
3.6–5.0	4.1	235–396	313	42–66	52
5.1–6.5	5.9	318–596	411	49–68	58
6.6–8.0	7.3	419–605	510	53–81	61
8.1–10.0	8.8	417–774	580	38–80	59
10.1–12.0	11.0	600–1106	766	36–68	57
12.1–14.0	12.8	556–1042	851	50–70	56
14.1–16.0	14.8	551–1228	951	30–82	55
16.1–20.0	17.8	695–1450	1167	40–69	53
20.1–24.0	21.6	885–1752	1323	40–74	58
24.1–28.0	26.1	1316–2295	1735	48–73	57
28.1–32.0	29.8	1226–2768	1759	38–75	57
32.1–36.0	34.5	1416–2718	2051	41–79	59

To obtain reference values for the albumin quotient in ventricular CSF and cisternal CSF, the age-related reference values of lumbar Q_{Alb} are multiplied by a factor of 0.4 to convert into ventricular Q_{Alb} and by a factor of 0.65 to convert to the cisternal Q_{Alb} reference range. In case of a blood contamination, a red blood cell-related correction of the protein concentration in CSF can be made (Appendix A).

A pathological increase in Q_{Alb} has limited power for differential diagnosis [17], but is, in general, a reliable sign of an active, acute process. The clinical relevance of an increased Q_{Alb} must be interpreted in the context of other pathological signs (see disease-related CSF data patterns in Section 5).

4.2. Comparison of IgG synthesis rate, IgG index and hyperbolic functions

The evaluation and interpretation of the intrathecal fraction (IF) of immunoglobulin synthesis (Ig_{IF}, i.e. IgG_{IF}, IgA_{IF} or IgM_{IF}) can now be reliably derived from the

hyperbolic discrimination function, which was empirically determined for a large range of the blood–CSF barrier dysfunctions based on findings in 4300 patients [2]. The resultant diagrams (Fig. 2), which also provide a uniform basis for IgA and IgM evaluation, are commonly referred to as Reiberdiagrams or Reibergrams in some countries [30,37]. Fig. 5 shows a comparison of the hyperbolic discrimination function with the IgG synthesis rate [44,51] and with the IgG Index [46].

Earlier studies comparing the clinical relevance of calculations for assessing intrathecal synthesis of IgG [51–55], frequently relied on multiple sclerosis patients with characteristically normal or only slightly increased albumin quotients (Fig. 1). Consequently, the serious limitations of linear approaches, when Q_{Alb} is strongly increased as in many neurological diseases, were not detected [54]. The hyperbolic discrimination line avoids the false-positive

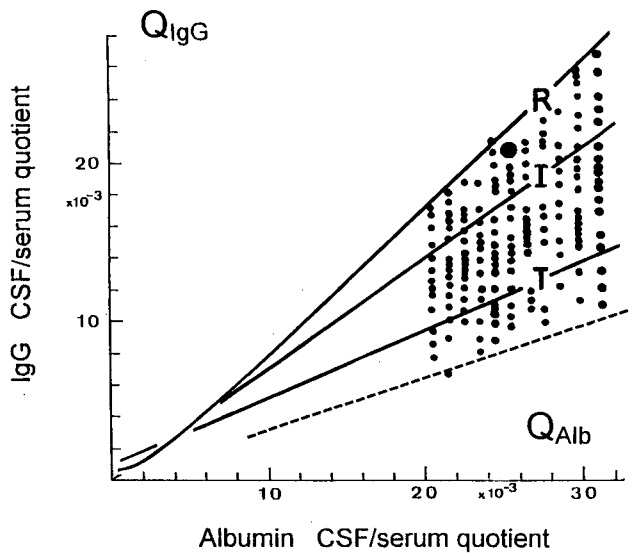


Fig. 5. Comparison of different discrimination lines (upper border of the reference range for blood-derived IgG in CSF) to detect intrathecal IgG synthesis. R, Reiber's hyperbolic discrimination line, Q_{Lim} (where $IgG_{IF}=0$) [2]. I, Link's IgG Index [46]. Graphical representation of the usually numerical evaluation with a discrimination line for $I=0.7$ (unitless). T, Tourtellotte's IgG synthesis rate [44,51]. The daily production rate (in 500 ml) can be multiplied by two to get the concentration per liter. The discrimination line for zero intrathecal synthesis ($IgG_{Syn}=0$) is calculated from the mathematically transformed function as: $Q_{IgG}=0.43\times Q_{Alb}+0.00084$. The data points represent the restricted range of $Q_{Alb}=20–30\times10^{-3}$ from the earlier clinical study with 4300 patients [2]. These are data from patients without an intrathecal IgG synthesis, e.g. cases of a Guillain–Barré polyradiculitis, bacterial meningitis (first day) or a spinal canal stenosis (typically without oligoclonal IgG). A representative patient (●) with a spinal canal stenosis without any inflammatory signs (normal cell count, no oligoclonal IgG) yields a false-positive result if the intrathecal IgG synthesis was evaluated by IgG synthesis rate (T) or by IgG Index (I), but would not be false positive with the hyperbolic discrimination line (R). The statistical re-evaluation of the data in Fig. 2 of Ref. [2] for larger albumin quotients $Q_{Alb}=60$ or 120×10^{3} showed that as many as 11/14 or 16/17 of the cases would be false positive with IgG synthesis rate and 6/14 or 8/17 with the IgG Index would be false positive for intrathecal IgG synthesis.

results yielded by the Tourtellotte formula and the Link Index when Q_{Alb} is elevated (Fig. 5).

The restricted data set in Fig. 5 is representative of the 4300 patients investigated in Ref. [2]. These patients, e.g. with Guillain–Barré polyradiculitis, early bacterial meningitis or spinal canal stenosis, had no oligoclonal IgG, that is, no intrathecal IgG synthesis. Fig. 5 shows that the evaluation of such patients based only on IgG synthesis rate or IgG Index gives many false-positive results for intrathecal IgG synthesis. The individual patient indicated in Fig. 5 was a typical patient with a spinal canal stenosis without any inflammatory signs in CSF (normal cell count, no oligoclonal IgG). The increased IgG synthesis rate and increased IgG Index offer misleading interpretations of the data in this case. The statistical re-evaluation (data in legend of Fig. 5) of the earlier data from patients with large albumin quotients (Fig. 2 in Ref. [2]) showed that up to 90% of samples with high albumin quotients (large barrier dysfunctions) had falsely increased results for IgG synthesis rate and up to 50% had false-positive IgG Indices.

A second deficiency of IgG synthesis rate involves the calculation of a daily amount of intrathecal IgG synthesis in a standard volume of 500 ml CSF produced/day — even though the mean CSF volume produced per day varies by a factor of three in patients between 5 and 80 years of age [56].

The IgG Index can give false-positive results not only for very large Q_{Alb} values, but also when Q_{Alb} values are low (e.g. in CSF of children) because only one reference value is used for the whole range. Linear reference ranges for an IgA or IgM Index are even less appropriate than for IgG due to the larger non-linear deviation of blood-derived IgA and IgM concentrations [2].

4.3. Quotient diagrams (Reibergrams) and numerical evaluation of intrathecal Ig fractions

Quotient diagrams, as demonstrated in Fig. 2, can help to evaluate intrathecal synthesis, the barrier dysfunction and immunoglobulin response patterns typical for neurological diseases. Diagrams (Fig. 2) show: normal range (1), blood–CSF barrier dysfunction (2) and intrathecal Ig synthesis either with (3) or without (4) barrier dysfunction. The details for interpretation are given in the legend of Fig. 2. Logarithmic scales [4,6,36] are used for practical reasons with a range up to $Q_{Alb}=150\times10^{-3}$ (Fig. 2). But the hyperbolic functions (see Appendix A for detailed functions and variables) are valid in the whole biological range up to the largest albumin quotients [5] measured so far: $Q_{Alb}=750\times10^{-3}$ which corresponds to a CSF albumin concentration which is 75% of serum concentration. In rare cases with $Q_{Alb}>150\times10^{-3}$, the numerical evaluation (Appendix A and Fig. 1) remains without graphical demonstration.

