

DIAGNOSTIC CHALLENGES IN CEREBROSPINAL FLUID ANALYSIS

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Abstract : The brain is surrounded and enclosed by a large volume of fluid known as cerebrospinal fluid (CSF). CSF is very vital as it gives important information about bleeding, inflammation and infection in brain, which is not available by other methods. 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 variable analysis of patterns, it can yield answers to clinical questions that aid differential diagnosis, suggestions for further specific analysis and sensitive controls for analysis. Innovative approaches to treatment of diagnostically challenging neurological diseases are being widely tested. All the currently available measurements, including CSF cytology, biochemistry, immunology and molecular markers suffer from poor sensitivity and specificity and often correlate poorly with each other. Thus, the need of the hour is a better understanding of not only the CSF but the newer diagnostic challenges posed and strategizing future diagnostics against all the challenges faced.

The brain and spinal cord are subjected to a varied number of physical processes quite different from those faced by other organs in the body as they lie in rigid body compartments of the skull and spinal canal. These physical processes depend on the fact that the brain is surrounded by and enclosed in a large volume of a fluid known as Cerebro Spinal Fluid (CSF). The analysis of CSF is very vital as it gives important information about bleeding, inflammation and infection in the brain, which is not available by other methods.

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 estimation variable analysis, the analysis of patterns can yield answers to clinical questions that aid differential diagnosis, and provides clue to suggestions for further specific analysis and sensitive controls for analysis (part of analytical quality assessment). CSF analysis can give detailed information about differentiation between acute inflammatory processes (viral v/s bacterial meningitis, encephalitis), detection of micro organisms (as cause of an inflammatory process), detection of an intracranial hemorrhage, early detection of a chronic inflammatory process or a polyspecific immune response, early detection of a post surgery infection, organic cause of psychiatric symptoms, differential diagnosis of dementia, detection of tumors or for efficiency of therapy.

All parts of the nervous system may be involved by infection: muscles (infectious polymyositis due to trichinosis, cysticercosis, certain viral infections), peripheral nerves (mononeuritis or mononeuritis multiplex due to leprosy, borreliosis), plexus (post infectious or post-vaccinnial Parsonage-Turner syndrome) spinal and/or cranial roots (radiculitis with or without associated meningitis i.e. menigo-radiculitis due to neuroborreliosis, varicella zoster, herpes simplex type 2, HIV, schistosomiasis), meninges (bacterial, viral, fungal, spirochetal meningitis), spinal cord (myelitis due to herpes viruses, HIV, HTLV-1, Mycoplasma

pneumoniae, Toxocara), cerebellum (post infectious cerebellitis due to varicella zoster), brain stem (rhombencephalitis) and the brain (encephalitis). The involvement of the brain also maybe diffuse or focal as in brain abscesses or in herpetic encephalitis.

Once the topographic diagnosis is made, the etiologic diagnosis will rely on blood analysis (inflammatory markers, leucocytosis, eosinophilia, serology), CSF analysis (pleocytosis, culture, PCR, intrathecal immune response), imaging of the target organ and in some cases, nerve or brain biopsy. Routine cerebrospinal fluid analysis includes estimation of total protein, albumin, immunoglobulins, glucose, lactate, cell count, cytological staining, bacterial culture and sensitivity and C reactive protein.

Changes in volume of blood, CSF or brain compartments produces compensatory changes in the other compartments which results in increased CSF pressure. Physiology explaining rise in CSF pressure in various diseases is: increased tissue volume in brain tumor and abscess, increased blood volume in hypercapnia, hypoxia and venous sinus occlusion, cytotoxic oedema in ischemia, trauma, toxins and metabolic diseases, vasogenic oedema in infection, brain tumours and inflammation and interstitial oedema in hydrocephalus with transependymal flow.

