CHAPTER 4

Routine cerebrospinal fluid (CSF) analysis

F. Deisenhammer,[a] A. Bartos,[b] R. Egg,[a]
N. E. Gilhus,[c] G. Giovannoni,[d] S. Rauer,[e]
F. Sellebjerg[f]

Background A great variety of neurological diseases require investigation of the cerebrospinal fluid (CSF) to prove the diagnosis or to rule out relevant differential diagnoses.

Objectives To evaluate the theoretical background and provide guidelines for clinical use in routine CSF analysis including total protein, albumin, immunoglobulins, glucose, lactate, cell count, cytological staining, and investigation of infectious CSF.

Methods Systematic Medline search for the above mentioned variables. Review of appropriate publications by one or more of the task force members. Grading of evidence and recommendations was based on consensus by all task force members.

CSF should be analysed immediately after collection. If storage is needed 12 ml of CSF should be partitioned into three to four sterile tubes. Albumin CSF/serum ratio (Q_{alb}) should be preferred to total protein measurement and normal upper limits should be related to patients' age. Elevated Q_{alb} is a non-specific finding but occurs mainly in bacterial, cryptococcal, and tuberculous meningitis, leptomingeal metastases as well as acute and chronic demyelinating polyneuropathies.

Pathological decrease of the CSF/serum glucose ratio or an increase in lactate concentration indicates bacterial or fungal meningitis or leptomeningeal metastases.

Intrathecal immunoglobulin G synthesis is best demonstrated by isoelectric focusing followed by specific staining.

Cellular morphology (cytological staining) should be evaluated whenever pleocytosis is found or leptomeningeal metastases or pathological bleeding is suspected. Computed tomography-negative intrathecal bleeding should be investigated by bilirubin detection.

Introduction

The cerebrospinal fluid (CSF) is a dynamic, metabolically active substance that has many

[a]Department of Neurology, Innsbruck Medical University, Austria; [b]Department of Neurology, Charles University, Prague, Czech Republic; [c]Department of Clinical Medicine, University of Bergen, Bergen, Norway, and Department of Neurology, Haukeland University Hospital, Bergen, Norway; [d]Department of Neuroinflammation, Institute of Neurology, University College London, Queen Square, London, UK; [e]Department of Neurology and Clinical Neurophysiology, Albert-Ludwigs University, Freiburg, Germany; [f]Department of Neurology, Copenhagen University Hospital, Denmark.

Huge: "ch04" — 2006/6/29 — 14:54 — page 14 — #1

Table 4.1 Typical constellation of CSF parameters in some neurological diseases.

	Total protein (g/l)	Glucose ratio	Lactate (mmol/l)	Cell count (per 3.2 μl)	Typical cytology
Normal values[a]	<0.45	>0.4–0.5	<1.0–2.9	<15	MNC
Disease					
Acute bacterial meningitis	↑	↓	↑	>1000	PNC
Viral neuro-infections (meningo/encephalitis)	= /↑	= /↓	=	10–1000	PNC/MNC
Autoimmune polyneuropathy	↑	=	=	=	
Infectious polyneuropathy	↑	=	=	↑	MNC
Subarachnoidal haemorrhage	↑	=	=	↑	erythrocytes, macrophages, siderophages MNC
Multiple sclerosis	=	=	=	= /↑	MNC
Leptomeningeal metastases	↑	= /↓	NA	= /↑	malignant cells, mononuclears

CSF, cerebrospinal fluid; MNC, mononuclear cells; PNC, polymorphonuclear cells. ↑/↓, increased/decreased; =, within normal limits; NA, evidence not available. [a]Normal values are given for lumbar CSF in adults.

important functions. It is invaluable as a diagnostic aid in the evaluation of inflammatory conditions, infectious or non-infectious, involving the brain, spinal cord, and meninges as well as in CT-negative subarachnoidal haemorrhage and in leptomeningeal metastases. CSF is obtained with relative ease by lumbar puncture (LP). Alterations in CSF constituents may be similar in different pathologic processes and cause difficulties in interpretation. Combining a set of CSF variables referred to as routine parameters (i.e. determination of protein, albumin, immunoglobulin, glucose, lactate, and cellular changes, as well as specific antigen and antibody testing for infectious agents) will increase the diagnostic sensitivity and specificity.

The aim of this guideline paper was to produce recommendations on how to use this set of CSF parameters in different clinical settings and to show how different constellations of these variables correlate with diseases of the nervous system (table 4.1) (Brainin *et al.*, 2004).

Search strategy

A Medline search using the search terms cerebrospinal fluid (CSF), immunoglobulin G (IgG) immunoglobulin M (IgM), immunoglobulin A (IgA), and albumin was conducted. Also, the key words 'cerebrospinal fluid' or 'CSF' were cross-referenced with 'glucose', 'lactate', 'cytology', 'cell* in title' excluding 'child*'. Furthermore, a search for 'cerebrospinal fluid' and 'immunoglobulin' and 'diagnosis' and 'electrophoresis' or 'isoelectric focusing' was performed limited to the time between 1 January 1980 and 1 January 2005, and returned only items with abstracts, and English language (274 references). A search for 'cerebrospinal fluid' AND 'infectious' limited for time (1 January 1980 until now) returned 560 abstracts. Abstracts that primarily did not deal with diagnostic issues and infectious CSF (e.g. non-infectious inflammatory diseases, vaccination, general CSF parameters, pathophysiology, cytokines and therapy) were excluded resulting in 60 abstracts.

Searching the items 'cerebrospinal fluid' AND 'serology' limited for time (1 January 1980 until now) and excluding abstracts not directly related to the topic returned 35 abstracts and a search for 'cerebrospinal fluid' AND 'bacterial culture' limited for time (1 Jan 1980 until now) resulted in 28 abstracts.

The abstracts were selected by the author who was in charge of the respective topic.

In addition, text books and articles identified in reference lists of individual papers were selected if considered appropriate.

There are no guidelines for CSF analysis published by the American Academy of Neurology (AAN). Individual task force members prepared draft statements for various parts of the manuscript. Evidence was classified as Class I–IV and recommendations as Level A–C according to the scheme agreed for EFNS guidelines (Brainin *et al.*, 2004). When only Class IV evidence was available but consensus could be reached, the Task Force has offered advice as Good Practice Points (Brainin *et al.*, 2004). The statements were revised and adapted into a single document that was then revised until consensus was reached.

