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Clinical Review

Diagnosis and clinical management of neurological disorders caused by cytomegalovirus in AIDS patients

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Cytomegalovirus (CMV) infections are common and severe complications of HIV infection. The virus involves the nervous system, causing encephalitis, polyradiculomyelitis and peripheral neuropathies. Due to their limited sensitivity, traditional virological approaches, such as virus isolation or antigen detection in the CSF are useful only in limited instances, e.g. CMV polyradiculopathy. The aetiological diagnosis of these disorders relies on the analysis of cerebrospinal fluid by PCR and quantitative PCR may be important to establish the extent of CNS lesions and to monitor the efficacy of antiviral treatments. CMV is susceptible to various antivirals, including ganciclovir, foscarnet and cidofovir. CMV infections of the nervous system, in particular encephalitis, however, show only a poor response to standard treatments. Drug combination treatments i.e. ganciclovir plus foscarnet, are currently under evaluation in clinical trials.

Keywords: CMV; encephalitis; nervous system; PCR; ganciclovir; foscarnet

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Introduction

Nervous system disorders occur frequently in patients with HIV infection, including those associated with vascular and metabolic disorders, tumours, opportunistic infections and infection of the nervous system by HIV itself.

Among the neurological complications associated with opportunistic infections, those caused by cytomegalovirus (CMV) are reported with high frequency (Anders *et al*, 1986; Petito *et al*, 1986; Kure *et al*, 1991). Nervous system lesions caused by CMV are often located in the brain, but may also be found in the spinal cord, nerve roots and peripheral nerves. The resulting clinical syndromes include encephalitis, myelitis, polyradiculitis, peripheral neuropathy, and various combinations of these entities (Vinters *et al*, 1989; Morgello *et al*, 1987; Said *et al*, 1991).

This consensus report considers the use of current diagnostic procedures and antiviral therapy in AIDS patients with suspected CMV infections of the nervous system.

Central nervous system: CMV encephalitis

CMV encephalitis accounts for the majority of the CMV induced pathology of the nervous system in

AIDS, and can be detected in brain tissues taken from up to one third of cases examined at autopsy (Anders *et al*, 1986; Petito *et al*, 1986; Kure *et al*, 1991). The main neuropathological features are of ventriculo-encephalitis, focal parenchymal necrosis and micronodular-encephalitis. In some cases, the meninges may also be infected. CMV ventriculo-encephalitis typically involves the periventricular areas, where necrotic lesions are found. Foci of necrosis can, however, also be found deep in the brain parenchyma, not associated with periventricular lesions. CMV inclusion-bearing cells are always present within or peripheral to the lesions. Micronodular-encephalitis is characterised by the presence of microglial nodules in the parenchyma of the brain, cerebellum, and spinal cord. These consist of aggregates of astrocytes, which usually surround inclusion-bearing cells, and are located in the grey matter (Vinters *et al*, 1989; Morgello *et al*, 1987; Schmidbauer *et al*, 1989; Setinek *et al*, 1995). A predominantly diffuse encephalopathy is the clinical correlate of CMV encephalitis. The prognosis is generally poor, with death ensuing within weeks of the diagnosis, often irrespective of antiviral treatment (Kalayjian *et al*, 1993; Holland *et al*, 1994; McCutchan, 1995; Salazar *et al*, 1995; Arribas *et al*, 1996).

Clinical diagnosis

CMV encephalitis usually occurs in severely immunocompromised AIDS patients, i.e., with a CD4+ cell count of less than 50 per mm^3. In patients with ventriculo-encephalitis the onset of neurological symptoms is subacute, developing over a period of weeks. Drowsiness, confusion, or lethargy dominate, but focal signs may also be present, including cranial nerve palsies, and motor deficits (Kalayjian *et al*, 1993; Salazar *et al*, 1995; Arribas *et al*, 1996) A syndrome of dementia has been associated with the micronodular form (Holland *et al*, 1994; McCutchan, 1995). The majority of patients with CMV encephalitis have a history of CMV viremia and/or CMV infection of other organs, including the retina, the gastrointestinal tract, and the lung. Blood analysis may also show serum electrolyte abnormalities consistent with an addisonian state, possibly induced by a CMV infection of the adrenal glands (Kalayjian *et al*, 1993; Holland *et al*, 1994; McCutchan, 1995; Salazar *et al*, 1995; Arribas *et al*, 1996).

