Infections of the central nervous system: Neuropathology

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Summary

Encephalitides include a large variety of diseases with high morbidity and mortality. Although the majority of identified pathogens are viruses, the cause of the disease remains unexplained in more than a half of the cases despite extensive testing. Neuropathology provides the bases of our understanding of the inflammatory lesions in the CNS. Cerebral biopsy proves to be necessary in cases of unknown etiology, which deteriorate despite treatment. Unexpected pathogens can be uncovered by untargeted transcriptomic analysis, based on deep sequencing of small quantities of pathological brain tissue. Combined with immunohistochemistry and *in situ* hybridization, using tailored antibodies and probes, this next generation sequencing (NGS) method opens perspectives in the diagnosis of encephalitides with a high efficiency particularly in, but not limited to, immunocompromised patients.

Key words: encephalitis, neuropathology, NGS, metagenomics, immunohistochemistry, in situ hybridization.

Introduction

Encephalitis is commonly defined as a rapidly progressive neurological disease associated with the evidence of an inflammatory process that impairs the central nervous system (CNS). This heterogeneous group includes diseases with high morbidity and mortality. The incidence of presumably infectious encephalitides is estimated at 1.5-7 cases /100,000 inhabitants per year [1]. Inectious encephalitides represent the most frequently identified cause, accounting for 20-50% of the cases [2]. Herpes simplex virus (HSV) is the most frequent reported pathogen in the

literature, followed by Varicella-zoster virus (VZV) [1]. Tuberculosis and listeriosis account for higher fatality rates in France than HSV and VZV [3]. The main alternative cause of encephalitis is auto-immunity, for which the treatment is based on immune suppression. The damaging consequences of such a therapy in cases caused by an undiscovered pathogen underline the need to differentiate between auto-immune and infectious causes of encephalitis early enough in the patient care. In addition the link between infection and auto-immunity is tight since, for example, HSV has been shown to trigger anti-NMDA auto-immune encephalitis [4].

Although a wide variety of pathogenic infectious organisms – bacteria, fungi, parasites, viruses, prions – may affect the central nervous system (CNS), the epidemiology of encephalitis has dramatically changed in the last decades primarily as a result of a decrease in vaccine-preventable diseases, although the troubling expansion of some of those which were under control until recently raises new challenges. Other factors influencing nowadays the etiology in encephalitis are the increasing number of immunocompromised patients at risk for opportunistic infections and a better knowledge and diagnosis of auto-immune diseases. Despite extensive testing and evaluation, the cause of more than half the cases remains unexplained [5,6]. Unknown etiology is associated with a high rate of mortality or severe handicap [7].

Bases of the neuropathology of infectious encephalitides

The brain and spinal cord are well protected from infective agents by the skull and vertebral column, by the meninges, and by the blood-brain-barrier. However infectious agents can enter the CNS through direct invasion after trauma, through the blood, or by retrograde spread via peripheral nerves [8]. Once they have penetrated into the CNS, the pathogens encounter a weak host defense compared to other organs, even weaker in immunodeficiency conditions.

This explains the continuing severity of CNS infections, despite the advances in diagnosis and treatment.

Neuropathology has provided most of our basic knowledge about the identification and behavior of pathogens. In the most severe cases, leading to death, the *post mortem* diagnosis, despite the decrease of autopsy rate, may help understanding the etiology and physiopathology and developing an appropriate care for future patients. Examination of brain tissues shows a variety of lesions that associate inflammatory changes and necrosis, the distribution of which may be suggestive of the causative agent. In addition, the lytic effects in host cells caused by some viruses, or the direct observation of the pathogen itself in bacterial, fungal and parasitic infections may lead to the diagnosis.

The pattern of inflammation provides some guidance to the origin of the encephalitis: predominance of neutrophils suggests a bacterial origin; presence of eosinophils a parasitic metazoal infection ; lymphocytes suggest a viral infection [Fig 1] ; vasculitis may be related to a fungal invasion; whereas pathogens such as Koch bacilli are associated to characteristic granulomas composed of lymphocytes, epithelioid and giant cells with caseous necrosis.