The amount of intrathecally synthesized immuno-

globulins released into CSF can be expressed either as contribution to the CSF concentration of immunoglobulins (Ig_{Loc} in mg/l) or preferably as an intrathecal fraction (Ig_{IF} in %) by comparing Ig_{Loc} to the total measured Ig concentration in CSF ($Ig_{IF}=Ig_{Loc}/Ig_{CSF}\times100$). Ig_{IF} is independent of changing Q_{Alb}. This is demonstrated in Table 3 (for formulas, see Appendix A). These intrathecal fractions can be conveniently and directly read from the quotient diagrams with lines for 20, 40, 60 and 80% intrathecal synthesis with the upper discrimination line (Q_{Lim}) as 0% synthesis (Fig. 2). Intrathecal Ig synthesis should be considered elevated if the intrathecal fraction Ig_{IF} is larger than 10%. Negative values of Ig_{IF} or Ig_{Loc}, calculated for quotients below the upper discrimination line Q_{Lim}, are reported as zero (mg/l or %), because a negative intrathecal synthesis makes no sense.

The relation between the intrathecal fractions (i.e. a one-, two- or three-class response) or predominance of one of the classes (among a two- or three-class response) constitutes the 'typical' disease-related immunoglobulin patterns (Table 1, Figs. 6–9). It is again the intrathecal fraction (IF) that most reliably indicates the dominance of intrathecal synthesis among the different immunoglobulin classes.

The reference ranges of the quotients in the quotient diagrams are method-independent as long as CSF and serum are analyzed in the same analytical run with the same method and in the same range of reliability of the standard curve, which is checked by serial dilution of a sample with a high concentration.

The diagrams are unreliable in rare cases in which blood contamination is combined with very low albumin quotients (e.g. in CSF from children); note that Q_{IgM} has a very flat slope of the hyperbolic discrimination line in this range. In addition, IgG, IgA, and IgM quotients should not be evaluated for CSF samples with blood contamination >7000 RBC/μl. Nevertheless, even with blood contamination, it should be noted that the IgG quotient could never exceed the albumin quotient (smaller molecule) in the absence of an additional intrathecal synthesis of IgG.

For statistical purposes when comparing groups, the mean line of the reference range (Q_{Mean} instead of Q_{Lim} [4]) is proposed. Values to calculate Q_{Mean} are given in Refs. [2,4]. The mean of the reference range, Q_{Mean} is asymmetrically nearer to Q_{Lim}, depending on the logarithmic scale.

Table 3
Variation of intrathecal fraction (Ig_{IF}) and locally synthesized Ig (Ig_{Loc}) with changing CSF flow rate (Q_{Alb}). The data originate from the patient in Fig. 3

	Puncture			
	2nd	3rd	4th	5th
$Q_{Alb}\times10^3$	80	68	32	20
IgM_{Loc} (mg/l)	85	77	29.2	14.2
IgM_{IF} (%)	60	62	61	59

Ventricular and cisternal CSF can also be evaluated reliably in these graphs to detect intrathecal synthesis of IgG, IgA, or IgM. The hyperbolic discrimination function is valid without any correction for CSF extracted at different sites. But, the interpretation of a blood–CSF barrier dysfunction requires different reference ranges, as described above.

4.4. Oligoclonal IgG

The analysis of oligoclonal IgG by isoelectric focusing (IEF) and immunofixation (IF) [12,54] represents the most frequently used, complementary method to detect inflammatory processes with intrathecal IgG synthesis. The high sensitivity of oligoclonal IgG detection by IEF/IF as opposed to agarose electrophoresis [55] is the basis of its relevance for diagnosis of multiple sclerosis [7,12,21] and other chronic inflammatory processes in the CNS [57]. Qualitative detection of oligoclonal IgA and IgM by electrophoretic separation and immunofixation are also available [58,59], but are less sensitive than IEF/IF detection of oligoclonal IgG.

The International Consensus for detection of oligoclonal IgG [12] proposed five types of results for paired analysis of CSF and serum:

Type 1: Normal CSF;
Type 2: Oligoclonal IgG restricted to CSF (example in Fig. 1);
Type 3: Oligoclonal IgG in CSF with additional identical bands in CSF and serum (combination of types 2 and 4);
Type 4: Identical oligoclonal bands in CSF and serum;
Type 5: Monoclonal bands in CSF and serum (myeloma or monoclonal gammopathy).

The location and number of bands generally have no importance for interpretation. Of most interest for neurological diagnosis, are Types 1, 2 and 3. Blood-derived, oligoclonal bands in CSF (Types 3 and 4) can give additional information, because these bands are typical of a broad spectrum of systemic diseases (GBS, polyneuropathies, tumors and inflammation [12]). A less frequent result is Type 5 with blood-derived monoclonal bands in CSF; this often reflects the higher sensitivity of IEF/IF than immunoelectrophoresis. A local paraprotein production restricted to the CNS is extremely rare.

4.5. Polyspecific immune response in the central nervous system and the Antibody Index

The Antibody Index (AI) is calculated to detect a pathological, brain-derived fraction of specific antibody in

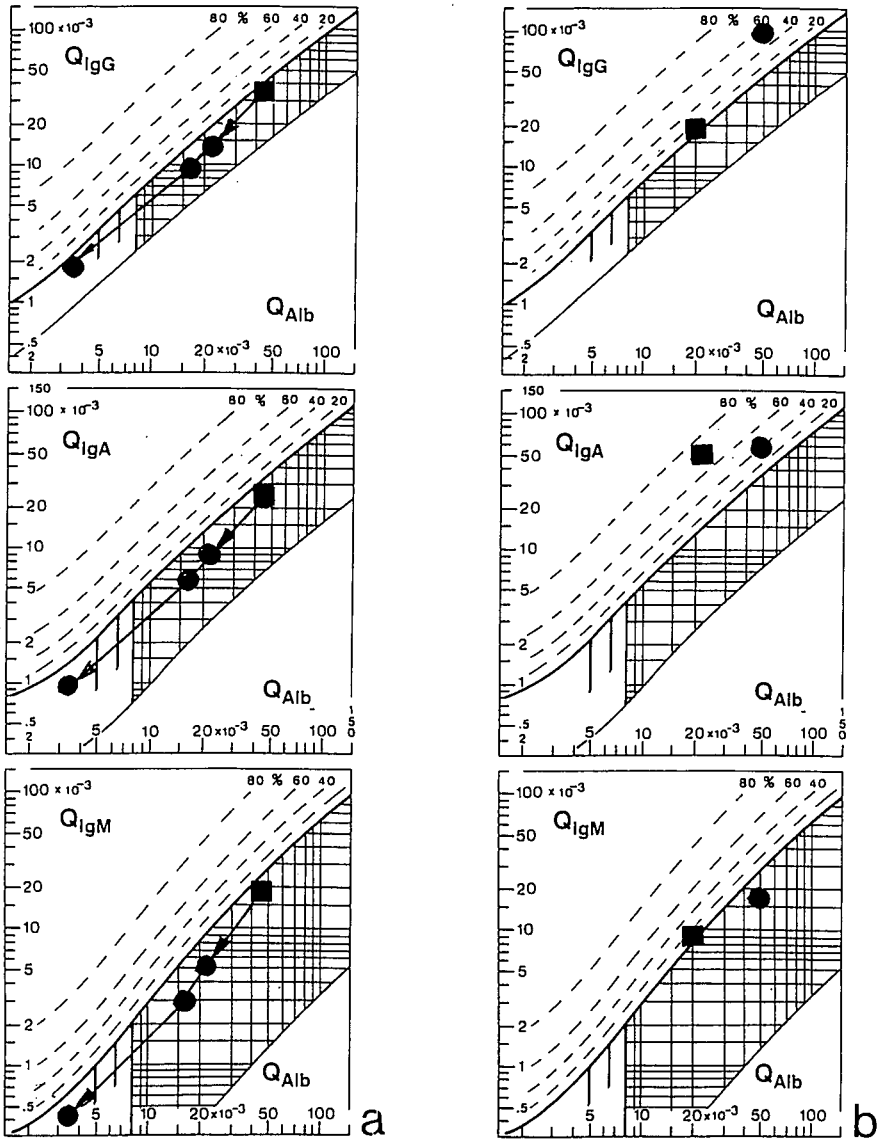


Fig. 6. Immunoglobulin pattern in different courses of bacterial meningitis. (a) Time course of data from a patient with meningococcal meningitis. Punctures were at days 1(■), 3, 6 and 13 after admission. Cell counts were 7250, 2730, 213 and 2/μl, respectively. Therapy started on the first day and the clinical course was uncomplicated. (b) Brain abscesses [26,66]: Example 1 (■) represents data from a patient with a brain abscess who had multiple congenital heart defects. Cell count 73/μl, 5% activated B-lymphocytes [64], oligoclonal IgG, dominant IgA synthesis (IgA_{IF}=67%). Example 2 (●): patient with multiple cortical abscesses after purulent meningitis. Cell count 13/μl, oligoclonal IgG. Two-class immune reaction with dominant IgG_{IF}=48% and IgA_{IF}=38%. The large Q_{Alb} value in this late phase of disease suggests extended meningeal adhesions, i.e. reduced CSF flow rate.