CSF CYTOLOGY

The timely detection of meningococcal meningitis epidemics is crucial, lowering the meningitis threshold obviously being necessary. Prevention strategies should be initiated as quickly as possible, full coverage of serogroup A (and W 135); a serogroup A containing meningococcal conjugate vaccine being one of the three highest priorities for new vaccine development. Nevertheless, bacterial meningitis can be diagnosed and treated even in rural areas employing simple diagnostic methods, the lack of laboratory facilities forcing the medical community to such simple methods as CSF turbidity, CSF leukocyte count or CSF- serum glucose ratio. Usual CSF **cytological findings** in various different types of meningitis are – *acute bacterial meningitis* several hundreds,

usually a few thousand but occasionally less than 100 cell seen, polymorphonuclear cells predominating; *Tuberculous meningitis* 25-100, rarely more than 500 cells, *Cryptococcal meningitis* 0-800 cells, average 50 with lymphocytic predominance; *viral meningitis* 5 to few hundred, lymphocytic predominance but there may be more than 80% polymorphs in the first few days; *Syphilitic meningitis* average of 500 cells, usually lymphocytes, rarely polymorphs. *Cysticercosis meningitis* has an increased mononuclear and polymorphonuclear cellular infiltration with 2-7% eosinophils; *Sarcoid meningitis* has 0 to fewer than 100 mononuclear cells; *Tumour* - 0 to several hundred mononuclear cells & malignant cells. Pleocytosis is also seen in various other diseases like *brain infarction*, *subarachnoid bleed*, *cerebral vasculitis*, *acute demyelination* and *brain tumours*.

CSF IN TOTAL PROTEINS

Total protein level of CSF ranges between 15-50mg/dl. While an elevated protein level lacks specificity, it is an index of neurological disease reflecting a pathological increase in permeability of endothelial cells. Increase in protein level to 5g/dl and above is seen in meningitis, blood stained CSF, and cord tumors with spinal block. Polyneuritis (Guillain barre syndrome), diabetic radiculopathy and myxoedema may increase level to 1 to 3 g/dl. Levels below 0.15g/dl occur most often with CSF leaks due to previous lumbar puncture or traumatic dural fistulas.

CSF GLUCOSE

Concentration of glucose in CSF depends on its concentration in the blood. Normal range of glucose concentration in CSF is between 2-5 and 4.5mmol/l in patients with normal blood glucose level. Hyperglycemia 4 hours prior to lumbar puncture results in parallel increase in CSF glucose. Decreased CSF glucose level is characteristic of acute purulent meningitis and is the usual finding in tuberculous and fungal meningitis; it is normal in viral meningitis, although reduced to 25% in mumps cases, and in some cases of herpes simplex and zoster meningoencephalitis. CSF glucose also decreases in other inflammatory meningitis including cysticercosis, amoebic meningitis, acute syphilitic meningitis, granulomatous arteritis and other vasculitis.

MICROBIOLOGICAL AND SEROLOGICAL REACTION

Use of appropriate stains and cultures is essential in cases of suspected infection. DNA amplification techniques using the polymerase chain reaction have improved diagnostic sensitivity.

DIAGNOSIS OF VARIOUS NEUROLOGICAL DISEASES ON THE BASIS OF CSF EXAMINATION

Bacterial meningitis

A prolonged increase of glutamate levels in the CSF may predict poor clinical outcome in patients with bacterial meningitis, possibly because of the sustained neurotoxic effects of this excitatory neurotransmitter. CSF cortisol levels in patients with bacterial meningitis are highly elevated and correlate with disease severity. Moreover, our findings also suggest that intrathecal cortisol may serve as a valuable marker in discriminating between bacterial and aseptic meningitis. Bacterial meningitis is a severe, comparatively frequent disease especially in tropical countries. The causative agent is usually identified by culture, which takes one or two days. Since the prognosis of the patient depends on the early onset of an apt therapy a more rapid diagnosis is highly desirable. Fluorescence in situ hybridization (FISH) is a quick test used for the diagnosis of bacterial meningitis. FISH allows specific visualization of bacteria by the fluorescent microscope by implementing fluorescently marked probes, which hybridize to specific complementary sequences on the bacterial ribosomal RNA. FISH has recently been introduced for the quick detection (3 hours) of pathogens directly in clinical samples like blood cultures, sputa and pyloric biopsies.