Quantitative analysis of total protein and albumin

The blood–CSF barrier is a physical barrier, consisting of different anatomical structures, for the diffusion and filtration of macromolecules from blood to CSF. The integrity of these barriers and CSF bulk flow determine the protein content of the CSF (Thompson, 1988; Reiber, 1994). In newborns, CSF protein concentrations are high, but decrease gradually during the first year of life, and are maintained at low levels in childhood. In adults, CSF protein concentrations increase with age (Eeg-Olofson *et al.*, 1981; Statz and Felgenhauer, 1983) (Class I). The CSF to serum albumin concentration quotient ($Q_{alb)}$ can also be used to evaluate blood–CSF barrier integrity (Andersson *et al.*, 1994). The Q_{alb} is not influenced by intrathecal protein synthesis, is corrected for the plasma concentration of albumin, and is an integral part of intrathecal immunoglobulin synthesis formulae. The Q_{alb} is a method-independent measure, allowing the use of the same reference values in different laboratories (Blennow *et al.*, 1993; Reiber, 1995). However, there are no conclusive data on how the Q_{alb} performs compared to total protein as a measure of blood–CSF barrier function in large cohorts of unselected patients.

There is a concentration gradient for total protein and the Q_{alb} along the neuraxis with the lowest concentrations in the ventricular fluid and the highest concentrations in the lumbar sac (Thompson, 1988; Fishman, 1992). A significant decrease of the Q_{alb} was observed from the first 0–4 ml of CSF to the last 21–24 ml of CSF obtained by LP (Blennow *et al.*, 1993) (Class I). The Q_{alb} is also influenced by body weight, sex, degenerative lower back disease, hypothyroidism, alcohol consumption (Class II) and smoking (Class III) (Kornhuber *et al.*, 1987; Skouen *et al.*, 1994; Nyström *et al.*, 1997; Seyfert *et al.*, 2002). Posture and physical activity may influence the CSF protein concentration, resulting in higher CSF protein concentrations in inactive, bed-ridden patients (Seyfert *et al.*, 2002) (Class III). Elevated CSF protein concentrations can be found in the majority of patients with bacterial (0.4–4.4 g/l), cryptococcal (0.3–3.1 g/l), tuberculous (0.2–1.5 g/l) meningitis and neuroborreliosis (Stockstill and Kauffman, 1983; Sabeta, 1985; Kaiser, 1998; Negrini *et al.*, 2000) (Class II). A concentration of >1.5 g/l is specific (99%), but insensitive (55%) for bacterial meningitis as compared to a variety of other inflammatory diseases (Lindquist *et al.*, 1988) (Class I).

In viral neuroinfections CSF protein concentrations are raised to a lesser degree (usually <0.95 g/l) (Negrini *et al.*, 2000) (Class II). The concentration in herpes simplex virus encephalitis is normal in half of the patients during the first week of illness (Koskiniemi *et al.*, 1984) (Class IV).

Non-infectious causes for an increased CSF protein and sometimes with an increased cell count include subarachnoidal haemorrhage, central nervous system (CNS) vasculitis, and CNS neoplasm (Jerrard *et al.*, 2001) (Class IV). Elevated total protein concentration with normal CSF cell count (albuminocytologic dissociation) is a hallmark in acute and chronic inflammatory demyelinating polyneuropathies but protein levels may be

normal during the first week (Segurado *et al.*, 1986; Senevirante, 2000) (Class IV). Total CSF protein is elevated in 80% of patients with leptomeningeal metastases to a median concentration of 1 g/l with a wide range (Twijnstra *et al.*, 1989) (Class III).

In conclusion, there is Class I evidence that increased Q_{alb} and total CSF protein concentrations are mainly supportive of bacterial, cryptococcal, and tuberculous meningitis as well as leptomingeal metastases. As Q_{alb} or protein is usually not the only CSF investigation the combination with other CSF variables will increase the diagnostic specificity, like albuminocytologic dissociation in Gullain–Barré syndrome.

Quantitative intrathecal immunoglobulin synthesis

Intrathecal Ig synthesis is found in various, mainly inflammatory CNS diseases (table 4.2). There is a close correlation between the Q_{alb} and the CSF-serum IgG concentration quotient (Q_{IgG}) which led to the development of the IgG index (Q_{IgG} /Q_{alb}) (Delpech and Lichtblau, 1972; Ganrot and Laurell, 1974; Link and Tibbling, 1977). Reiber's hyperbolic formula and Öhman's extended immunoglobulin indices are based on the demonstration of non-linear relationships between the Q_{alb} and CSF-serum concentration quotients for IgG, IgA and IgM (Öhman *et al.*, 1989 and 1993; Reiber, 1994).

Table 4.2 Percentage of patients in different categories of disease with elevated IgA-index, IgG-index, IgM-index, or non-linear intrathecal synthesis formula values (data from Schipper *et al.,* 1988; McLean *et al.* 1990; Öhman *et al.,* 1992; Sellebjerg *et al.,* 1996; Korenke *et al.,* 1997). Unexpected increases are more common with the IgA index, IgG index and IgM index than with corresponding non-linear formulae.

	IgG (%)	IgA (%)	IgM (%)
No inflammatory and no CNS disease	<5	<5	<5
Non-inflammatory CNS disease (including degenerative and vascular diseases)	<25[a]	<5	<5
Infections of the nervous system	25–50	25	25
Bacterial infections	25–50	25–50	<25
Viral infections	25–50	<25	<25
Lyme neuroborreliosis	25–50	<25	75
Multiple sclerosis	70–80	<25	<25
Clinically isolated syndromes	40–60	<10	<25
Inflammatory neuropathies	25–50[a]	25–50[a]	25–50[a]
Neoplastic disorders (in general)	<25[a]	ND	ND
Paraneoplastic syndromes	<25	ND	ND
Meningeal carcinomatosis	25–50	ND	ND
Other neuroinflammatory diseases	25–50[b]	ND[c]	ND

CNS, central nervous system; ND, not determined in larger studies using non-linear immunoglobulin formulae. [a]Usually not associated with oligoclonal bands (artefact in presence of barrier impairment); [b]rare in biopsy-proven neurosarcoidosis; [c]prominent IgA synthesis in adrenoleukodystrophy.