Contrast-enhanced computed tomography (CT) may show diffuse, ill-defined low attenuation lesions in the brain parenchyma. By magnetic resonance (MR) examination, low signal intensity on T1-weighted images, with high signal intensity on T2-weighted MR images can be observed. These patterns are the likely radiological equivalent of the focal necrosis induced by CMV. In cases with ventriculo-encephalitis, the lesions are observed in the periventricular white matter, with surrounding oedema and ill-defined periventricular enhancement after injection of contrast medium (Post *et al*, 1986; Walot *et al*, 1996). In many patients with advanced CMV ventriculo-encephalitis, however, no abnormalities are observed by MRI (Clifford *et al*, 1996).

Laboratory diagnosis

Because of frequent systemic CMV infection, standard virological investigations to identify CMV in blood, virus isolation in cell culture, pp65 antigenemia and DNA PCR are often positive in patients with CMV encephalitis, but are not diagnostic. An aetiological diagnosis of CMV infection of the nervous system requires the identification of the virus in brain tissue or in the cerebrospinal fluid (CSF).

In patients with CMV encephalitis, the CSF profile is variable. Hypoglycorrhachia, increased total protein or mild pleocytosis are often present (Holland *et al*, 1994; Singh *et al*, 1993; Miller *et al*, 1996).

CMV encephalitis can be diagnosed by the detection of CMV-DNA in the CSF using the polymerase chain reaction (PCR). The detection of microbial genomes in the CSF by DNA amplification techniques now represents the major approach to the laboratory diagnosis of several infections of the CNS (Darnell, 1993; Tyler, 1994; Weber *et al*, 1996; Cinque *et al*, 1997). There have been several reports on the use of CSF PCR for the detection of CMV-DNA in AIDS-related neurological complications (Holland *et al*, 1994; Cinque *et al*, 1992, 1995, 1996a; Gozlan *et al*, 1992, 1994; Wolf and Spector, 1992; Clifford *et al*, 1993; Fillet *et al*, 1993; Weber *et al*, 1994; Revello *et al*, 1994; Achim *et al*, 1994; Arribas *et al*, 1995; Fox *et al*, 1995; Shinkai and Spector, 1995; Cohen, 1996; Vogel *et al*, 1996).In these studies the diagnostic potential of CSF PCR was evaluated by comparing PCR data with histopathological observations at autopsy, virus isolation from the CSF, or clinical findings. In the most relevant studies, which compared CSF findings to histopathology, the sensitivity and specificity of CSF PCR for the diagnosis of CMV infection of the nervous system were higher than 80 and 90%, respectively (Gozlan *et al*, 1994; Cinque *et al*, 1996a). Positive and negative predictive values ranged between 86–92% and 95–98% (Gozlan *et al*, 1994; Cinque *et al*, 1996a). CMV-DNA has rarely been detected in CSF of patients without CMV neurological complications, including AIDS patients with extra-cerebral CMV disease, CMV viraemia, or CMV-DNA in serum (Cinque *et al*, 1992; Gozlan *et al*, 1992)

By allowing a diagnosis '*in vivo*', CSF PCR has also led to a better characterization of the clinical syndromes associated with CMV infections of the nervous system; CMV ventriculo-encephalitis being an example. Furthermore, the application of this technique has contributed, and will probably

continue to contribute, to the recognition of atypical forms of CMV brain involvement (e.g., transient neurological dysfunction possibly related to vasculitis of the vertebrobasilar arteries, or CMV encephalitis during acute primary HIV-1 infection (Berger *et al*, 1996; Klein *et al*, 1996).