Neoplastic meningitis

CSF cytology is used as a diagnostic gold standard for neoplastic meningitis Positive CSF cytology and the presence of multifocal neurological deficits are compatible with the diagnosis of neoplastic meningitis.

Serious doubt about the validity and reliability of the technique have recently been raised. Whereas, false-positive studies are rare or nonexistent in cases of solid tumour neoplastic meningitis; false-negative results are common, particularly when small CSF volumes (<10 cm³) are submitted, or when specimens are not processed by the cytology laboratory immediately, when CSF is obtained from a site distant from the location of active CSF disease (i.e., from the lumbar region in patients with cranial nerve or cerebral signs and symptoms or from a ventricular reservoir in patients with predominantly spinal disease), or when a second CSF specimen is not obtained after an initial specimen is cytologically negative.

Multiple sclerosis

Increased CSF Ig (predominantly IgG but IgM and IgA may also increase) is found in more than 90% patients of multiple sclerosis. Linked to the elevation of IgG is the finding of oligoclonal bands in the cathodal region of electrophoresis CSF osteopontin levels are higher in patients with active disease.

Syphilis

CSF examination is recommended in patients with syphilis with ophthalmic symptoms. CSF mononuclear pleocytosis (more than 5 cells per vol.) and increased protein support the

diagnosis of neurosyphilis. CSF-VDRL is more sensitive in meningovascular syphilis than asymptomatic neurosyphilis and tabes dorsalis. False positive CSF-VDRL may also occur if blood contaminates CSF as it occurs in a traumatic lumbar puncture.

CNS viral infections

CSF by PCR is virtually diagnostic of viral CNS infection (in asymptomatic immunocompetent individuals the CSF does not amplify viral nucleic acid). False positive tests are very rare when the test is performed by a reliable laboratory.

Acute poliomyelitis

CSF shows pleocytosis polymorphonuclear cells predominating during the acute stage. CSF protein is mild to moderately increased. CSF polio virus specific IgM antibody test enables an accurate immunological diagnosis.

Cerebral malaria

Protozoan infections like malaria and African trypanosomiasis are major killers in tropical countries. Every year, more than 1 million people, primarily children, die from malaria due to severe anaemia, multi-organ system failure or cerebral complications. Cerebrospinal fluid analysis has diagnostic and prognostic values which are routinely applied for trypanosomiasis, but not for cerebral malaria. The use of toxic drugs in trypanosomiasis emphasises the need for standardized criteria to determine CNS involvement in this disease. However, in cerebral malaria, clinical diagnosis is not specific enough, leading to false diagnosis and over diagnosis. Systematic lumbar puncture and standardized data collection could improve treatment strategies in both diseases. In children with cerebral malaria, central nervous system TNF alpha production is associated with subsequent neurologic and cognitive morbidity.

Creutzfeldt- Jakob disease

Creutzfeldt-Jakob disease (CJD) is a spongiform encephalopathy that affects about 1 in 10⁶ inhabitants per year. In 1994, a variant of CJD (vCJD) was described in the United Kingdom; further studies are indicated to establish causal relation with bovine spongiform encephalopathy (BSE). In a recent WHO publication a call has been made for addressing surveillance and diagnosis of not only BSE but also CJD and vCJD in Third world countries. To assist the clinical diagnosis of CJD and vCJD various cerebrospinal fluid (CSF) biomarkers are available.

The 14-3-3 protein assay involves Western blot followed by immunodetection. A commercial ELISA is used to measure tau and amyloid-beta in CSF. Transport conditions of the CSF samples have no effect on the detection of the biomarkers. Elevation of IgG to total CSF protein ratio, sometimes with oligoclonal bands has been reported to occur in up to 20% cases. Immunoassays that detect the class of 14-3-3 proteinase inhibitor proteins released into CSF from damaged neurons have proved extremely useful in diagnosis in difficult cases.