For the detection of intrathecal IgG synthesis, the detection of IgG oligoclonal bands is superior to the IgG index and the non-linear formulae both in terms of diagnostic sensitivity and specificity. However, the detection of IgG oligoclonal bands is technically more demanding than the quantitative measures, and it has been suggested that in the setting of suspected multiple sclerosis (MS), oligoclonal bands analysis may be omitted in patients with an IgG-index value above 1.1, as almost 100% of such patients turn out to have intrathecally synthesized IgG oligoclonal bands (F. Deisenhammer, unpublished data).

In studies comparing CSF findings in patients with MS and other neurological diseases, non-linear formulae were superior (Öhman *et al.*, 1992; Sellebjerg *et al.*, 1996). Intrathecal IgA, IgG and IgM synthesis formulae may be helpful in discriminating between different infectious diseases of the nervous system (Felgenhauer, 1982; Felgenhauer and Schädlich, 1987) (Class III). However, one study suggested that increased values of the Reiber formula do not always reflect intrathecal IgM synthesis as increased values were observed in several patients with non-inflammatory diseases without IgM oligoclonal bands in CSF (Sharief *et al.*, 1990) (Class II). In conclusion, there is no evidence to support the routine use of quantitative assessment of intrathecal immunoglobulin synthesis in the diagnosis of neurological diseases, but in the setting of suspected MS the IgG index may be used as a screening procedure to determine intrathecal IgG synthesis.

Qualitative (oligoclonal) intrathecal IgG synthesis

The detection of intrathecal oligoclonal IgG in the CSF is useful diagnostically, particularly as it is one of the laboratory criteria supporting the clinical diagnosis of MS (McDonald *et al.*, 2001). In addition, it can be used to assist in the diagnosis of other putative autoimmune disorders of the CNS, such as paraneoplastic disorders and CNS infections (Rauer and Kaiser, 2000; Stich *et al.*, 2003; Storstein *et al.*, 2004).

Using electrophoresis techniques it is possible to classify the humoral responses according to the number of antibody clones produced (i.e. monoclonal, oligoclonal and polyclonal responses; figure 4.1). Earlier methods have now been superseded by the development of the more

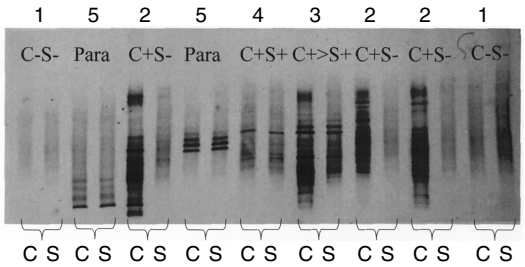

Figure 4.1. IEF immunoblots of the five consensus patterns of various CSF and serum isoelectric focusing patterns for local/systemic synthesis. The pattern number is given above the paired samples.

Type 1 (C-S-): No bands in CSF and serum. Normal.

Type 2 (C+S-): Oligoclonal IgG is present in the CSF with no apparent corresponding abnormality in serum, indicating local intrathecal synthesis of IgG. Typical example: MS.

Type 3 (C+>S+): There are IgG bands in both the CSF and serum, with additional bands present in the CSF. The oligoclonal bands that are common to both CSF and serum imply a systemic inflammatory response, whereas the bands that are restricted to the CNS suggest that there is an additional CNS-only response. Typical examples: MS, systemic lupus erythematodes (SLE), sarcoid etc.

Type 4 (C+S+): There are oligoclonal bands present in the CSF, which are identical to those in serum. This is not indicative of local synthesis, but rather, the pattern is consistent with passive transfer of oligoclonal IgG from a systemic inflammatory response. Typical examples: Guillain–Barre syndrome, acute disseminated encephalomyelitis (ADEM) and systemic infections.

Type 5 (Para): There is a monoclonal IgG pattern in both CSF and serum, the source of which lies outside the CNS. Typical examples: Myeloma, monoclonal gammopathy of undetermined significance (MGUS).

sensitive technique of isoelectric focusing (IEF) and immunofixation (Andersson *et al.*, 1994).

Isoelectric focusing uses a pH gradient to separate IgG populations on the basis of charge, which are then transferred onto a nitro-cellulose or other membrane before immunostaining using an anti-human immunoglobulin (Keir *et al.*, 1990). Some laboratories continue to use silver staining to detect oligoclonal bands (OCBs) with good results (Blennow and Fredman, 1995).

As CSF is an ultrafiltrate of plasma, it contains immunoglobulins that are passively transferred from the plasma, as well as immunoglobulins synthesized locally. Any systemic pattern of immunoglobulin production seen in plasma or serum will therefore be mirrored in the CSF. It is imperative that any CSF analysis for oligoclonal bands is accompanied by a paired blood analysis.

An oligoclonal intrathecal IgG antibody response is not specific. Table 4.3 provides a list with the proportion of cases with oligoclonal bands (for a more detailed list please see McLean *et al.* (1990)). Local synthesis of oligoclonal bands is therefore not diagnostic and has to be interpreted in the clinical context. A recently published recommendation regarding detection of oligoclonal bands concluded as follows (Freedman *et al.*, 2005):

> The single most informative analysis is a qualitative assessment of CSF for IgG, best performed using IEF together with some form of immunodetection (blotting or fixation). This qualitative analysis should be performed using unconcentrated CSF and must be compared directly with serum run simultaneously in the same assay in an adjacent track. Optimal runs utilize similar amounts of IgG from paired serum and CSF. Recognised positive and negative controls should be run with each set of samples.

In putative non-infectious inflammatory disorders of the CNS there is Class I evidence to support the use of CSF IEF for both predictive and diagnostic testing in the diagnosis of MS. In other non-infectious inflammatory disorders of the CNS Class II and III evidence exists to support the use of CSF IEF to supplement other diagnostic tests (table 4.3).