CSF [7,8,21,43]. The Antibody Index is the ratio between CSF/serum quotient of the specific antibody (Q_{spec}) and the total CSF/serum immunoglobulin quotient, (Q_{IgG}), i.e. AI=Q_{spec}/Q_{IgG}. In case of a large intrathecal immunoglobulin synthesis ($Q_{IgG}>Q_{Lim}$), Q_{spec} refers to the upper limit of the reference range (Q_{Lim}) with AI=Q_{spec}/Q_{Lim} to avoid false-negative results (Formulas for Q_{Lim}, etc., see Appendix A) [8]. Theoretically, the normal AI value must be AI=1.0. Depending on methodological accuracy, the reference range of AI is 0.7–1.3 and clinically relevant, pathological AI values are AI>1.4 [7]. Examples of AI values in different neurological diseases are shown in Table 4. The relevance is discussed with the disease-related data patterns below.

The Antibody Index is a relative value and does not indicate the absolute amount in serum or in CSF (as titers do). AI values for HSV AI and VZV AI can be similar (see legend to Fig. 8b or Table 4), but the amount of HSV and VZV antibodies might be very different. The higher concentration might reflect the predominant causative antigen. The absolute amount (in this case titers) can sometimes also help to determine whether an increased Toxoplasma AI value represents co-stimulation (multiple sclerosis) or causative antigen (opportunistic infection). In

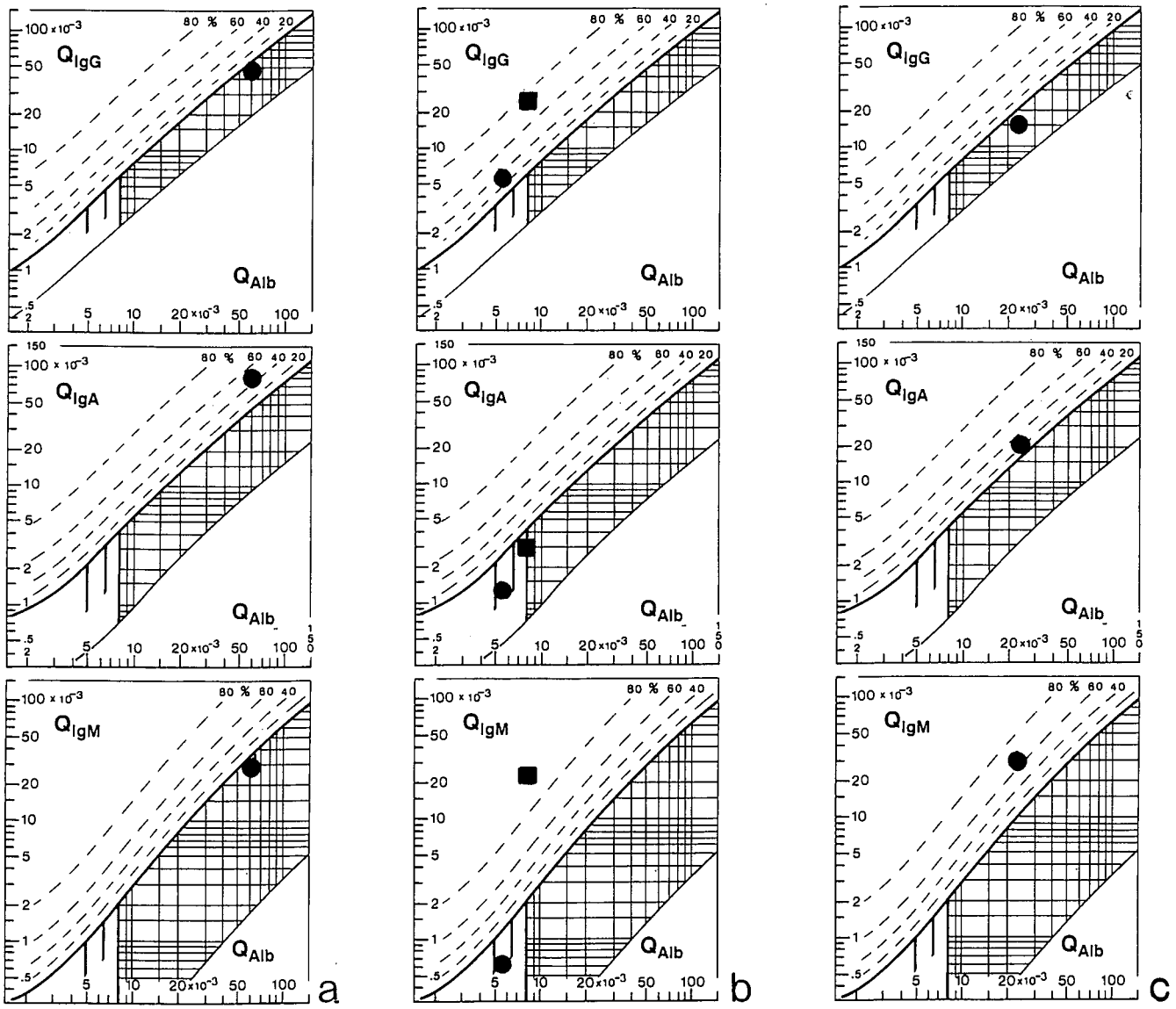


Fig. 7. Immunoglobulin patterns in neurotuberculosis, neuroborreliosis and neurosyphilis. (a) Neurotuberculosis with a dominant IgA synthesis (IgA_{IF} = 45%) and oligoclonal IgG; cell count 90/μl, increased lactate 5.7 mmol/l. Together with the large albumin quotient (sometimes much larger than shown), this combination is highly indicative for this diagnosis. (b) Two cases of neurosyphilis. Patient 1 (●) with a meningovascular type of disease shows intrathecal IgG synthesis. Patient 2 (■) with a parenchymal type of disease shows intrathecal IgG (IgG_{IF} = 75%) and a dominant intrathecal IgM fraction (IgM_{IF} = 85%). Both patients were in an acute state of disease with increased cell counts, a normal age-related albumin quotient and the typical absence of IgA synthesis in neurosyphilis. (c) Neuroborreliosis. Typical pattern of three-class immune response with dominant humoral and cellular IgM class [19,64], blood–CSF barrier dysfunction and normal lactate in CSF. Cell count 336/μl. Such a pattern has a clinical sensitivity of 70% and specificity of 96% even before detection of specific antibodies (see also Figs. 2 and 3 and Table 4).

principle, the Antibody Index can also be calculated from the ratio of antibody titers in CSF and serum. However, the discontinuous nature of titration results in imprecision and low sensitivity of detection for titers and only an AI>4 would be recognized as certainly pathological. For detection of the MRZ reaction (see below) in chronic diseases, this imprecision is unacceptable; therefore, titers should be replaced by the ELISA technique [8] which yields continuous values and allows efficient and more precise analysis of CSF and serum.

4.6. MRZ antibodies and multiple sclerosis

Eighty-four to 94% of MS patients have intrathecal antibody synthesis against one, two or three of the measles (M), rubella (R) and zoster (Z) viruses (MRZ antibodies or MRZ reaction), and slightly more if herpes simplex (H) is included (MRZH) [21]. Examples are shown in Fig. 1 and Table 4. The frequencies and magnitudes of the antibody indices rise with increased intrathecal total IgG synthesis as recently reported in detail [21]. Combinations M+R or