Quantitative and semi-quantitative PCR assays (Cinque *et al*, 1995; Arribas *et al*, 1995; Shinkai *et al*, 1995; Cohen, 1996), and also a branched chain DNA assay (Drew *et al*, 1995a), have been evaluated in patients with CMV neurological complications. In patients with CMV encephalitis, quantitative PCR has demonstrated that the CSF may contain up to 10^7 copies of CMV-DNA per ml. A correlation has been shown between the amount of CMV-DNA in the CSF and the pattern and extent of the lesions in the brain. The more extensive forms of ventriculo-encephalitis being associated with a higher CMV-DNA CSF load (Cinque *et al*, 1995; Arribas *et al*, 1995; Shinkai and Spector, 1995). Furthermore, CMV-DNA titres may vary as a consequence of antiviral treatment. A decrease or a clearance of CMV-DNA from the CSF has been observed in patients with CMV encephalitis receiving ganciclovir, foscarnet, or a combination of these two drugs (Revello *et al*, 1994; Cinque *et al*, 1995, 1996b; Arribas *et al*, 1995; Shinkai *et al*, 1995; Cohen, 1996; Drew *et al*, 1995a).

Both single PCR assays followed by DNA hybridization with an internal probe, and nested PCR assays, have been described for amplification of CMV-DNA in the CSF. CSF preparation prior to amplification has usually consisted of simple heating of small aliquots (e.g., 10 μl) to 95°C. Alternatively, nucleic acid purification from larger volumes can also be used. Irrespective of the amplification and nucleic acid extraction protocols utilised, the sensitivity of all the PCR assays described is of the order of 10–100 copies of CMV-DNA per reaction. CSF specimens can be analysed either immediately after sampling or even after long-term storage. In most laboratories a clinical sample can be processed within one day. All the studies described used 'in-house' developed PCR assays.

Virus isolation from the CSF is only rarely successful in patients with CMV encephalitis (Vinters *et al*, 1989; Morgello *et al*, 1987; Wolf and Spector, 1992; Gozlan *et al*, 1994; Dix *et al*, 1985). This is probably due to the low numbers of infectious virus particles in the CSF. CMV pp65 or other antigens, and CMV-DNA have been identified in CSF cells using immunocytochemistry or *in situ* hybridization (Revello *et al*, 1994; Stark *et al*, 1993; Musiani *et al*, 1994). As noted for virus isolation, the sensitivity of antigen or DNA detection in CSF cells is infrequent in patients with CMV encephalitis, and appears to be dependent upon the number of cells in the CSF (Wolf and Spector, 1992).

Because of the profound levels of immunosuppression, a sustained intrathecal antibody response is not achieved in AIDS patients with nervous system disease (Cinque and Linde, unpublished data). In addition, production of a low level of non-specific or polyspecific intrathecal antibodies, as a result of HIV-induced immunoactivation, can be demonstrated (Chiodo *et al*, 1988; Buffet *et al*, 1991). However, a specific intrathecal antibody response has been demonstrated in a few patients with CMV neurological complications. In these cases, the appearance of a CNS humoral immune response was preceded by the detection of CMV-DNA in the CSF by PCR, and associated with a good clinical outcome, either spontaneously or following antiviral therapy (Weber *et al*, 1994).

Brain biopsy is not considered to be a useful diagnostic procedure in patients with CMV encephalitis, because focal brain lesions are rarely revealed by neuroradiological examination. The examination of CNS tissue has therefore been mainly limited to the study of autopsy material. The histopathological diagnosis of CMV infection of the nervous system is based on the examination of tissue preparations stained with haematoxylin-eosin. Demonstration of typical cytomegalic cells with intranuclear inclusions is diagnostic of CMV infection. Cytomegalic inclusions are found in glial, neuronal, and endothelial cells (Vinters *et al*, 1989; Morgello *et al*, 1987; Schmidbauer *et al*, 1989) The presence of inflammatory infiltrates, microglial reaction, or necrosis, further characterise CMV induced lesions. Immunocytochemistry and in situ hybridisation, for the detection of CMV antigens and DNA, respectively, can be useful to confirm a diagnosis in cases where other pathogens (e.g., HIV, *Toxoplasma gondii*) might be responsible for or contributory to the lesions.