CSF glucose concentration, CSF/serum glucose ratio and lactate

As glucose is actively transported across the blood–brain barrier the CSF glucose levels are directly proportional to the plasma levels and therefore simultaneous measurement in CSF and blood is required. Normal CSF glucose concentration is 50–60% of serum values (Jerrard *et al.*, 2001) (Class IV). A CSF/serum glucose ratio less than 0.4–0.5 is considered to be pathological (Feigin *et al.*, 1992) (Class IV). CSF glucose takes several hours to equilibrate with plasma glucose; therefore, in unusual circumstances levels of CSF glucose can actually be higher than plasma levels for several hours. During CSF storage glucose is degraded. Therefore, glucose determination must be performed immediately after CSF collection.

A high CSF glucose concentration has no specific diagnostic importance and is related to an elevated blood glucose concentration, for example, in diabetics.

The behaviour of the CSF/serum glucose ratio in different neurological diseases is shown in table 4.1.

The relevance of CSF lactate is similar to that of CSF/serum glucose ratio. CSF lactate is independent of blood concentration (Watson and Scott, 1995) (Class IV). The normal value is considered to be $<$2.8–3.5 mmol/l (Jordan, 1983) (Class II). Except for mitochondrial disease CSF lactate correlates inversely with CSF/serum glucose ratio. An increased level can be detected earlier than the reduced glucose concentration.

Decreased CSF/serum glucose ratio or increased CSF lactate indicate bacterial and fungal infections or leptomeningeal metastases.

Cytological examination

Cytological evaluation should be performed within 2 h after puncture, preferably within 30 min because of a lysis of both red blood cells and white blood cells (Steele *et al.*, 1986) (Class IV).

Cerebrospinal fluid leukocytes are usually counted in a Fuchs-Rosenthal chamber (volume 3.2 μl) and therefore, counts are reported as '/3'

Table 4.3 Inflammatory diseases of the CNS associated with CSF oligoclonal IgG bands (McLean *et al.*, 1990).

Disorder	Incidence of oligoclonal bands (%)	Evidence
Multiple sclerosis	95	Class I[a]
Auto-immune		
Neuro-SLE	50	Class III
Neuro-Behcet's	20	Class II
Neuro-sarcoid	40	Class III
Harada's meningitis-uveitis	60	Class III
Infectious		
Acute viral encephalitis (<7 days)	<5	Class II
Acute bacterial meningitis (<7 days)	< 5	Class II
Subacute sclerosing panencephalitis (SSPE)	100	Class I
Progressive rubella panencephalitis	100	Class I
Neurosyphilis	95	Class I
Neuro-AIDS	80	Class II
Neuro-borrelliosis	80	Class I
Tumour	<5	Class III
Hereditary		
Ataxia-telangiectasia	60	Class III
Adrenoleukodystrophy (encephalitic)	100	Class II

CNS, central nervous system; CSF, cerebrospinal fluid; IgG, immunoglobulin G; SLE, systemic lupus erythematodes. [a]This is based on studies using the Poser diagnostic criteria (Poser *et al.*, 1983) that were validated against the original Schumacher criteria (Schumacher *et al.*, 1965). None of these criteria have been validated using population-based studies. Therefore, it could be argued that the diagnostic 'gold standard' is a flawed standard.

cells to correct for a standard volume of 1 μl. A cytocentrifuge (cytospin), the Sayk sedimentation chamber, or membrane filtration can be used to obtain a sufficient number of cells for cytology (Lamers and Wevers, 1995). For cellular differentiation May–Gruenwald–Giemsa staining is widely used but specific methods may be performed, especially for the detection of malignant cells (Roma *et al.*, 2002; Adam *et al.*, 2001) (Class II).

Lymphocytes and monocytes at the resting phase and occasionally ependymal cells are found in normal CSF.

An increased number of neutrophilic granulocytes can be found in bacterial and acute viral CNS infections (Spanos *et al.*, 1989; Adam, 2001) (Class II). In the postacute phase a mononuclear transformation occurs.

Upon activation lymphocytes can enlarge or become plasma cells indicating an unspecific inflammatory reaction (Adam, 2001; Zeman *et al.*, 2001) (Class IV). Resting monocytes enlarge and display vacuoles when activated. Macrophages are the most activated monocytes. These cell forms can occur in a great variety of diseases.

Erythrophages occur 12–18 h after haemorrhage. Siderophages containing haemosiderin are seen as early as 1–2 days after haemorrhage and may persist for weeks. Macrophages containing haematoidin (crystallized bilirubin) degraded from haemoglobin may appear about 2 weeks after bleeding and are a sign of a previous subarachnoid

Table 4.4 List of infectious agents responsible for the vast majority of infectious CNS diseases.

Pathogen	Symptoms, Comments	Recommended diagnostic method*
Bacteria		
Should be considered in first line		
Neisseria meningitides		Microscopy, culture
Streptococcus pneumoniae		Microscopy, culture
Haemophilus influenzae	Rare due to vaccination	Microscopy, culture
Staphylococcus aureus	Neurosurgical intervention, trauma	Microscopy, culture
Escherichia coli	Newborns	Microscopy, culture
Borrelia burgdorferi sensu lato		Serology
Treponema pallidum	Syphilis in the past	Serology
Mycobacterium tuberculosis		PCR[a], culture, positive tuberculin test
Should be considered especially in immunosuppressed patients		
Actinobacter species		Culture
Bacteroides fragilis		Culture
JC-virus	Progressive multifocal leukoencephalopathy	PCR
Listeria monocytogenes		Microscopy, culture
Nocardia asteroides		Microscopy (modified Ziehl-Neelsen stain and culture from brain biopsy)
Pasteurella multocida		Culture
Streptococcus mitis		Culture
Should be considered in special situations		
Brucella spp.	Ingestion of raw milk (products) from cows, sheep or goats	Culture
Campylobacter fetus		Microscopy, culture
Coxiella burnetti (Q-fever)	Contact with infected parturient animals (sheep, goat, cattle) or inhalation of dust contaminated by the excrements of infected animals or ticks	Serology
Leptospira interrogans	Exposure to contaminated water or rodent urine	Culture, serology
Mycoplasma pneumoniae	Children and young adults	Serology
Rickettsia	Tick exposure, exanthema	Serology
coagulase-negative staphylococci	Patients with ventricular shunts or drainages	Culture
group B streptococci	(preterm) newborns	Microscopy, culture
Tropheryma whipplei (M. Whipple)	Patients with gastrointestinal symptoms (malabsorption)	PCR
Viruses		
Should be considered in first line		
Herpes simplex virus (HSV) type 1 and 2		PCR, serology
Varicella–Zoster virus (VZV)		PCR, serology
Enteroviruses (Echovirus, Coxsackievirus A, B)		PCR, serology
Human immunodeficiency virus (HIV) type 1 and 2		PCR, serology

Continued

Table 4.4 Continued.