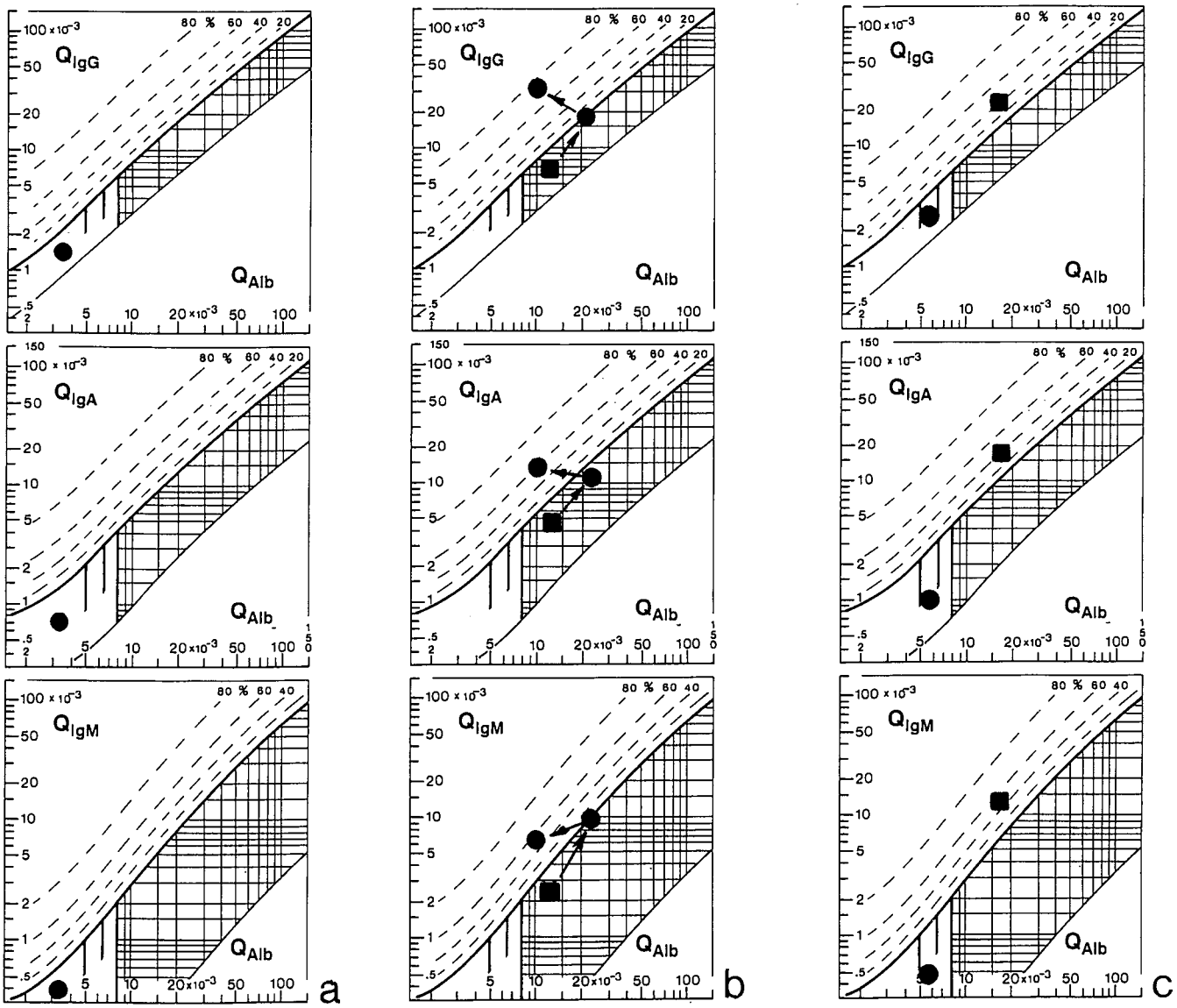


Fig. 8. Immunoglobulin pattern of viral CNS infections. (a) Zoster ganglionitis (facial nerve palsy). The VZV Antibody Index was increased (VZV AI = 2.4, HSV AI = 1.0). Another example is shown in Table 3. (b) Herpes simplex virus encephalitis with an increased albumin quotient at time of first diagnostic puncture. (■) 1st day of admission without any humoral immune response, cell count 57/μl, no oligoclonal IgG in CSF (type 4), HSV AI = 0.7, and VZV AI = 1.0, HSV PCR = positive. The 2nd puncture, 7 days after admission was oligoclonal IgG-positive (type 3); HSV AI = 10.5, VZV AI = 1.6, cell count 280/μl, PCR positive. The 3rd puncture 30 days after admission, three-class immune response (IgG_{IF}, IgA_{IF}, $IgM_{IF} > 0$), oligoclonal IgG-positive, HSV AI = 97, VZV AI = 65, cell count 30/μl (see also example in Table 4). Further examples with a more typical, isolated IgG class response are shown in Refs. [3–5,18,30]. (c) HIV encephalitis of a 30-year-old patient in an early stage (●) with 22 cells/μl, no oligoclonal IgG, HIV AI = 1.0 and *Toxoplasma* AI = 0.9. Another patient (■) with chronic HIV encephalitis and opportunistic toxoplasmosis with increased albumin quotient and three-class humoral immune response. Cell count 140/μl, Toxoplasma AI = 9.2, HIV AI = 5.7; CMV AI = 1.0.

M+Z or R+Z that are rarely seen in other diseases (e.g. acute infections) are clues to the presence of a chronic, in particular autoimmune-type disease. In other neurological diseases, the frequency of MRZ reactions is below 1% for the single species (compared to 78% frequency of elevated measles AI in MS) and far below the 0.1% frequency for M+R+Z, except for very rare instances in which a disease follows a chronic course (e.g. chronic neuroborreliosis, see Table 4). Although much less frequent than the MRZ reaction, increased intrathecal synthesis of HSV antibodies (28% frequency in MS patients) is still higher than other antibody species in MS (e.g. intrathecal toxoplasma antibodies [10% of 84 MS patients] or intrathecal autoantibody synthesis against dsDNA [20% of 60 MS patients]).

The relevance of the MRZ reaction is different from that of oligoclonal IgG (legend to Fig. 1) for evaluating possible MS. The absence of oligoclonal IgG in CSF is strong evidence against a diagnosis of MS. However, the

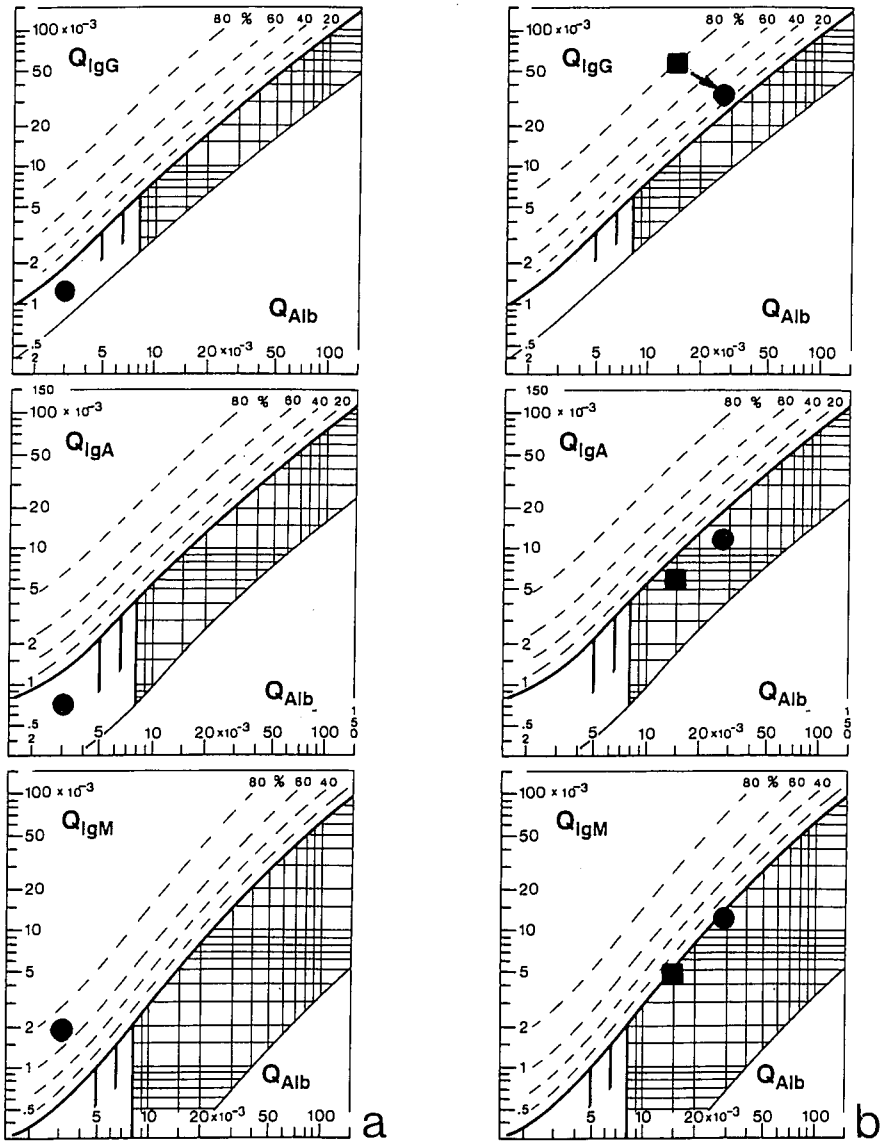


Fig. 9. Intrathecal lymphoma and therapy control in the case of a meningeal lymphoma (meningeal lymphomatosis): (a) Intrathecal lymphoma of a patient with AIDS, stage WR6. Cell count 18/μl, no oligoclonal IgG, HIV AI=4.5, IgM_{IF}=65% [67]. (b) Therapeutic control as seen by comparing (■) first puncture before treatment with (●) second puncture 6 days after treatment with 15 mg methotrexate. The initial intrathecal IgG fraction (IgG_{IF}=80%) was reduced to 30% and the cell count was reduced from 114 to 5/μl. Tumor cells had disappeared.

presence of oligoclonal IgG is not at all specific for MS. The presence of an MRZ reaction is predictive of an autoimmune-type chronic inflammatory disease even at the time of first clinical symptoms. This is of particular relevance in a monosymptomatic disease like optic neuritis [72]. However, the MRZ reaction is not specific for MS; it is detected in a number of autoimmune diseases with CNS involvement [57], such as lupus erythematosus, Sjögren syndrome or Wegener granulomatosis.

