parasites of Nervous system

Introduction

Central nervous system (CNS) infections of parasitic organisms represent a minor problem . When present, however, early recognition is essential if an adequate therapeutic response is expected. For this reason, clinical microbiology laboratories should develop an approach for handling this less comm on diagnostic problem .
Cerebrospinal fluid (CSF) analysis is a set of laboratory tests that examine a sample of the fluid surrounding the brain and spinal cord. This fluid is an ultrafiltrate of plasma. It is clear and colorless. It contains glucose, electrolytes, amino acids, and other small molecules found in plasma, but has very little protein and few cells. CSF protects the central nervous system from injury, cushions it from the surrounding bone structure, provides it with nutrients, and removes waste products by returning them to the blood. CSF is withdrawn from the subarachnoid space through a needle by a procedure called a lumbar puncture or spinal tap. CSF analysis includes tests in clinical chemistry, hematology, immunology, and microbiology.

Several protozoan and helminth parasites can affect the central nervous system. In some cases the parasite may have a tropism for the central nervous system (CNS) and this represents the primary affect of the pathogen. In many cases, though, the pathogen does not exhibit a specific tropism for the CNS and affects many other organs and tissues. However, the symptoms associated with the invasion of the CNS tend to be more pronounced.

Protozoa Affecting the Central Nervous System

Toxoplasma gondii : associated with congenital defects and AIDS
African trypanosomes African sleeping sickness
Plasmodium falciparum: cerebral malaria
Entamoeba histolytica: rare invasion of the brain
Free-living ameba: Naegleria fowelleri and Acanthamoeba

Helminthes Affecting the CNS

Cysticercosis : Taenia solium (pork tape Worm) muscle and brain
Hydatid Disease :Echinococcus species liver (75%) and lungs (15%)
Schistosomiasis : Schistosoma species liver and bladder
Paragonimiasis : Paragonimus lung

Routine examination of CSF includes visual observation of color and clarity and tests for glucose, protein, lactate, lactate dehydrogenase, red blood cell count, white blood cell count

GROSS EXAMINATION.: Color and clarity are important diagnostic characteristics of CSF. Straw, pink, yellow, or amber pigments (xanthochromia) are

abnormal and indicate the presence of bilirubin, hemoglobin, red blood cells, or increased protein. Turbidity (suspended particles) indicates an increased number of cells. Gross examination is an important aid to differentiating a subarachnoid hemorrhage from a traumatic tap. The latter is often associated with sequential clearing of CSF as it is collected; streaks of blood in an otherwise clear fluid; or a sample that clots.

Sample collection

Lumbar puncture is performed by inserting the needle between the fourth and fifth lumbar vertabrae (L4-L5). This location is used because the spinal cord stops near L2, and a needle introduced below this level will miss the cord. In rare instances, such as a spinal fluid blockage in the middle of the back, a physician may perform a spinal tap in the cervical spine

After the procedure, the site of the puncture is covered with a sterile bandage.The puncture site should be observed for signs of weeping or swelling for 24 hours.

SPECIMEN TRANSPORT AND STORAGE

time between specimen collection and processing

Specimens should be transported and processed as soon as possible. Specimens should be cultured as soon as possible after receipt, ideally within 10 minutes and within a maximum of two hours. Cells disintegrate and a delay may produce a cell count that does not reflect the clinical situation of the patient.

Do not refrigerate specimen until after microscopy and bacterial culture have been performed. The specimen should then be refrigerated pending further investigation

Examination of CSF

The diagnosis of meningitis from the examination of CSF involves the following

- Complete cell count
- Differential leucocyte count
- Examination of Gram-stained smear
- Culture
- Determination of glucose and protein concentrations (usually performed bychemical pathology departments)
- PCR where appropriate
- Antigen testing

Examination of the deposit by cytocentrifugation (eg Cytospin) is the most accurate method of cell differentiation but may not be routinely available.

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MICROSCOPY

STANDARD TOTAL CELL COUNT

Perform total WBC and RBC counts on the uncentrifuged specimen, preferably the last specimen taken, in a counting chamber. Cell counts should not be performed on specimens containing a clot (which would invalidate the result).

Differential leucocyte count

1. Counting chamber method (recommended for lower WBC counts)

a) Non- or lightly-bloodstained specimens

Stain the unspun CSF with 0.1% stain solution such as toluidine, methylene or Nile blue. These stain the leucocyte nuclei aiding differentiation of the cells. If the CSF is diluted when adding the stain, remember to take the dilution factor into account when calculating the final cell count.Count and record the actual numbers of each leucocyte type.

b) Heavily bloodstained specimens

Dilute specimen with WBC diluting fluid and leave for 5 min before loading the counting chamber. This will lyse the RBCs and stain the leucocyte nuclei for differentiation. Count and record the actual numbers of each leucocyte type. Taking the dilution factor into account, express as number of cells per litre

Examination for amoebae

Examine both uncentrifuged and centrifuged deposits as wet preparations. Place a drop of specimen on a clean microscope slide, cover with a coverslip and examine for amoebic trophozoites