Pathogen	Symptoms, Comments	Recommended diagnostic method*
Epstein–Barr virus (EBV)	Lymphadenitis, splenomegaly	PCR
Cytomegalovirus (CMV)	Very rare in immunocompetent patients	PCR
Should be considered in special situations		
Adenovirus	Children and young adults	PCR, culture, antigen detection
Human T-cell leukaemia virus type I (HTLV-I)	Spastic paraparesis	Serology
Influenza - and Parainfluenza virus		Serology
Lymphocytic chorio-meningitis (LCM)		Serology
Mumps virus		Serology
Poliovirus	Flaccid paresis	PCR
Rabies virus	Contact with rabies-infected animals	PCR from CSF, root of hair, cornea
Rotavirus	Diarrhoea, febrile convulsions in children	Antigen detection in stool specimens
Rubella virus		Serology
Sandfly Fever	Endemic region: Italy	Serology
Fungi		
Aspergillus fumigatus		Antigen detection in CSF, where required culture from brain biopsy
Cryptococcus neoformans		Antigen detection in CSF, india ink stain, less sensitive than antigen detection, culture
Parasites		
Toxoplasma gondii		CSF: PCR, serology; brain biopsy: PCR
Strongyloides stercoralis		Pathogen detection in stool

The following pathogens should be considered in acute myelitis [Recommendation Level B]: HSV type 1 and 2 (PCR), VZV (PCR), Enteroviruses (PCR), *Borrelia burgdorferi sensu latu* (serology, AI), HIV (serology), tick-borne encephalitis virus (only in endemic areas) (serology, AI). [a]Nested PCR technique has been shown to be substantially more sensitive and specific than conventional single step PCR techniques (Takahashi *et al.*, 2005).

bleeding (Adam, 2001) (Class IV). However, spectrophotometry of CSF involving bilirubin quantitation has been recommended as the method of choice to prove CT-negative subarachnoid bleeding up to 2 weeks after onset (UK National External Quality Assessment Scheme for Immunochemistry Working Group, 2003).

Lipophages indicate CNS tissue destruction. The presence of macrophages without detectable intracellular material is a non-specific finding, occurring in disc herniation, malignant meningeal infiltration, spinal tumours, head trauma, stroke, MS, vasculitis, infections and subarachnoid haemorrhage (Adam, 2001) (Class IV).

Eosinophils are normally not present in CSF. The presence of 10 or more eosinophils/μl in CSF or eosinophilia of at least 10% of the total CSF leukocyte count is associated with a limited number of diseases, including parasitic infections, and coccidioiodomycosis. It can occur in malignancies

and react to medication and ventriculoperitoneal shunts (Lo Re, 2003). Malignant CSF cells indicate leptomeningeal metastases. False positive results often occur when inflammatory cells are mistaken for tumour cells or due to contamination with peripheral blood (Twijnstra *et al.*, 1987). False negative detection of malignant cells on cytologic examination of CSF is common. Factors increasing the detection rate of malignant cells include a volume of at least 10.5 ml and repeating this procedure once if the cytology is negative. The detection rate of 50–70% after the first investigation can be increased to 85–92% after a second puncture (Glantz *et al.*, 1998) (Class III). Further LPs will only slightly increase the diagnostic sensitivity (Wasserstrom *et al.*, 1982; Kaplan *et al.*, 1990) (Class III).

In conclusion, cell count is generally useful because most of the indications for CSF analysis include diseases that are associated with elevated numbers of various cells. Cytological staining can be helpful in distinguishing CNS diseases when the cell count is increased.

Investigation of infectious CSF

There are many small to medium-sized studies investigating the diagnostic sensitivity and specificity of tests for various infectious agents but no controlled study evaluating a work-up of infectious CSF in general. Therefore, there are no valid data on the indication, sensitivity and specificity of microbiological procedures in general (i.e. how to proceed with CSF in obvious CNS infections). Existing proposals for the general work-up of infectious CSF are based on clinical practice and theoretically plausible procedures (Schlossberg, 1990; Kniehl *et al.*, 2001; Kaiser, 2002).

There is a great number of methods for antigen or specific antibody detection and their use depend mainly on the type of antigen (table 4.4).

In neuroinfections specific antigen or antibody detection should be performed depending on the clinical presentation and the results of basic CSF analysis. The formula for the estimation of the relative intrathecal synthesis of specific antibodies in the CSF (Antibody Index [AI]) is as follows:

Estimation of intrathecal synthesis of specific antibodies in the CSF (Antibody Index [AI])

$$\text{Antibody ratio} = \frac{\text{Antibody} - \text{concentration}_{\text{CSF}}}{\text{Antibody} - \text{concentration}_{\text{serum}}}$$

$$\text{IgG ratio} = \frac{\text{IgG} - \text{concentration}_{\text{CSF}}}{\text{IgG} - \text{concentration}_{\text{serum}}}$$

$$\text{AI} = \text{Antibody ratio/IgG ratio(positive} > 1,5)$$

Cerebrospinal fluid polymerase chain reaction can be performed rapidly and inexpensively and has become an integral component of diagnostic medical practice. A patient with a positive PCR result is 88 times more likely to have a definite diagnosis of viral infection of the CNS as compared to a patient with a negative PCR result. A negative PCR result can be used with moderate confidence to rule out a diagnosis of viral infection of the CNS (the probability of a definite viral CNS infection was 0.1 in case of a negative PCR result compared to a positive PCR result) (Jeffery *et al.*, 1997). It should be considered that false negative results are most likely if the CSF sample is taken within the first 3 days after the illness or 10 days and more after the onset of the disease (Davies *et al.*, 2005; Kennedy, 2005).