In acute infections, intrathecal synthesis of antibodies to Z or H or the combination of Z+H (which have a combined frequency of only 5% in MS [21]) can be relevant diagnostically (Table 4). For example, in facial nerve palsy caused by varicella-zoster virus (Fig. 8a), an increased VZV AI was seen in 11/11 cases at the time of first diagnostic puncture [5,38]. In the case of acute disease, the AI has higher diagnostic sensitivity than the oligoclonal IgG [7].

4.7. Microorganisms in CSF (PCR)

The detection of organism-specific nucleic acids in CSF by polymerase chain reaction (PCR) is most useful for rapid diagnosis of certain CNS infections prior to onset of antibody response [70]. PCR has a clinical sensitivity of 98% in HSV-1 encephalitis [13]; 80–95% in cytomegaloviral encephalitis and polyradiculitis [14] and a sensitivity up to 76% for JC virus is reported in progressive multifocal leukoencephalopathy [60]. PCR is the key relevant diagnostic tool for the diagnosis of tuberculous

Table 4
Examples of Antibody Index values (AI) in inflammatory neurological diseases[a]

	SSPE	VZV-G	HSV-Enceph		HIV-Enceph		MS	Neuroborreliosis	
			A	B	WR2	WR6		Acute	Chronic
Measles V AI	**89.5**	1.0	0.9	1.0	0.9	1.1	**4.3**	0.8	**3.2**
Rubella V AI	0.9	1.0	0.8	0.9	1.0	0.9	**2.0**	1.0	**7.9**
VZV AI	1.0	**9.0**	0.7	**9.4**	1.1	1.0	**3.1**	0.9	0.9
HSV AI	1.1	**1.5**	1.2	**27.0**	0.9	**2.3**	1.2	1.0	**2.6**
HIV AI	–	–	–	–	**5.4**	**4.2**	–	–	–
Borrelia (IgG) AI	–	1.0	–	–	–	–	**0.7**	**8.6**	**19.3**
Borrelia (IgM) AI	–	0.9	–	–	–	–	0.8	**4.3**	**10.1**

[a] SSPE (Subacute Sclerosing Panencephalitis), the high AI values far exceed the polyspecific response in multiple sclerosis with measles AI<40. VZV-G (Zoster ganglionitis), a biological co-reaction is seen in many cases but is not a cross-reactivity in the assay. A higher antibody titer in CSF and serum is observed for the causative virus. HSV-Enceph (Herpes Simplex Encephalitis) with data from the same patient: A, initial diagnostic puncture; B, 11 days later. Another example is shown in Fig. 8b. For co-stimulation, see comment to VZV-G. HIV-Enceph (HIV-Encephalitis) – WR2 and WR6 (Walter Reed 2 and 6). MS, multiple sclerosis. Neuroborreliosis, an acute type and a rare chronic type of the disease. The chronic type does not develop from an acute form of the disease. Pathologic values are the bold numbers. For a corresponding set of data from different patients, see Ref. [8].

meningitis [61]. After early reports of false-positive results due to amplification of common sequences, the specificity is now 100% and diagnostic sensitivity is 90% by 'nested' PCR [31].

In many other inflammatory neurological diseases, the clinical sensitivity of PCR for specific DNA or RNA in CSF is too low for routine CSF analysis (e.g. in neuroborreliosis diagnostic sensitivity is <40%). Detailed references for EBV, HTLV-1 and others are summarized in Ref. [3].

4.8. Cells in CSF [3,18,26,30]

Total cell count in CSF represents the most sensitive parameter for characterization of an acute inflammatory disease of the central nervous system.

- In normal CSF, 0–4 WBC/μl are observed. A pathological RBC count in CSF can be due to hemorrhage or artefactual or a post-surgical blood contamination of CSF. The white blood cell count can be corrected to some extent by subtracting 1 WBC/μl for each 1000 erythrocytes/μl;
- Differential cell count reports percentages of lymphocytes, monocytes and granulocytes, but plasma cells μl;
- The timing of CSF puncture in the course of a disease is critical for relevant interpretation of differential cell patterns. Dynamics of cell patterns describe:
  – Neutrophilic reaction;
  – Lymphocytic reaction;
  – Mixed cell population;
  – Cells after hemorrhage;
- Particular observations like mitosis or tumor cells are rare events;
- The subdifferentiation in B/T-lymphocytes or subclasses of T-lymphocytes has not gained sufficient clinical relevance for routine analysis;
- A promising recent development is cytochemistry for activated B-lymphocytes [64] indicating an acute or chronic inflammatory process in early disease of patients with normal or very low total cell counts. The appearance of activated B-cells corresponds well with the frequency of the morphologically defined plasma cells (H. Reiber, unpublished data from 600 CSF samples).

5. Disease-related data patterns in CSF

5.1. Patterns of disease-related immune response in quotient diagrams

The unambiguous value of immunoglobulin patterns for differential diagnosis of neurological diseases has greatly improved the general relevance of CSF analysis compared to earlier reports of single analyte parameters [1,34]. It is in particular, the IgA and IgM analyses combined with IgG that have improved the clinical relevance.

Clinical specificity and sensitivity of single data patterns are also critically dependent on the time of puncture during the course of the disease. The patterns in Figs. 1, 2 and 6–9 refer to the diagnostically relevant first puncture, usually shortly after onset of clinical symptoms. The onset of clinical symptoms varies from very rapid in bacterial meningitis or a few days for viral meningitis to a delay of 2–3 weeks for subacute tuberculous meningitis [17,26]. In conditions such as brain abscess (Fig. 6b), the diagnostic sensitivity varies with the distance of the pathological process from CSF space. The specificity of immunoglobulin patterns increases with the number of complementary data available. The frequencies of single pathological variables as part of these typical patterns are summarized in Ref. [4], together with additional discriminative variables. In all cases, the specificity of CSF immunoglobulin

pattern depends critically on, and increases significantly with a clear differential diagnostic question. This differential diagnostic context provides discriminative power to the patterns of CSF data sets in general and in particular to the immunoglobulin class response.

The following representative CSF data patterns in Reibergrams represent new examples or are selected from earlier reports of statistically evaluated groups of diseases [3–5,7,18,19,23,38], clinical case reports [7,17] and observations of rare findings [22,24,26]. An earlier summary of pathological signs in CSF for many neurological diseases is given in Ref. [4].

5.1.1. Immune-response patterns in bacterial infections

At the time of first diagnostic puncture, bacterial infections (Figs. 6 and 7) show large, disease-dependent differences in the patterns of immunoglobulin response that can vary from complete absence of a humoral immune response (bacterial meningitis, Fig. 6a) to a one-, two- or three-class immunoglobulin response (Table 1). Bacterial infections at presentation are also characterized by strong cellular immune response and prominent blood/CSF barrier dysfunction (i.e. stronger reduction of CSF flow rate) than viral infections. Increased lactate in CSF [26,65] and a decrease in the CSF-to-plasma ratio of glucose are other relevant analytical variables for discriminating among acute diseases.

