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EFNS TASK FORCE ARTICLE

# Guidelines on routine cerebrospinal fluid analysis. Report from an EFNS task force

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A great variety of neurological diseases require investigation of cerebrospinal fluid (CSF) to prove the diagnosis or to rule out relevant differential diagnoses. The objectives were 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. The methods included a Systematic Medline search for the above-mentioned variables and 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. It is recommended that 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 increased 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 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 computed tomography (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 pathological processes and cause interpretation difficulties. 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 1) [1].

## 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

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**Table 1** Typical constellation of CSF parameters in some neurological diseases

| | Total protein (g/l) | Glucose ratio (mmol/l) | Lactate | Cell count | Typical cytology (per 3.2 $\mu$l) |
|---|---|---|---|---|---|
| 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.

fluid' and 'immunoglobulin' and 'diagnosis' and 'electrophoresis' or 'isoelectric focusing' was performed limited to the time between 1 January 1980 and 1 January 2005, 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 which 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 January 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 [1]. When only class IV evidence was available but consensus could be reached, the task force has offered advice as good practice points [1]. The statements were revised and adapted into a single document which 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 [2,3]. 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 [4,5] (class I). The CSF to serum albumin concentration quotient ($Q_{alb}$) can also be used to evaluate blood-CSF barrier integrity [6]. 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 [7,8]. 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 ventricular fluid and the highest concentrations in the lumbar sac [3,9]. 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 [10] (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) [11–14]. Posture and physical activity may influence the CSF protein concentration, resulting in higher CSF protein concentrations in inactive, bed-ridden patients [13] (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 [15–18] (class II). A concentration of >1.5 g/l is specific (99%), but insensitive (55%) for bacterial meningitis when compared with a variety of other inflammatory diseases [19] (class I).

In viral neuroinfections CSF protein concentrations are raised to a lesser degree (usually $< 0.95$ g/l) [15] (class II). The concentration in herpes simplex virus encephalitis is normal in half of the patients during the first week of illness [20] (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 [21] (class IV). Elevated total protein concentration with normal CSF cell count (albuminocytological dissociation) is a hallmark in acute and chronic inflammatory demyelinating polyneuropathies but protein levels may be normal during the first week [22,23] (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 [24] (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 albuminocytological dissociation in Gullain–Barré syndrome.

## Quantitative intrathecal immunoglobulin synthesis

Intrathecal Ig synthesis is found in various, mainly inflammatory CNS diseases (Table 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 ($Q_{IgG}/Q_{alb}$) [25–27]. 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 [2,28,29]. 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 [30,31]. Intrathecal IgA, IgG and IgM synthesis formulae may be helpful in discriminating between different infectious diseases of the nervous system [32,33] (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 [34] (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.

**Table 2** Percentage of patients in different categories of disease with elevated IgA index, IgG index, IgM index, or non-linear intrathecal synthesis formula values [30,31,40,62,63]. 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.

## Qualitative (oligoclonal) intrathecal Ig 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 [35]. 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 [36–38].

Using electrophoresis techniques it is possible to classify the humoral response according to the number of antibody clones produced (i.e. monoclonal, oligoclonal and polyclonal responses; Fig. 1). Earlier methods have now been superseded by the development of the more sensitive technique of isolelectric focusing (IEF) and immunofixation [6].

Isolelectric focusing uses a pH gradient to separate IgG populations on the basis of charge, which are then transferred onto a nitrocellulose or other membranes before immunostaining using an anti-human immunoglobulin [39]. Some laboratories continue to use Sliver staining to detect oligoclonal bands (OCBs) with good results [7].

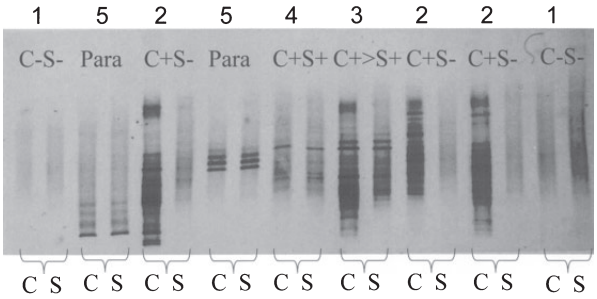


**Figure 1** IEF immunoblots of the five consensus patterns of various CSF and serum isoelectric focusing patterns for local/systemic synthesis. The pattern number is given above the paired samples. Type 1 (C−S−): No bands in CSF and serum. Normal. Type 2 (C + S−): Oligoclonal IgG is present in the CSF 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 which are common to both CSF and serum imply a systemic inflammatory response, whilst the bands which 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–Barré 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).

As CSF is an ultrafiltrate of plasma, it contains immunoglobulins which 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 3 provides a list with the proportion of cases with oligoclonal bands (for a more detailed list please see 40). 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 [41]: '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.'