Viral encephalitides

The neuropathology of viral infections although relatively stereotyped [9], is influenced by the type of the virus, its site of entry into the body, the age and immune status of the patient. Topography of the lesions may be itself indicative, like temporal lobe involvement in HSV, ventriculitis in cytomegalovirus (CMV) [Fig2], associated peripheral nerve impairment in rabies. The most specific lesions are related to the cytopathic effect that refers to structural changes in host cells [Fig 2, 3]. Some viruses induce specific alteration within the cell, for instance owl's eye nuclear inclusions in CMV infection [Fig2]; cytoplasmic inclusions in rabies (Negri's body)

[Fig3,4]; enlarged oligodendroglial nuclei in progressive multifocal encephalopathy (PML) due to polyomavirus; eosinophilic inclusion bodies in measles. Electron microscopy, widely used in the past to identify viruses, is no longer routinely available. It is largely replaced by immunohistochemistry and *in situ* hybridization.

Most viral infections of the CNS cause meningeal inflammation. In many cases a lymphocytic infiltrate around the vessels, in the Virchow-Robin subarachnoid space, is the only histological abnormality.

Some viruses affect exclusively the grey matter causing *polioencephalitis* or *poliomyelitis*. Lymphocytic inflammation includes perivascular cuffs and parenchymal infiltration, associated with neuronophagia. The principal viruses in that category are the enteroviruses, rabies and some arboviruses. The main differential diagnosis is auto-immune encephalitis that preferentially develops in the limbic cortex or in the cerebellum.

By contrast other viruses affect the white matter and cause *leucoencephalitis*. Polyomaviruses (JC, BK, SV40), that cause PML in a context of immunosuppression belong to this group. Differential diagnoses include multiple sclerosis and acute disseminated encephalomyelitis (ADEM).

Most viruses are able to affect both grey and white matter, causing *panencephalitis*. Necrotizing panencephalitides are most often due to herpes viruses (HSV, CMV, VZV). These viruses produce recognizable cytopathic effects in various types of cells in the CNS including neurons, glia, endothelial and ependymal cells. Non necrotizing panencephalitis may be caused by a large number of viruses including HIV, CMV, HTLV-1, arboviruses, measles, and probably many other still unidentified pathogens. Measles, incidence of which has recently increased in relation with decreased vaccination rate, may induce distinct disorders. Subacute sclerosing panencephalitis (SSPE) is a widespread chronic inflammation, with gliosis and progressive

atrophy, associated with sparse neuronal intranuclear inclusion bodies. measles inclusion-body encephalitis (MIBE) has a subacute course and develops in immunocompromised patients. It is characterized by numerous intranuclear and cytoplasmic inclusions in neurons and glia [10].

The increased number of immunocompromised patients, either affected by acquired immunodeficiency syndrome (AIDS), or more frequently nowadays treated by immunosuppressive drugs, led to substantial changes in the pathological profile of encephalitides. Already known encephalitides may be encountered with less inflammatory reaction, or more necrosis; new pathogens are emerging; restoration of immune response may itself cause a severe brain disorder as part of an immune restoration inflammatory syndrome (IRIS) [11].

Diagnostic tools

Neuroimaging, electroencephalography (EEG) and cerebrospinal fluid (CSF) cytology may facilitate the diagnosis by showing suggestive patterns [12]. Up to recently, diagnostic techniques relied on prior knowledge of the most usual causative agents [12, 13]. Informed by a bundle of clinical and epidemiological information, relying on guidelines and local resources, a laboratory will perform tests targeted to specific diseases such as polymerase chain reaction (PCR) or serological assays in blood and CSF [12]. These techniques are routinely used to detect the most frequent infectious causes worldwide, headed by HSV and VZV. Nevertheless, this approach has fundamental limitations and contributes to the high proportion of encephalitis cases that remain undiagnosed [6]. Aside from the difficulties of testing for a multitude of rare pathogens expected to cause encephalitis, this approach does not permit the identification of new or unexpected pathogens.

Brain biopsy

Although some time considered as a standard diagnostic procedure for HSV encephalitis [14, 15], the indication of brain biopsy tends to be limited in more recent guidelines to cases of unknown etiology that deteriorate despite empirical treatment. The biopsy should target a region with neuroimaging abnormalities in a non-eloquent area of the brain [12] The diagnosis of encephalitis represents the third most common diagnosis, after malignant lymphoma and prion diseases in HIV negative patients, with rapidly deteriorating neurologic condition [16]. Let us underline here that brain biopsies are contraindicated in prion diseases. Although nonspecific inflammatory changes were found in about 40 % of the biopsies in rapidly progressive neurologic diseases [17], the diagnosis of encephalitis was retained in less than 4% in the same series [17, 18]. "Encephalitis not otherwise specified" (ENOS) is currently considered the most common category of encephalitis diagnosed by brain biopsy [19], the specific cause of the disease being rarely uncovered after hypothesis-driven investigations using the limited panel of available immunohistochemistry and PCR tests. It must be underlined, however, that the diagnosis of ENOS after brain biopsy is much more frequent than after autopsy [19], primarily because the latter permits to sample a larger number of areas.