- **Bacterial meningitis** (*Streptococcus, Hemophilus, Neisseria*): classically shows a large albumin quotient, increased white cell count [4–6] with dominant neutrophils and increased lactate. An intrathecal humoral immune response, if seen at all, appears 3 days after initial clinical symptoms. For this reason, immunoglobulin patterns are not helpful for therapy decisions in the early phase of this disease. Depending on the causative bacterium, some patients can initially demonstrate IgA synthesis (meningococcal and pneumococcal meningitis) [5,38], which is probably explained by a preceding systemic infection [17]. In cases of early successful treatment without complications, the initial pattern with a large albumin quotient normalizes in a few days without development of a humoral immune response (Fig. 6a).
- **Brain abscess:** Continuing intrathecal synthesis after bacterial meningitis commonly reflects complications such as multiple abscesses (Fig. 6b). Analysis of immunoglobulin patterns can be helpful, in particular, the detection of an IgA synthesis points to abscess as the cause of the hypodense zones in Computed Tomography rather than tumors. Felgenhauer et al. [26] provide a detailed description of differential diagnostic aspects in bacterial meningitis with reference to quotient diagrams. As perforation of the abscess into CSF is usually lethal, this analysis can save lives. The usual patterns in the quotient diagrams show isolated or predominant IgA synthesis (Fig. 6b). The extent of the increase of Q_{Alb} depends on the localization of the abscess. In particular, a spinal abscess that causes a restriction of CSF turnover in the lumbar CSF can lead to increased Q_{Alb}. In parenchymatous localization, a normal Q_{Alb} can be associated with an intrathecal IgA synthesis ($IgA_{IF}>0$) [26].
- **Tuberculous meningitis:** A distinctive pattern (Fig. 7a) that can contribute to the rapid diagnosis of neurotuberculosis, includes dominant IgA synthesis, intermediate pleocytosis and major blood/CSF barrier dysfunction (Q_{Alb} up to 400×10^{-3}), frequently combined with increased lactate (or decreased glucose CSF/serum ratio) and occasionally with oligoclonal IgG. IgA synthesis is common at time of admission (85%), and is detected either by an $IgA_{IF}>10\%$ or $Q_{IgA}>Q_{IgG}$. In patients with suspected neurotuberculosis, PCR is the analysis of choice [3,4,26,30,31,61,70], but it would not be efficient to analyse all patients for the specific antigens or antibodies; the basic analysis starts with quotient diagrams. A few examples of intrathecal IgM synthesis are reported in the spinal variety of the disease [26,38].
- **Neuroborreliosis** (Figs. 2 and 7c): Dominant IgM synthesis ($IgM_{IF}>IgA_{IF}>IgG_{IF}$), a cellular IgM response (activated B cells) and blood/CSF barrier dysfunction yield a high specificity (96%) [19] with a sensitivity of 70% for this disease — even without a *Borrelia*-specific Antibody Index [19]. Nevertheless, an elevated *Borrelia*-specific Antibody Index (Table 4) is very useful for diagnosis [19], in particular by increasing the sensitivity from 70 to 80%. There are very rare cases of chronic neuroborreliosis which do not start as an acute disease and which show an MRZ antibody synthesis typical of autoimmune type diseases (Table 4 and Refs. [21,57]). The PCR for *Borrelia burgdorferi* in CSF has a diagnostic sensitivity below 40% and does not improve the overall sensitivity obtained with the basic program. Qualitative analysis with Western blot is less sensitive than *Borrelia* AI for detection of neuroborreliosis. Lactate is normal.
- **Neurosyphilis** (Fig. 7b). CSF data from patients with parenchymal or meningovascular neurosyphilis have in common the absence of IgA synthesis. A normal or only slightly increased Q_{Alb} value and occasionally a concomitant IgM synthesis complete the pattern. Indeed, intrathecal IgA synthesis contradicts the diagnosis of neurosyphilis. Strong similarities in the immunoglobulin response patterns of neurosyphilis and MS include frequently normal Q_{Alb}, as well as obligatory intrathecal IgG synthesis that is occasionally combined with intrathecal IgM synthesis. In contrast to neurosyphilis, an $IgA_{IF}>0$ is occasionally seen in MS (9% frequency [21]), but this is not a

differential diagnostic problem, because *Treponema* antibodies and the clinical as well as MRI information easily discriminate between these diseases.

5.1.2. Immune response patterns in viral infections

Virus-caused inflammatory processes (Fig. 8) have in common the absence of an early intrathecal humoral immune response, normal lactate and a predominant mononuclear cell pattern. But among the viral infections of the CNS, there are several interesting exceptions, including mumps infections [17], which even at the time of the initial diagnostic puncture can exhibit a humoral immune response with dominant IgM synthesis similar to the neuroborreliosis pattern (Fig. 7c).

- **HSV encephalitis** (Fig. 8b): An increased intrathecal IgG, IgA or IgM fraction would rule out the diagnosis of early HSV encephalitis. But, 6–10 days after first clinical symptoms, oligoclonal IgG and, in particular, an elevated HSV-specific or VZV-specific Antibody Index is confirmation for the diagnosis (Table 4). For early diagnosis of HSV encephalitis, PCR has priority and high diagnostic sensitivity [13]. The dynamics of the immunoglobulin patterns in late HSV encephalitis [7] include a humoral immune response. For the patient in Fig. 8b, oligoclonal IgG and HSV AI=10.5 were detectable at day 7 after admission, and a three-class immune response IgG_{IF}, IgA_{IF}, $IgM_{IF}>0$ was apparent at day 30 after start of clinical symptoms. This intrathecal immune response, in particular of the IgG class (oligoclonal IgG and HSV-specific AI), can last many years [17] with a very slow diminution, similar to that shown in Fig. 4 for neurosyphilis. But it is typical for the albumin quotient to be normal in these cases. Additional examples of HSV encephalitis are shown in Table 4 and in Refs. [30,38].
- **VZV ganglionitis** (Fig. 8a) represents one of the cases in which CSF analysis sometimes provides the only source of information for correct diagnosis. In this situation, the high frequency of intrathecal antibody response (increased VZV-specific AI) with a normal CSF protein pattern [5,38] highlights the importance of communicating the differential diagnostic question to the laboratory. A normal protein pattern (Fig. 8a) does not exclude the need for VZV-specific Antibody Index if zoster infection as a cause of facial nerve palsy is being considered. By contrast, facial nerve palsy with dominant intrathecal IgM synthesis (Fig. 2) might point to the need for *Borrelia burgdorferi*-specific Antibody Index rather than VZV-specific Antibody Index. On the other hand, in the presence of a normal protein profile (e.g. Fig. 8a), analysis for *Borrelia*-specific antibodies would not be the first choice for complementary analytical efforts.
- **HIV encephalitis** (Fig. 8c) is characterized by the weak immune response [67] with a pleocytosis and with oligoclonal IgG often not detectable in the CSF/serum diagrams. In particular, the appearance of opportunistic infections provides the clinical relevance to investigate immunoglobulin patterns in this disease.
- **Opportunistic infections of the brain** [14] frequently induce a three-class reaction accompanied by a blood–CSF barrier dysfunction; a similar pattern can reflect viral (CMV) or toxoplasma etiology (Fig. 8c). Obviously, the pattern of an opportunistic CMV infection at the time of diagnostic puncture differs from a primary virus infection and represents a more general pattern, which is typical for opportunistic infection independent of the causative microorganism. In the case of suspected opportunistic infection, PCR analysis for CMV is often useful [14].
- **Intrathecal lymphoma** (Fig. 9a). A normal CSF turnover (i.e. normal Q_{Alb}), a normal cell count and exclusive synthesis of IgM should suggest the possibility of this unusual opportunistic disease.
- **Meningeal lymphomatosis** (Fig. 9b) is shown as an example using quotient diagrams to demonstrate therapeutic efficacy of the drug via the reduction in the intrathecal IgG fraction.

5.1.3. Relevance of IgA and IgM analysis in CSF

Analysis of IgA and IgM in CSF contributes importantly to the immunoglobulin patterns. The dynamics of intrathecal immune response are seemingly more related to the special pathomechanisms of the disease or to the causative antigen than to the standard maturation of the immune reaction seen in blood (Fig. 3). An intrathecal IgM class response ($IgM_{IF}>0\%$) can be seen with different frequencies and intensities in acute as well as in chronic diseases, including neuroborreliosis, mumps, meningitis, parenchymatous neurosyphilis and multiple sclerosis. Detection of an IgM response offers little information on its own, but in combination with other CSF data, an intrathecal IgM synthesis can contribute to a more or less typical pattern in neuroborreliosis, mumps meningitis or non-Hodgkin lymphoma.

An intrathecal IgA response at the time of first diagnostic puncture typically indicates a bacterial origin of the disease, but, again, this response has vastly different frequencies ranging from 0% IgA synthesis in neurosyphilis to 90% in neurotuberculosis. An initial IgA synthesis can be seen transiently in meningococcal and pneumococcal meningitis but is characteristically absent in staphylococcal or streptococcal meningitis [5,38]. The detection of an intrathecal IgA synthesis can provide vital information about a brain abscess (two cases shown in Fig. 6b).

In viral infections, an intrathecal IgA synthesis later in the course of the disease combined with IgM synthesis can be seen, for example, in herpes simplex encephalitis (Fig. 8b), but is not helpful for diagnosis or prognosis. However, the detection of a three-class immune response (sometimes

combined with barrier dysfunction) is an important, highly plausible indication of an opportunistic disease in the case of HIV encephalopathy.