**Table 3** Inflammatory diseases of the CNS associated with CSF oligoclonal IgG bands [40]

| Disorder | Incidence of oligoclonal bands (%) | Evidence |
|---|---|---|
| Multiple sclerosis | 95 | Class I[a] |
| Autoimmune | | |
| 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 | 100 | Class I |
| Progressive rubella panencephalitis | 100 | Class I |
| Neurosyphilis | 95 | Class I |
| Neuro-AIDS | 80 | Class II |
| Neuroborelliosis | 80 | Class I |
| Tumour | < 5 | Class III |
| Hereditary | | |
| Ataxia-telangectasia | 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 [64] that were validated against the original Schumacher criteria [65]. 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.

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 CSF IEF to supplement other diagnostic tests (Table 3).

## CSF glucose concentration, CSF/serum glucose ratio and lactate

Because 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 [21] (class IV). A CSF/serum glucose ratio less than 0.4–0.5 is considered to be pathological [42] (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, e.g. in diabetics.

The behaviour of the CSF/serum glucose ratio in different neurological diseases is shown in Table 1.

The relevance of CSF lactate is similar to that of CSF/serum glucose ratio. CSF lactate is independent of blood concentration [43] (class IV). The normal value is considered to be $< 2.8$–$3.5$ mmol/l [44] (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 [45] (class IV).

Cerebrospinal fluid leucocytes are usually counted in a Fuchs-Rosenthal's chamber (volume 3.2 $\mu$l) and therefore, counts are reported as '/3' cells in order to correct for a standard volume of 1 $\mu$l. A cytocentrifuge (cytospin), the Sayk sedimentation chamber, or membrane filtration can be used to obtain a sufficient number of cells for cytology [46]. For cellular differentiation May-Grünwald–Giemsa staining is widely used but specific methods may be performed, especially for the detection of malignant cells [47,48] (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 [48,49] (class II). In the postacute phase a mononuclear transformation occurs.

Upon activation lymphocytes can enlarge or become plasma cells indicating an unspecific inflammatory reaction [48,50] (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 bleeding [48] (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 [51].

Lipophages indicate CNS tissue destruction. The presence of macrophages without detectable intracellular material is a non-specific finding, occurring in disc hernation, malignant meningeal infiltration, spinal tumours, head trauma, stroke, MS, vasculitis, infections and subarachnoid haemorrhage [48] (class IV).

Eosinophils are not normally present in CSF. The presence of 10 or more eosinophils/$\mu$l in CSF or eosinophilia of at least 10% of the total CSF leucocyte count is associated with a limited number of diseases, including parasitic infections, and coccidioiodomycosis. It can occur in malignancies and reactive to medication and ventriculoperitoneal shunts [52]. 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 [53]. False-negative detection of malignant cells on cytological 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 [54] (class III). Further LPs will only slightly increase the diagnostic sensitivity [55,56] (class III).

In conclusion, cell count is generally useful because most of the indications for CSF analysis include diseases which are associated with elevated numbers of

**Table 4** List of infectious agents responsible for the vast majority of infectious CNS diseases

| Pathogen | Symptoms, comments | Recommended diagnostic method[a] |
|---|---|---|
| **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 leucoencephalopathy | 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 |
| 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 choriomeningitis (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 |

**Table 4** (Continued)

| Pathogen | Symptoms, comments | Recommended diagnostic method[a] |
|---|---|---|
| **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 [level B recommendation]: 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 [66].

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 [18,57,58].

There is a great number of methods for antigen or specific antibody detection and their use depends mainly on the type of antigen (Table 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:

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

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

$$\text{AI} = \frac{\text{Antibody ratio}}{\text{IgG ratio (positive: } >1.5)}$$

Cerebrospinal fluid polymerase chain reaction (PCR) 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 than 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 than a positive PCR result) [59]. 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 [60,61].

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 and good practice points

Cerebrospinal fluid should be immediately (i.e. $<1$ h) analysed 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 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, PCR and antigen detection. Bigger 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 CSF collection (level B recommendation). Exact upper normal limits of protein concentration differ according to technique and 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 recommendation).

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 support bacterial or fungal meningitis or leptomeningeal metastases (level B recommendation).

Intrathecal IgG synthesis can be measured by various quantitative methods, but at least for the diagnosis of multiple sclerosis the detection of oligoclonal bands by appropriate methods is superior to any existing formula (level A recommendation). Patients with other diseases associated with intrathecal inflammation, e.g. 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 recommendation).

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

For standard microbiological examination sedimentation at $3000 \times g$ for 10 min is recommended (level B recommendation). Microscopy should be performed using Gram or methylene blue, Auramin O or Ziehl-Nielsen (*Mycobacterium tuberculosis*), or Indian ink stain (*Cryptococcus*). Depending on clinical presentation incubation with bacterial and fungal culture media can be useful. Anaerobic culture media are only recommended 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. 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 reportedly have no conflicts of interest.

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