Alzheimer's disease

CSF pressure, cell count, sugar and protein are with in normal

limits. Presence of Ubiquitin and tau protein levels in CSF have been reported to be raised in Alzheimers disease, also the level of A Beta 4 are reduced and of this in combination with tau has been suggested as a diagnostic marker.

Acquired immunodeficiency syndrome (AIDS)

Central nervous system (CNS) opportunistic complications in patients with HIV infection include CNS toxoplasmosis, cryptococcal and tuberculous meningitis, cytomegalovirus (CMV), herpes encephalitis, and progressive multifocal leuco-encephalopathy (PML). Some of these conditions can be diagnosed relatively easily by the examination of the cerebrospinal fluid (CSF) such as cryptococcal meningitis. For other conditions, CSF findings may suggest the diagnosis such as in the case of tuberculous meningitis. In the presence of CNS toxoplasmosis with cerebral oedema surrounding the lesions; performing a spinal tap is contraindicated.

The interpretation of CSF findings in those with HIV infection is complicated by the fact that HIV itself may cause CSF abnormalities, even in the absence of HIV encephalitis or AIDS dementia complex. CNS tuberculosis generally occurs in patients with still a relatively good immune function (CD4 count less than 350), while all other opportunistic infections only occur at very low CD4 count: CNS toxoplasmosis (less than 100), cryptococcal meningitis (less than 100), CMV encephalitis (less than 00) and PML (less than 50).

Human African Trypanosomiasis(HAT)

The detection of trypanosomes in blood, lymphatic gland juice and/or cerebrospinal fluid, remains the unique and certain criterium of human African trypanosomiasis diagnosis. However, the study of CSF modifications is necessary to establish and assess efficacious treatment. The white blood cells count (WBC) and protein level are used as an indicators to determine the in CSF stage of the disease and evaluate the efficacy of treatment.

Neuroborreliosis

A combination of basic CSF variables and *Borrelia burgdorferi* (Bb) specific IgG and IgM antibody index (AI) values are used for the diagnosis of early neuroborreliosis. Combined analysis of Bb-specific AI values and basic CSF variables give the highest sensitivity of 80% and specificity of 98%.

Lymes disease

Presence of CSF pleocytosis and PCR testing of CSF are helpful in diagnosis.

Rhinocerebral mucormycosis

It is a severe opportunistic infection caused by moulds belonging to the *mucoraceae* family. These organisms are saprophytic in the respiratory and digestive tracts in 2% of normal individuals. However, conditions such as diabetes mellitus, hematological cancers, renal insufficiency, organ transplantation and chemotherapy can predispose to disease. The CSF analysis shows pleocytosis and high concentration of protein.

Paracoccidioidomycosis (PCM)

It is a chronic granulomatous infectious disease, endemic in

subtropical areas of Central and South America. The diagnosis of the central nervous system (CNS) involvement with paracoccidioidomycosis is frequently difficult. A definitive diagnosis is usually made by the isolation of the *P. brasiliensis* from CNS biopsy or necropsy material. The presence of antibodies anti-gp43 in CSF of patients with CNS involvement in PCM may indicate disease.

Guillain barre syndrome

In the first week of neurological symptoms the CSF protein may be normal but then becomes elevated on subsequent examination. In 10% cases, CSF protein remains normal throughout the illness. Transient oligoclonal IgG bands and elevated myelin basic protein may be detected in some patients.

Subarachnoid hemorrhage

Cerebrospinal fluid adrenomedullin concentration correlates with hyponatremia and delayed ischemic neurological deficits after subarachnoid hemorrhage.

Dementia

Measuring proteins in cerebrospinal fluid (CSF) has gained wide acceptance for the differential diagnosis of dementia. Median Abeta 42 level increases in dementia and decreases in Alzheimers. S-100B protein increases during follow-up in both the diseases.

Neurocysticercosis(NCC)

It is a parasitic disease of the nervous system caused by the larva of taenia solium. The disease is an important public health problem in developing countries. The diagnostic criteria

for NCC are related to the clinical and epidemiological data, neuroimaging studies and the reactivity of immunosorbent assay (ELISA) for NCC in the serum and CSF.