The IgA synthesis observed in adrenoleukodystrophy [24], a metabolic disease, is still unexplained. From a pathophysiological point of view it is remarkable that even in neurological diseases caused by parasites like the nematode angiostrongylus, an immunoglobulin response is detectable in CSF [22].

With the invention of sensitive assays for analysis of IgA and IgM in CSF by particle-amplified nephelometry (Dade–Behring and Beckman–Coulter) or by ELISA [38] together with the PC-supported evaluation programs, this extension of a basic CSF analysis is affordable and should not be neglected in appropriate situations.

5.2. Non-inflammatory diseases of the CNS and diagnostic relevance of brain proteins

Non-inflammatory conditions such as stroke typically show a CSF data pattern without a humoral immune response, except in the case of complications or an inflammatory cause like a zoster angiitis with IgG and IgM synthesis [26]. In degenerative diseases, there is usually no oligoclonal IgG detectable. Exceptions are reported for Alzheimer disease as well as in Creutzfeldt–Jacob disease [74]. In tumors, an immune response is absent except in rare cases of secondary inflammation or a lymphoma. The finding of oligoclonal IgG in CSF must always be regarded seriously. Disc prolapse should not be regarded as cause of oligoclonal IgG and a search for the real cause is indicated. Brain proteins in CSF are sometimes important for diagnosis of non-inflammatory CNS diseases. Although under normal conditions about 20% of CSF proteins are derived from brain [29], only a few CSF proteins are exclusively brain-derived [10,11]. Examples of conditions in which brain proteins are diagnostically relevant include degenerative diseases [33,35,68,69,71,75], tumors [10,73], hypoxias and brain infarction [15] or identification of CSF in nasal secretions [32]. The analysis of brain proteins in blood represents a particularly interesting development, as it supplies information about brain processes without a lumbar puncture and allows serial analysis from blood.

Neuron-specific enolase (gamma, gamma dimer), one of the first proteins introduced for serial analysis in blood to characterize destructive processes in brain, is particularly useful for prognosis of clinical outcome after cerebral hypoxia [15]. The high concentration of NSE in neurons enables blood-based detection of even very small amounts of neuron destruction. Other brain cell-specific proteins like S-100b or tau protein require much higher analytical sensitivity for detection of brain-derived changes in blood.

5.2.1. Tumor marker proteins in CSF

The molecular flux/CSF flow theory is valid for all proteins and not restricted to immunoglobulins. The evaluation of tumor marker proteins like carcinoembryonic antigen (CEA) is possible in the same type of diagrams with hyperbolic discrimination lines. Brain metastasis of a CEA-synthesizing tumor [23,73] can be evaluated in the IgA diagrams, because CEA and IgA have similar diffusion coefficients. Intrathecal fractions with up to $CEA_{IF}=99\%$ and Q_{CEA} up to 1600×10^{-3} represent the most extreme values of pathological, intrathecal fractions in CSF. The extent of brain-derived CEA released into CSF depends critically on the distance of the metastasis from the CSF space [73]; hence, a normal CEA quotient cannot exclude the presence of a metastasis in the forebrain. In contrast to CEA in CSF, which normally originates exclusively from blood, angiotensin-converting enzyme (ACE) in CSF originates with a mean fraction of 70% from brain [10]. Introduced as a serum marker protein for sarcoidosis, ACE can also be used as a marker protein in CSF for detection of neurosarcoidosis but with a very low sensitivity. In this case, because the ACE in CSF is predominantly brain-derived, the absolute value of ACE (instead of the CSF/serum quotient) is evaluated as a function of Q_{Alb} [10]. Values of ACE activity in CSF with $(ACE)>0.5+90Q_{Alb}$ μmol/min per ml reflect pathologically increased brain-derived fractions.

Sometimes an intrathecal lymphoma can be identified by an isolated IgM synthesis (Fig. 9a), an unusual pattern for inflammatory disease. When lymphoma is an opportunistic disease related to HIV encephalitis (Fig. 9a), an HIV-dependent increase in cell count and the eventual appearance of oligoclonal IgG can be misleading. Quotient diagrams are also relevant for monitoring therapy as seen in meningeal lymphomatosis when treatment with methotrexate reduced the intrathecal IgG fraction in CSF significantly (Fig. 9b).

5.2.2. Degenerative diseases of the CNS

Early discrimination of different causes of dementia (Alzheimer disease, Creutzfeldt–Jakob disease, multi-infarct dementia or depressive pseudodementia) is facilitated by a set of brain proteins, detectable in CSF and serum [33,35,68–71,75]. Laboratory evaluation of possible Alzheimer disease typically includes increased tau protein concentration and decreased β-amyloid 1-42 proteins that have high sensitivity and specificity [33,70] in the relevant differential diagnostic group.

- **Alzheimer disease** (AD) can be differentiated from non-AD diseases like multi-infarct dementia, if tau protein values are increased and combined β-amyloid values are decreased in CSF. Tau protein is non-specifically increased in other diseases [33].
- **Creutzfeldt–Jakob disease** (CJD) exhibits rapidly progressive dementia and at least two of the following clinical findings: (1) myoclonus, (2) tic and cerebellar signs, (3) pyramidal and extrapyramidal signs, (4) akinetic mutism. The detection of protein 14-3-3 can

change the diagnosis from possible to probable CJD. The EEG-signs are equivalent but some patients without a typical EEG are positive for protein 14-3-3 [15,17,35]. Very high tau protein values (>1200 pg/ml) point to this diagnosis. Increased concentrations of neuron-specific enolase in CSF further suggest rapidly progressive neural degeneration [68].

CSF data patterns in the Reibergrams are normal and oligoclonal IgG is rare in AD and CJD [74]; however, the detection of oligoclonal IgG is not sufficient cause to exclude the diagnosis of Creutzfeldt–Jakob disease or Alzheimer Disease [74].

6. Summary of interpretations and particular comments

Evaluation of the CSF data based on the reference ranges can result in the following standard interpretations:

- normal CSF (including cell counts);
- normal CSF protein concentrations;
- increased cell count;
- blood/CSF barrier dysfunction;
- intrathecal specific antibody synthesis;
- inflammatory process in CNS;
- intrathecal tumor/metastasis.

These interpretations are based on the following definitions:

Blood–CSF barrier dysfunction: Increased (age-related) Q_{Alb} values can reflect mechanical and inflammatory restrictions of CSF turnover as well as hemorrhage that are distinguished via differential cell counts.

Inflammatory process: Includes all specimens that show either increased cell count >20/μl or activated B-lymphocytes (>0.1% of lymphocytes) and any humoral immune reaction in CNS;

A humoral immune reaction in CNS is defined as $Q_{IgG}>Q_{Alb}$, $IgG_{IF}>10\%$, oligoclonal IgG in CSF (type 2 and 3) or any Organism-specific Antibody Index AI>1.4; both of the latter are possible in spite of $Ig_{IF}=0\%$. A local IgA or IgM synthesis in CNS is suggested if $IgA_{IF}>10\%$, $IgM_{IF}>10\%$ or $Q_{IgA}>Q_{IgG}$ or $Q_{IgM}>Q_{IgA}$.

6.1. Particular analytical comments

The CSF Report (Fig. 1) can include or omit specific CSF analysis data and/or interpretation depending on local preferences. A set of method-oriented comments is in use:

- Analysis of oligoclonal IgG can be omitted if $Q_{IgG}>Q_{Alb}$.
- Intrathecal IgA synthesis is indicated by $Q_{IgA}>Q_{IgG}$ (even when $IgA_{IF}=0\%$).
- Total cell count (WBC) is corrected for blood contamination (subtraction of 1 WBC/1000 RBC in CSF).
- Albumin and IgG CSF concentrations are corrected for blood contamination in the range of 1000–7000 RBC/μl (Blood contamination >7000 cells/μl does not allow a reliable evaluation of immunoglobulin- and albumin-quotients). Mean corrections for albumin are 18 or 36 mg/l for 2000 or 4000 RBC/μl; IgG corrections in CSF are 2.5/5.0/10.0 mg/l for 1000/2000/4000 RBC/μl.
- A normal CSF data set suggests that analyses for *Borrelia*-specific AI are not necessary.