Treatment

Ganciclovir (9-(1,3-dihydroxy-2-propoxymethyl)-guanine; DHPG) and foscarnet (trisodium phosphonoformate hexahydrate; PFA) are currently licensed for the treatment of CMV infections in AIDS patients. Both are of established value for the treatment of CMV retinitis and gastro-intestinal disease (Palestine *et al*, 1991; Spector *et al*, 1993; Blanshard *et al*, 1995) Recently, cidofovir ((S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)-cytosine; HPMPC) has also been approved for use in patients with CMV retinitis (Lalezari *et al*, 1996).

Ganciclovir

Ganciclovir is a nucleoside analogue which, following sequential phosphorylation to ganciclovir triphosphate, inhibits DNA polymerases of CMV and other herpesviruses. Its use is associated with toxic effects, granulocytopenia being the most frequent (Crumpacker, 1996). The standard dosage for treatment of CMV infection in AIDS patients is

10 mg/kg/day for 2 – 3 weeks given intravenously in two doses. Following a 3 week course of treatment, long-term intravenous maintenance therapy at lower doses (5 mg/kg/day) is recommended. An oral form of ganciclovir has recently been approved for maintenance treatment of CMV retinitis, and for primary prophylaxis in HIV-infected patients 'at risk' of developing CMV disease, i.e., those with less than 100 CD4+ lymphocytes per mm^3 (Drew *et al*, 1995b; The Oral Ganciclovir European and Australian Cooperative Study Group, 1995; Spector *et al*, 1996). CMV strains resistant to ganciclovir may develop as a consequence of mutations in the CMV phosphotransferase gene UL97, leading to less efficient ganciclovir phosphorylation, alone or in combination with mutations in the DNA polymerase gene (Sullivan *et al*, 1992; Chou *et al*, 1995; Lurain *et al*, 1992; Baldanti *et al*, 1995). Resistance to ganciclovir has been reported in HIV-infected patients receiving ganciclovir for more than 3 months (Drew *et al*, 1991; Boivin *et al*, 1996).

To date, only a limited number of studies have been carried out to determine ganciclovir levels in the CSF and CNS. In two patients receiving 2.5 mg/kg/day of ganciclovir. The penetration to the CSF after intravenous administration was 31 and 67% of plasma levels. CSF concentrations reached were equal to or just below the minimum inhibitory concentration for CMV (Shepp *et al*, 1985). In an autopsy study of 6 cases, the average ganciclovir concentration in the CNS was 38% of that observed in cardiac blood, and much lower than that detected in the kidneys (Fletcher *et al*, 1986).

Ganciclovir appears to be of only low efficacy in the treatment of CMV ventriculo-encephalitis. Retrospective studies clearly demonstrate that CMV encephalitis often occurs in patients undergoing maintenance therapy (Kalayjian *et al*, 1993; Schwarz *et al*, 1990; Berman and Kim, 1994). Prospective studies indicate only a limited response in patients with CMV encephalopathy (Cinque *et al*, 1995; Cohen, 1996; Fiala *et al*, 1988; Laskin *et al*, 1987; Price *et al*, 1992). Using PCR, it has been demonstrated that CMV-DNA is cleared from the CSF in a proportion of patients with CMV infection of the CNS treated with ganciclovir (Cinque *et al*, 1995; Cohen, 1996). However, in many patients with overt encephalitis, CMV-DNA is still detectable following treatment, though at lower levels than observed prior to the initiation of treatment (Cinque *et al*, 1995; Drew *et al*, 1995b). A peripheral response to therapy for example, disappearance of CMV pp65 antigen from blood polymorphonuclear leukocytes, is generally observed in cases of treatment failure of the CNS disease (Cinque *et al*, 1995). This observation suggests that drug levels adequate to treat CMV infection are not attained in the CNS when using the currently recommended dosage for the treatment of systemic CMV disease. This may be explained by the low lipophilicity of ganciclovir, which limits its penetration of the blood-brain-barrier. The development of CMV strains resistant to ganciclovir may be an additional explanation for treatment failure (Holida *et al*, 1995). Because of the low rate of isolation of CMV from the CSF in patients with CMV encephalitis, onset of drug resistance is difficult to assess by conventional biological assays. By sequencing of CMV-DNA amplified from the CSF, mutations in the CMV-UL97 gene (which confer resistance to ganciclovir) have been detected in patients, with CMV neurological diseases, who had received ganciclovir for more than one year (Wolf and Spector, 1995).