Neuroimaging diagnosis of parasitic infections of the CNS

Neuroimaging studies (CT scan and MRI) play an important role in early diagnosis; however, there is a wide range of neuroimaging findings in parasitic infections of the CNS, often with considerable overlap, which makes determination of a specific diagnosis difficult. Therefore, correlation with laboratory tests, especially CSF analysis, is considered to be fundamental in establishing a definitive diagnosis. In addition to conventional CT and MRI, advanced neuroimaging techniques, such as fluid attenuation inversion recovery (FLAIR), diffusion MR, perfusion MR, and MR spectroscopy have been employed, as well as the 3D MRI sequences [Fast Imaging Employing Steady-state Acquisition (FIESTA) and Spoiled Gradient Recalled Echo (SPGR)] which provide more clues for differentiation of CNS parasitic diseases,

Immunological and molecular diagnosis of the main parasitic infections of central nervous system

Direct visualization of the parasite, as for cerebral malaria, HAT (human african trypanosomiasis) and toxoplasmosis, is clearly definitive. In many parasite infections, however, this is not a feasible option, and so the development of specific and sensitive serodiagnostic and molecular biological [polymerase chain reaction (PCR)] assays for viable parasites is an urgent priority that will complement and confirm clinical examination.
The detection of parasite DNA through the PCR is an experimentally simple approach and is currently receiving much more attention than serological detection of secreted products of viable parasites. Unfortunately, parasite DNA can originate from both the live and dead organism and thus, a positive PCR is not necessarily definitive proof of a viable parasite infection. The strength of the PCR, however, is its sensitivity and exquisite specificity, which are characteristics that provide a powerful tool for the differential diagnosis of parasite subtypes and polymorphisms and for molecular epidemiological investigations.

Anti-parasite antibodies are synthesized soon after host invasion and so their detection is still the most frequently employed diagnostic tool. Once again, however, their detection is not definitive proof of a current, viable infection as antibodies can persist for months, even years, after elimination of the parasite, for example by drug treatment.

For antibody diagnosis of cerebral parasitic infections, both serum and CSF samples are commonly employed. The presence of antibodies in CSF is a clear indication of cerebral involvement and damage, but does not necessarily exclude the presence of the parasite in other body locations. Similarly, the presence of serum antibodies may, or may not, indicate a cerebral infection with consequent impact on the blood–brain barrier. Importantly, the demonstration of anti-parasite antibodies, being clear evidence of exposure to the parasite, is an extremely useful tool for the evaluation of parasite endemicity at the level of a population before, during and after control programs.

There has been considerable investigation into the development of serological and molecular biological procedures for the diagnosis. therefore, a variety of recombinant metacestode antigens and synthetic peptides have been tested as target for antibody detection. Amongst these, particularly useful examples are the cathepsin L-like protease and the T24H and Ts8B2 recombinant proteins. Immunodiagnosis of *Taenia solium* taeniosis/cysticercosis. Molecular cloning and characterisation of Ts8B1, Ts8B2 and Ts8B3, three new members of the *Taenia solium* metacestode 8 kDa diagnostic antigen family. Simple, sensitive, specific and economic enzyme-linked

immunosorbent assay (ELISA) assays detecting antibodies to reproducible, recombinant antigens are clearly a preferred alternative to the enzyme-linked immunotransfer blot (EITB), which is expensive, technically more complicated and requires parasite material.

Detection of Naegleria foweleri

Microscopic Methods

Premortem diagnosis is rare, but when CSF pressure is low, lumbar puncture can be performed. CSF is purulent, and when bacteria are not found, amoebic meningoencephalitis should be considered. The CSF is cloudy and slightly hemorrhagic with increased cellularity composed mainly of neutrophils.

CSF is characterized by low glucose and elevated protein. Direct microscopic examination of CSF as a wet mount is the method of choice in the diagnosis of PAM because CSF contains motile amoebae which can be recognized by light microscopic observation. If present in CSF, amoebae can be identified by staining fixed preparations with Wright's, Giemsa, or hematoxylin and eosin (H & E). Although Gram stain is used in clinical laboratories for detection of bacteria in CSF, Gram stain is not useful for diagnosis of amoebae because it does not depict the characteristic nuclear morphology of the amoebae. Amoebae can be mistaken as macrophages, but N. fowleri nucleus contains a large, central, round nucleolus which should distinguish A commercially available enzyme-linked immunosorbent assay (Indicia, Oulin, France) based on the use of a monoclonal antibody (5D12) that recognizes a glycosylated epitope on N. fowelleri can be used to diagnose infections it from host cells

Culture Methods

CSF or biopsied brain tissue should be kept and transported at room temperature to the diagnostic laboratory. This material can be inoculated onto tissue culture cells (Vero, fibroblasts) and incubated at in the presence of the antibiotics, penicillin-streptomycin. Fungicides are lethal to the amoebae. Amoebae that are present will multiply and destroy the monolayer in 24 to 48 hours. Biopsy tissue also can be placed on 1.5% nonnutrient agar coated with a layer of bacteria (Escherichia coli). The amoebae will emerge from the tissue, ingest the bacteria, and divide. The amoebae, then, can be observed on the agar using an inverted light microscope

Polymerase Chain Reaction (PCR) Assays

More rapid molecular techniques are now available in research laboratories, but these methods generally are not available in most clinical laboratories. Highly specific and sensitive PCR and real-time PCR assays have been developed for the detection of N. fowleri in clinical and environmental samples