In general, PCR is indicated in the following situations:

when microscopy, culture or serology is insensitive or inappropriate;
when culture does not yield a result despite clinical suspicion of infectious meningitis/meningoencephalitis; and
in immunodeficient patients.

RECOMMENDATIONS

CSF should be analysed immediately (i.e. <1 h) after collection. If storage is required for later investigation this can be done at 4–8°C (short term) or at −20°C (long term). Only

continued

protein components and RNA (after appropriate preparation) can be analysed from stored CSF (Good Practice Point).

The Level B recommendation regarding CSF partitioning and storage states that 12 ml of CSF should be partitioned into three to four sterile tubes. It is important that the CSF is not allowed to sediment before partitioning. Store 3–4 ml at 4°C for general investigations, cultivation and microscopic investigation of bacteria and fungi, antibody testing, polymerase chain reaction (PCR), and antigen detection. Larger volumes (10–15 ml) are necessary for certain pathogens like *Mycobacterium tuberculosis*, fungi or parasites.

Normal CSF protein concentration should be related to the patient's age (higher in the neonate period and after age of 60 years) and the site of LP (Level B). Exact upper normal limits of protein concentration differ according to the technique and the examining laboratory.

The Q_{alb} should be preferred to total protein concentrations, partly because reference levels are more clearly defined and partly because it is not confounded by changes in other CSF proteins (Level B).

The glucose concentration in CSF should be related to the blood concentration. Therefore CSF glucose/serum ratio is preferable. Pathological changes in this ratio or in lactate concentration are supportive for bacterial or fungal meningitis or leptomeningeal metastases (Level B).

Intrathecal IgG synthesis can be measured by various quantitative methods, but at least for the diagnosis of MS the detection of oligoclonal bands by appropriate methods is superior to any existing formula (Level A). Patients with other diseases associated with intrathecal inflammation, for example, patients with CNS infections, may also have intrathecal IgA and IgM synthesis as assessed by non-linear formulae (Reiber hyperbolic formulae or extended indices), which should be preferred to the linear IgA and IgM indices (Level B).

Cellular morphology (cytological staining) should be evaluated whenever pleocytosis is found or leptomeningeal metastases or pathological bleeding is suspected (Level B). If cytology is inconclusive in case of query CSF bleeding measurement of bilirubin is recommended up to 2 weeks after the clinical event.

For standard microbiological examination sedimentation at $3000 \times g$ for 10 min is recommended (Level B). Microscopy should be performed using Gram or methylene blue, Auramin O or Ziehl-Nielsen (*M. tuberculosis*), or Indian ink stain (*Cryptococcus*). Depending on the clinical presentation incubation with bacterial and fungal culture media can be useful. Anaerobic culture media are recommended only if there is suspicion of brain abscess. A viral culture is generally not recommended. A list of infectious agents and their association with different diseases as well as the recommended method of detection is provided in table 4.4. The results of bacterial antigen detection have to be interpreted with respect to the microscopical CSF investigation and culture results. It is not routinely recommended in cases of negative microscopy. A diagnosis of bacterial nervous system infection based on antigen detection alone is not recommended (risk of contamination).

Conflicts of interest

The authors have reported no conflicts of interest.

Acknowledgment

We are grateful to Professor Christian Bogdan (Director of the Department for Microbiology and Hygiene, Albert Ludwigs-Universität Freiburg, Germany) and to Professor Rüdiger Dörries (Head of the Department of Virology, Institute of Medical Microbiology und Hygiene Ruprecht-Karls-Universität Heidelberg, Germany) for critical review of the microbiological part of the manuscript (infectious CSF).

References

Adam P, Taborsky L, Sobek O *et al.* (2001). Cerebrospinal fluid. *Adv. Clin. Chem.* **36:**1–62.

Andersson M, Alvarez-Cermeño J, Bernadi G *et al.* (1994). Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report. *J Neurol Neurosurg Psychiatry* **57:**897–902.

Blennow K, Fredman P, Wallin A, Gottfries C-G, Långström G, Svennerholm L (1993). Protein analyses in cerebrospinal fluid. I. Influence of concentration gradients for proteins on cerebrospinal fluid/serum albumin ratio. *Eur Neurol* **33:**126–128.

Blennow K, Fredman P (1995). Detection of cerebrospinal fluid leakage by isoelectric focusing on polyacrylamide gels with silver staining using the PhastSystem. *Acta Neurochir* **136:**135–139.

Brainin M, Barnes M, Baron JC, Gilhus NE, Hughes R, Selmaj K, Waldemar G (2004). Guidance for the preparation of neurological management guidelines by EFNS scientific task forces–revised recommendations 2004. *Eur J Neurol* **11:**577–581.

Davies NW, Brown LJ, Gonde J, Irish D, Robinson RO, Swan AV, Banatvala J, Howard RS, Sharief MK, Muir P (2005). Factors influencing PCR detection of viruses in cerebrospinal fluid of patients with suspected CNS infections. *J Neurol Neurosurg Psychiatry* **76:** 82–87.

Delpech B, Lichtblau E (1972). Étude quantitative des immunoglobulines G et de l'albumine du liquide cephalo rachidien. *Clin Chim Acta* **37:**15–23.

Donald PR, Malan C, van der Walt A (1983). Simultaneous determination of cerebrospinal fluid glucose and blood glucose concentrations in the diagnosis of bacterial meningitis. *J Pediatr* **103:**413–415.

Eeg-Olofson O, Link H, Wigertz A (1981). Concentrations of CSF proteins as a measure of blood brain barrier function and synthesis of IgG within the CNS in 'normal' subjects from the age of 6 months to 30 years. *Acta Paediatr Scand* **70:**167–170.

Feigin RD, McCracken GH Jr, Klein JO (1992). Diagnosis and management of meningitis. *Pediatr Infect Dis J* **11:**785–814.

Felgenhauer K (1982). Differentiation of the humoral immune response in inflammatory diseases of the central nervous system. *J Neurol* **228:** 223–237.