Combining neuropathology and metagenomics

To decrease the number of unknown causes a combination of adequate sampling and improved diagnostic technology is needed. The analysis of neuropathological alteration can be completed by metagenomics, i.e. the study of genetic material recovered from tissue samples. Next generation sequencing (NGS) methods, also known as "Deep sequencing", open perspectives for comprehensive and unbiased detection of pathogens in clinical samples [20]. Applied to the diagnosis of encephalitides, this approach is aimed to detect exogenous sequences within a

high number of host sequences. RNAs transcripts are mandatory intermediates of the replication of viruses and of any kind of pathogens having a transcriptional activity. After conversion into DNA by a reverse transcriptase, all the DNA sequences are analyzed. Downstream analysis allows differentiation between human fragments and extraneous sequences, possibly specific of a micro-organism. Any reads mapping to the human genome are removed, after which all remaining nonhuman sequences are compared to a database of known sequences. An increasing number of case reports and studies in the literature provide evidence of the rapid development of this technique. A recent review listed 25 articles describing 44 cases in which NGS on brain tissue or CSF provided a diagnosis in otherwise undiagnosed cases of encephalitis [20]. In 63%, unexpected organisms were detected which had not been detected using targeted PCR and, in several cases, were not considered among possible agents. Some were indeed known human pathogens (parvovirus 4; coronarovirus OC-43; astrovirus MLB1; mumps vaccine virus) but novel causes of encephalitis. In other cases, rare causes of encephalitis were found, due to bacteriae (*Brucella melitensis*, *Leptospira santarosai*); fungi (*Candida tropicalis*); protozoans (*Balamuthia mandrillaris*). But in most of the cases (18/28) a novel pathogen was uncovered: arenaviruses, bornaviruses, astroviruses, cycloviruses, gemycircularviruses, densovirus.

We have had, for example, the opportunity to observe one of them, in a 14 year-old boy, suffering from X-linked agammaglobulinemia who underwent a progressive psychomotor regression [21]. The brain biopsy showed microglial nodules and lymphocytic cuffs [Fig1]. NGS performed in collaboration with the Pasteur Institute revealed large fragments of the VA1/HMO-C astrovirus, confirmed by tailored RT-PCR. The clinical state improved under ribavirin treatment. Astroviruses have since been recognized as emerging causes of encephalitis in immunosuppressed patients. Untargeted transcriptomic analysis requires small quantities of

pathological brain tissue, and shows an outstanding efficiency in immunocompromised patients with a high level of suspicion of infection.

But finding viral expression is not enough: in the case of a novel or emerging cause of encephalitis for which the clinical significance may be unclear, proving causality is particularly important. Organism-specific immunostaining or *in situ* hybridization in affected tissues provides additional evidence of the cellular distribution of the infection and excludes the possibility of an artefactual contamination. In this context brain biopsies are more informative specimens than CSF. CSF is more readily available in clinical practice; metagenomic sequencing in the CSF is developing with promising results [22]. Nevertheless, the pathogenicity of some viruses (e.g. densovirus, gemycircularvirus, cyclovirus) only detected in the CSF, remains doubtful. In addition the pathogen may not be detected in the CSF in diseases caused by mutated pathogens like SSPE in chronic measles or mumps vaccine encephalitis [20].

The most recent French Guidelines on the management of infectious encephalitis in adults introduced the use of this technique in case of negative results of targeted diagnostic procedures [23]. One can expect that in a near future NGS will be considered a front-line diagnostic tool in encephalitides. Combination with neuropathology offers a tremendous opportunity to be of help in the most severe cases, in particular in immunocompromised patients, but not only.

Figures

1- Brain biopsy sample. H&E. Frontal cortex of a 14-year-old immunocompromised boy: inflammatory infiltrates and microglial activation, suggestive of viral encephalitis.

2- Autopsy sample. H&E. Fourth ventricle in a 34 year-old woman with AIDS: enlarged ependymal cells with owl's eye nuclear inclusions (arrow), characteristic of CMV ventriculitis.

3- Autopsy sample. H&E. Hippocampus in a 10-year-old boy: Negri's body characteristic of rabies. X40

4- Autopsy sample. Anti-rabies polyclonal antibody in a 41-year old man.

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