CONCLUSION

Innovative approaches to treatment of diagnostically challenging neurological diseases are being widely tested. Unfortunately, research on diagnostic strategies and outcome measures on which any advances in treatment ultimately depends, has not been avidly pursued. All the currently available measurements, including CSF cytology, biochemistry, immunology and molecular markers suffer from poor sensitivity and specificity and often correlate poorly with each other. Although CSF cytological examination, performed according to a rigorous, research supported protocol, may be the optimum diagnostic and outcome measure at this time, additional research is a prerequisite for any further advances in the diagnosis of challenging CSF's.

RECOMMEDED READING

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Drug Profile

VORICONAZOLE

Voriconazole is a triazole antifungal agent, chemically, it is designated as (2R,3S)-2(2,4-difluorophenyl)-3-(5-fluoro-4-pyrimidinyl)-1(1H-1,2,4- triazol-1-yl)-2-butanol.

Indications: The Drug is indicated for use in the treatment of . (a) invasive aspergillosis; (b) fluconazole- resistant serious invasive candida infections (including *C. Krusei*); (c)esophageal candidiasis; (d) Serious fungal infections caused by *scedosporium apioserum* (a sexual form of *pseudallescheria boydia*)

Mecanism of Action : Voriconazole is a triazole antifungal agent; its primary mode of action is by inhibition of fungal Cytochrome P-450-mediated 14 alpha-alamosterol demethylation - an essential step in fungal ergosterol biosynthesis. The accumulation of 14 alpha-methyl sterols correlates with the subsequent loss of ergosterol in the fungal cell wall and may be responsible for the antifungal activity of voriconazole. The drug has been shown to be more selective for fungal cytochrome P- 450 enzymes than for various mammalian cytochrome p-450 enzyme systems.

Dosage and Administration: The pharmacokinetics of orally administered voriconazole is not affected by renal impairment. Therefore, no adjustment is necessary for oral dosing for patients with mild to severe renal impairment.

Table 1 A: Recommended Dosing Regimen in Adults and Adolescents (12 to 16 years of age)

Dose Regimen	Intravenous	Oral (tablets)	
		Patients 40 Kg and above	Patients less than 40 Kg
Loading Dose (for the first 24 hrs)	6 mg/kg every 12 hrs	400 mg every 12 hrs.	200 mg every 12 hrs.
Maintenance Dose (after first 24 hrs)	4 mg/kg twice daily	200 mg twice daily	100 mg twice daily

Voriconazole is haemodialysed with a clearance of 121 ml/min. A four hour haemodialysis session does not remove a sufficient amount of Voriconazole to warrant dose adjustment. After intravenous administration, cyclodextrin can accumulate in the kidneys, unlike after oral administration. Accumulation of the intravenous vehicle, hydroxypropyl B cyclodextrin may occurs in patients with moderate or severe renal insufficiency (creatinine clearance <50 mL/min). Oral voriconazole should be administered to these patients, unless an assessment of the benefit/risk to the patient, justifies the use of intravenous voriconazole. Serum creatinine levels should be closely monitored in these patient, and if increase occurs, consideration should be given to changing to oral voriconazole therapy.

Precautions: (1) Pregnancy- teratogenic effects on the foetus. (2) Women of childbearing potential should use effective contraception with increased dose during co-administration of voriconazole with oral contraceptive. (3) The excretion of voriconazole in breast milk has not been investigated. Voriconazole should not be used by nursing mothers unless the benefit clearly out weights the risk.

Undesirable Effects : The most frequently reported adverse events in the therapeutic trials include fever, rash, vomiting, nausea, diarrhoea, headache, sepsis, peripheral edema, abdominal pain and respiratory disorder. The treatment- related adverse events which may often need discontinuation of voriconazole therapy include (i) elevated liver enzymes, (ii) rash, (iii) visual disturbances .(colour vision change, photophobia, decrease in visual field- 20% cases). (iv) dermatological reaction like photosensitivity.