Felgenhauer K, Schädlich H-J (1987). The compartmental IgM and IgA response within the central nervous system. *J Neurol Sci* **77:**125–135.

Freedman MS, Thompson EJ, Deisenhammer F *et al.* (2005) Recommended standard of cerebrospinal fluid analysis in the diagnosis of multiple sclerosis. *Arch Neurol* **62:**865–870.

Fishman RA (1992). *Cerebrospinal Fluid in Diseases of the Nervous System*. Philadelphia, PA: W.B. Saunders, 1992.

Ganrot K, Laurell C-B (1974). Measurement of IgG and albumin content of cerebrospinal fluid, and its interpretation. *Clin Chem* **20:**571–573.

Genton B, Berger JP (1990). Cerebrospinal fluid lactate in 78 cases of adult meningitis. *Intensive Care Med* **16:**196–200.

Glantz MJ, Cole BF, Glantz LK, Cobb J, Mills P, Lekos A, Walters BC, Recht LD (1998). Cerebrospinal fluid cytology in patients with cancer: minimizing false-negative results. *Cancer* **82:**733–739.

Jeffery KJ, Read SJ, Peto TE, Mayon-White RT, Bangham CR (1997). Diagnosis of viral infections of the central nervous system: clinical interpretation of PCR results. *Lancet* **349:**313–317.

Jerrard DA, Hanna JR, Schindelheim GL (2001). Cerebrospinal fluid. *J Emerg Med* **21:**171–178.

Jordan GW, Statland B, Halsted C (1983). CSF lactate in diseases of the CNS. *Arch Intern Med* **143:** 85–87.

Kaiser R (1998). Neuroborreliosis. *J Neurol* **245:**247–255.

Kaiser R (2002). Entzündliche und infektiöse Erkrankungen. In: Hufschmidt A, Lücking CH, eds. *Neurologie Compact, Leitlinie für Klinik und Praxis*. Georg Thieme Verlag, Stuttgart.

Kaplan JG, DeSouza TG, Farkash A, Shafran B, Pack D, Rehman F, Fuks J, Portenoy R (1990). Leptomeningeal metastases: comparison of clinical features and laboratory data of solid tumors, lymphomas and leukemias. *J Neurooncol* **9:**225–229.

Keir G, Luxton RW, Thompson EJ (1990). Isoelectric focusing of cerebrospinal fluid immunoglobulin G: an annotated update. *Ann Clin Biochem* **27:** 436–443.

Kennedy PG (2005). Viral encephalitis. *J Neurol* **252:**268–272.

Kniehl ER, Dörries HK, Geiss B, Matz D, Neumann-Häfelin HW, Pfister H, Prange D, Schlüter B, Spellerberg FB, Specker (2001). *MiQ 17: Qualitätsstandards in der mikrobiologisch-infektiologischen Diagnostik*. Mauch H, Lütticken R eds. München, Jena: Urban & Fischer, 2001.

Korenke GC, Reiber H, Hunneman DH, Hanefeld F (1997). Intrathecal IgA synthesis in X-linked cerebral adrenoleukocystrophy. *J Child Neurol* **12:** 314–320.

Kornhuber J, Kaiserauer, CH, Kornhuber AW, Kornhuber ME (1987). Alcohol consumption and blood-cerebrospinal fluid barrier dysfunction in man. *Neurosci Lett* **79:**218–222.

Koskiniemi M, Vaheri A, Taskinen E (1984). Cerebrospinal fluid alterations in herpes simplex virus encephalitis. *Rev Infect Dis* **6:**608–618.

Lamers KJB, Wevers RA (1995). Cerebrospinal fluid diagnostics: biochemical and clinical aspects. *Klin Biochem Metab* **3:**63–75.

Lindquist L, Linne T, Hansson LO, Kalin M, Axelsson G (1988). Value of cerebrospinal fluid analysis in the differential diagnosis of meningitis: a study in 710 patients with suspected central nervous system infection. *Eur J Clin Microbiol Infect Dis* **7:** 374–380.

Link H, Tibbling G (1977). Principles of albumin and IgG analyses in neurological disorders. III. Evaluation of IgG synthesis within the central nervous system in multiple sclerosis. *Scand J Clin Lab Invest* **37:**397–401.

Lo Re V 3rd, Gluckman SJ (2003). Eosinophilic meningitis. *Am J Med* **114:**217–223.

McDonald WI, Compston A, Edan G *et al.* (2001). Recommended diagnostic criteria for multiple sclerosis: guidelines from the international panel on the diagnosis of multiple sclerosis. *Ann Neurol* **50:**121–127.

McLean BN, Luxton RW, Thompson EJ (1990). A study of immunoglobulin G in the cerebrospinal fluid of 1007 patients with suspected neurological disease using isoelectric focusing and the Log IgG-Index. A comparison and diagnostic applications. *Brain* **113:**1269–1289.

Negrini B, Kelleher KJ, Wald ER (2000). Cerebrospinal fluid findings in aseptic versus bacterial meningitis. *Pediatrics* **105:**316–319.

Nyström E, Hamberger A, Lindstedt G, Lundquist C, Wikkelsö (1997). Cerebrospinal fluid proteins in subclinical and overt hypothyroidism. *Acta Neurol Scand* **95:**311–314.

Öhman S, Forsberg P, Nelson N, Vrethem M (1989). An improved formula for the judgement of intrathecally produced IgG in the presence of blood brain barrier damage. *Clin Chim Acta* **181:**265–272.

Öhman S, Ernerudh J, Forsberg P, Henriksson A, von Schenck H, Vrethem M (1992). Comparison of seven formulae and isoelectrofocusing for determination of intrathecally produced IgG in neurological diseases. *Ann Clin Biochem* **29:**405–410.

Öhman S, Ernerudh J, Forsberg P, von Schenck H, Vrethem M (1993). Improved formulae for the judgement of intrathecally produced IgA and IgM in the presence of blood CSF barrier damage. *Ann Clin Biochem* **30:**454–462.

Poser CM, Paty W, Scheinberg LC *et al.* (1983). New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol* **13:**227–231.