6.2. Diagnosis-related comments

- The MRZ reaction indicates a chronic inflammatory process (autoimmune type). Differential diagnosis includes multiple sclerosis or autoimmune disease with involvement of CNS.
- A large Q_{Alb} ($>20\times10^{-3}$) or a large cell count (>90/μl) or the absence of oligoclonal IgG are not consistent with the suggested diagnosis of multiple sclerosis.
- Large Q_{Alb}, dominant intrathecal IgA synthesis (frequently combined with oligoclonal IgG), increased CSF lactate (>3.4 mmol/l) and intermediate pleocytosis indicate a high probability of tuberculous meningitis. PCR is recommended.
- Lactate >3.4 mmol/l and cell count >500/μl suggest bacterial infection.
- Intrathecal three-class reaction with HIV-specific AI>1.5 is indicative of an opportunistic infection. Appropriate antibody analysis or PCR for the suspected organism is recommended.
- Isolated intrathecal IgM synthesis without further inflammatory signs (normal cell count, no oligoclonal IgG) suggests lymphoma.
- Intrathecal synthesis of carcinoembryonic antigen, CEA ($Q_{CEA}>Q_{Lim}$ (IgA)) indicates tumor metastasis in brain.
- Beta amyloid 1–42 is decreased (<500 pg/ml) and tau protein concentrations (>360 pg/ml) are increased in Alzheimer disease.
- Tau protein values >1200 pg/ml are observed in Creutzfeldt–Jacob disease. Analysis of protein 14.3.3 and neuron-specific enolase in CSF is recommended.

- Protein 14.3.3 is associated with Creutzfeldt–Jakob disease.

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Appendix A

A.1. Drawing hyperbolic functions in quotient diagrams ('Reibergrams')

The graphs shown in Fig. 1 are available as PC programs from different sources (CSF-COM from Dade–Behring, Marburg, Germany and CSF Report from Beckman–Coulter, Krefeld, Germany, or TR-CSF program of Wormek (www.wormek.de). The Beckman and Wormek solutions integrate the knowledge-based evaluation program of Faber and Trendelenburg [16].

With the different laboratory data systems in use and with the easy access to suitable PC software, development of customized PC-supported CSF reports is now straightforward.

The reference ranges for the CSF/serum quotient diagrams are subtended by the upper discrimination line (Q_{Lim}) and the lower borderline (Q_{Low}). These lines for IgG, IgA, IgM are hyperbolic functions. The diagrams use logarithmic scales covering the range of most frequent values: $Q_{\mathrm{Alb}}=1.5\times10^{-3}$–$150\times10^{-3}$ and Q_{IgG}, Q_{IgA}, Q_{IgM} between 0.3×10^{-3} and 150×10^{-3} (Fig. 2).

The dashed lines which indicate the magnitude of the intrathecally synthesized fractions (IF=20, 40, 60, 80%) are calculated from $Q_{\mathrm{Lim}}=0\%$. For example, for IF=20% and with $Q(20\%)$ on the 20% intrathecal synthesis line, the ratio $Q(20\%):Q_{\mathrm{Lim}}=1:(1-0.2)$ or $Q(20\%)=Q_{\mathrm{Lim}}/0.8$.

The upper limit of the age-related reference range of Q_{Alb} for patients older than 5 years is calculated from $Q_{\mathrm{Alb}}=(4+\mathrm{age}/15)\times10^{-3}$ [16]. This function allows integration of fixed bars as shown in Fig. 1 and Figs. 6–9 for 15, 40 and 60 years of age or can be used in a flexible program to relate the reference limit according to the individual age (Fig. 2). Q_{Alb} values of the newborn <4 months require reference to the particular ranges, as reported [6].

A.2. Hyperbolic functions of the reference range

1. **The general hyperbolic function:**

$$Q\mathrm{Ig}=a/b\sqrt{Q(\mathrm{Alb})^2+b^2}-c$$

has the values of a/b, b^2, c as reported in Ref. [2] for Q_{Lim}, Q_{Mean} (mean line) and Q_{Low} (lower limit) of the reference range for blood-derived IgG, IgA and IgM in CSF. The upper limit $Q_{\mathrm{Lim}}(\mathrm{Ig})$ of the reference range in the CSF/serum quotient diagrams is described as:

$$Q_{\mathrm{Lim}}\,(\mathrm{IgG})=0.93\sqrt{(Q_{\mathrm{Alb}})^2+6\times10^{-6}}-1.7\times10^{3}$$

$$Q_{\mathrm{Lim}}\,(\mathrm{IgA})=0.77\sqrt{(Q_{\mathrm{Alb}})^2+23\times10^{-6}}-3.1\times10^{3}$$

$$Q_{\mathrm{Lim}}\,(\mathrm{IgM})=0.67\sqrt{(Q_{\mathrm{Alb}})^2+120\times10^{-6}}-7.1\times10^{3}$$

2. **Quantitation of intrathecal synthesis:** Values for Q_{IgG}, Q_{IgA}, Q_{IgM} above the hyperbolic discrimination line, Q_{Lim}, indicate an intrathecal synthesis (Fig. 2). The amount of locally synthesized immunoglobulins released into CSF can be expressed either as the contribution to the CSF Ig concentration:

$$\mathrm{Ig}_{\mathrm{Loc}}=[Q_{\mathrm{Ig}}-Q_{\mathrm{Lim}}(\mathrm{Ig})]\times\mathrm{Ig}_{\mathrm{serum}}\qquad[\mathrm{mg/l}]$$

or as the intrathecal fraction, $\mathrm{Ig}_{\mathrm{IF}}$, referring $\mathrm{Ig}_{\mathrm{Loc}}$ to the total Ig concentration in CSF ($\mathrm{Ig}_{\mathrm{Loc}}/\mathrm{Ig}_{\mathrm{CSF}}$), and rearranged with $Q_{\mathrm{Ig}}=\mathrm{Ig}_{\mathrm{CSF}}/\mathrm{Ig}_{\mathrm{serum}}$:

$$\mathrm{Ig}_{\mathrm{IF}}=[1-Q_{\mathrm{Lim}}(\mathrm{Ig})/Q_{\mathrm{Ig}}]\times100\qquad[\%]$$

$\mathrm{Ig}_{\mathrm{IF}}$ is preferred for routine analysis, because the locally (in CNS) synthesized contribution to CSF IgG concentration (i.e. $\mathrm{IgG}_{\mathrm{Loc}}$ in mg/l) depends on the blood–CSF barrier function (Q_{Alb}) (Table 3). In contrast, the intrathecal fraction (i.e. $\mathrm{IgG}_{\mathrm{IF}}$ as % of total CSF IgG) is independent of the CSF flow rate (Fig. 3) and offers the better term to determine dominance of intrathecal synthesis among the different immunoglobulin classes ($\mathrm{IgG}_{\mathrm{IF}}$; $\mathrm{IgA}_{\mathrm{IF}}$; $\mathrm{IgM}_{\mathrm{IF}}$). A calculation example for Q_{Lim} with $Q_{\mathrm{Alb}}=5\times10^{-3}$ follows: $Q_{\mathrm{Lim}}(\mathrm{IgG})=0.93\sqrt{(5\times10^{-3})^2+6\times10^{-6}}-1.7\times10^{-3}=3.48\times10^{-3}$; the same result is obtained more practically by $Q_{\mathrm{Lim}}=0.93\sqrt{5^2+6}-1.7=3.48$ when $Q_{\mathrm{Alb}}\times10^{3}$ is inserted and evaluated as $Q_{\mathrm{Lim}}\times10^{3}=3.48$. Calculation examples for $\mathrm{Ig}_{\mathrm{IF}}$ or $\mathrm{Ig}_{\mathrm{Loc}}$ are reported in Refs. [4,6,38].
3. **Dominance** amongst intrathecal fractions: $\mathrm{IgG}_{\mathrm{IF}}>\mathrm{IgM}_{\mathrm{IF}}$ means dominant intrathecal IgG synthesis.
4. **Antibody Index:** the specific intrathecal immune response of a certain antibody species (Ab) is calculated with the Ab CSF/Ab serum concentration quotient (Q_{spec}) and the total IgG (or IgM class) quotient either as the empirical Q_{IgG} or in case of greatly increased polyspecific immune response by $Q_{\mathrm{Lim}}(\mathrm{IgG})$ as follows: $\mathrm{AI}=Q_{\mathrm{spec}}/Q_{\mathrm{IgG}}$ when ($Q_{\mathrm{IgG}}<Q_{\mathrm{Lim}}$) and $\mathrm{AI}=Q_{\mathrm{spec}}/Q_{\mathrm{Lim}}$ (IgG) when ($Q_{\mathrm{IgG}}>Q_{\mathrm{Lim}}$).

   Reference range AI=0.7–1.3; pathological values AI>1.4. Calculation examples are shown in Refs. [4,6,38].

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