Rauer S, Kaiser R (2000). Demonstration of anti-HuD specific oligoclonal bands in the cerebrospinal fluid from patients with paraneoplastic neurological syndromes. Qualitative evidence of anti-HuD specific IgG-synthesis in the central nervous system. *J Neuroimmunol* **111:**241–244.

Reiber H (1994). Flow rate of cerebrospinal fluid (CSF) – a concept common to normal blood-CSF barrier function and to dysfunction in neurological diseases. *J Neurol Sci* **122:**189–203.

Reiber H (1995). External quality assessment in clinical neurochemistry: survey of analysis for cerebrospinal fluid (CSF) proteins based on CSF/serum quotients. *Clin Chem* **41:**256–263.

Reiber H, Thompson EJ, Grimsley G *et al.* (2003). Quality Assurance for Cerebrospinal Fluid Protein Analysis: International Consensus by an Internet-Based Group Discussion. *Clin Chem Lab Med* **41:**331–337. Available at: www.teamspace.net/CSF.

Roma AA, Garcia A, Avagnina A, Rescia C, Elsner B (2002). Lymphoid and myeloid neoplasms involving cerebrospinal fluid: comparison of morphologic examination and immunophenotyping by flow cytometry. *Diagn Cytopathol* **27:**271–275.

Sabetta JR, Andriole VT (1985). Cryptococcal infection of the central nervous system. *Med Clin North Am* **69:**333–344.

Schipper HI, Bardosi A, Jacobi C, Felgenhauer K (1988). Meningeal carcinomatosis: origin of local IgG production in the CSF. *Neurology* **38:**413–416.

Schlossberg D (1990*). Infections of the Nervous System*. Springer-Verlag, Berlin.

Schumacher FA, Beebe GW, Kibler RF *et al.* (1965). Problems of experimental trials of therapy in multiple sclerosis. *Ann NY Acad Sci* **122:**552–568.

Segurado OG, Kruger H, Mertens HG (1986). Clinical significance of serum and CSF findings in the Guillain-Barre syndrome and related disorders. *J Neurol* **233:**202–208.

Sellebjerg F, Christiansen M, Rasmussen LS, Jaliachvili I, Nielsen PM, Frederiksen JL (1996). The cerebrospinal fluid in multiple sclerosis. Quantitative assessment of intrathecal immunoglobulin synthesis by empirical formulae. *Eur J Neurol* **3:**548–559.

Seneviratne U (2000). Guillain-Barre syndrome. *Postgrad Med J* **76:**774–782.

Seyfert S, Kunzmann V, Schwertfeger N, Koch HC, Faulstich A (2002). Determinants of lumbar CSF protein concentration. *J Neurol* **249:**1021–1026.

Sharief MK, Keir G, Thompson EJ (1990). Intrathecal synthesis of IgM in neurological diseases: a comparison between detection of oligoclonal bands and quantitative estimation. *J Neurol Sci* **96:** 131–143.

Skouen JS, Larsen JL, Vollset SE (1994). Cerebrospinal fluid protein concentrations related to clinical findings

in patients with sciatica caused by disk herniation. *J Spinal Disord* **7:**12–18.

Spanos A, Harrell FE Jr, Durack DT (1989). Differential diagnosis of acute meningitis. An analysis of the predictive value of initial observations. *JAMA* **262:** 2700–2707.

Statz A, Felgenhauer K (1983). Development of the blood-CSF barrier. *Develop Med Child Neurol* **25:**152–161.

Steele RW, Marmer DJ, O'Brien MD, Tyson ST, Steele CR (1986). Leukocyte survival in cerebrospinal fluid. *J Clin Microbiol* **23:**965–966.

Stich O, Graus F, Rasiah C, Rauer S (2003). Qualitative evidence of anti-Yo-specific intrathecal antibody synthesis in patients with paraneoplastic cerebellar degeneration. *J Neuroimmunol* **141:**165–169.

Stockstill MT, Kauffman CA (1983). Comparison of cryptococcal and tuberculous meningitis. *Arch Neurol* **40:**81–85.

Storstein A, Monstad SE, Honnorat J, Vedeler CA (2004). Paraneoplastic antibodies detected by isoelectric focusing of cerebrospinal fluid and serum. *J Neuroimmunol* **155:**150–154.

Takahashi T, Nakayama T, Tamura M, Ogawa K, Tsuda H, Morita A, Hara M, Togo M, Shiota H, Suzuki Y, Minami M, Ishikawa H, Miki K, Shikata E, Takahashi S, Kuragano T, Matsumoto K, Sawada S, Mizutani T (2005). Nested polymerase chain reaction for assessing the clinical course of tuberculous meningitis. *Neurology* **64:**1789–1793.

Thompson EJ (1988). *The CSF Proteins: A Biochemical Approach*. Amsterdam, Netherlands: Elsevier.

Twijnstra A, Ongerboer de Visser BW, van Zanten AP (1987). Diagnosis of leptomeningeal metastasis. *Clin Neurol Neurosurg* **89:**79–85.

Twijnstra A, Ongerboer de Visser BW, van Zanten AP, Hart AA, Nooyen WJ (1989). Serial lumbar and ventricular cerebrospinal fluid biochemical marker measurements in patients with leptomeningeal metastases from solid and hematological tumors. *J Neurooncol* **7:**57–63.

UK National External Quality Assessment Scheme for Immunochemistry Working Group. (2003) National guidelines for analysis of cerebrospinal fluid for bilirubin in suspected subarachnoid haemorrhage. *Ann Clin Biochem* **40:**481–488.

van Oostenbrugge RJ, Twijnstra A (1999). Presenting features and value of diagnostic procedures in leptomeningeal metastases. *Neurology* **53:**382–385.

Wasserstrom WR, Glass JP, Posner JB (1982). Diagnosis and treatment of leptomeningeal metastases from solid tumors: experience with 90 patients. *Cancer* **49:**759–772.

Watson MA, Scott MG (1995). Clinical utility of biochemical analysis of cerebrospinal fluid. *Clin Chem* **41:**343–360.

Zeman D, Adam P, Kalistova H, Sobek O, Andel J, Andel M (2001). Cerebrospinal fluid cytologic findings in multiple sclerosis. A comparison between patient subgroups. *Acta Cytol* **45:**51–59.