To increase delivery of ganciclovir to the brain, the use of a chemical delivery system has been proposed. In animal models, high uptake of ganciclovir in the brain and sustained levels lasting for several hours after administration have been described (Brewster *et al*, 1994).

Foscarnet

Foscarnet is a non-nucleoside inhibitor of viral DNA polymerases. It is active against herpesviruses and HIV. Like ganciclovir, foscarnet has low oral bioavailability, and therefore requires intravenous administration. Nephrotoxicity is the main dose-limiting factor, although this may be controlled by prolonged infusion times (Oberg, 1989). The standard treatment is 180 mg/kg/day in two doses, for 2 – 3 weeks, whilst maintenance life-long therapy is recommended at 90 mg/kg/day. Foscarnet-resistant strains have been identified in the laboratory and have also been detected *in vivo*. Foscarnet resistance is associated with mutations in the DNA polymerase gene and resistant mutants generally exhibit cross-resistance with other DNA polymerase inhibitors (Sullivan *et al*, 1992; Baldanti *et al*, 1996; Sarasini *et al*, 1995).

The CSF:plasma distribution of foscarnet in the CSF has been studied in more detail and in larger groups of patients than for ganciclovir. In two different studies CSF to plasma ratios were highly variable (range 0 to 3.4 times plasma concentration), with mean or median values of 23 and 27%, respectively (Hengge *et al*, 1993; Raffi *et al*, 1993). Drug levels equal to, or higher than, the 50% inhibitory concentration for CMV, were achieved in the CSF in most of the cases studied (Hengge *et al*, 1993; Raffi *et al*, 1993; Sjovall *et al*, 1989).

Much less data is available regarding the efficacy of foscarnet in patients with CMV encephalitis. As with ganciclovir, CMV encephalitis may develop in patients receiving foscarnet. In patients who were switched from ganciclovir to foscarnet after development of CMV encephalitis, quantitative CMV-DNA PCR in the CSF showed only low or no reduction of CSF CMV loads after three weeks of treatment (Cinque *et al*, 1996).

Other treatments

New strategies have been proposed for the treatment of CMV encephalitis. Included among these is the use of more aggressive treatment regimes, such as combination ganciclovir and foscarnet therapy. The rationale for the concurrent use of these two drugs is their different mechanism of action and the difference in their toxicity profiles. Furthermore, a synergistic effect of ganciclovir and foscarnet has been demonstrated *in vitro* (Freitas *et al*, 1989). Combined ganciclovir and foscarnet was tolerated and effective in patients with gastrointestinal disease or recurrent retinitis, and in the maintenance treatment of patients with retinitis (Dieterich *et al*, 1993; Studies of Ocular Complications of AIDS Research Group, 1996; Jacobson *et al*, 1994). Evidence supporting the therapeutic efficacy of this treatment regimen in patients with CMV encephalitis is limited to individual case reports (Peters *et al*, 1992; Enting *et al*, 1992). Clinical trials using concurrent ganciclovir and foscarnet in patients with CMV neurological disease are currently being performed in Europe (Cinque *et al*, 1996b; Van der Meer *et al*, 1996) and in the United States (ACTG 305). These studies are designed to evaluate both the efficacy of the combined treatment and the patient's tolerance of this therapy using clinical, virological, and pharmacokinetic profiles, in both first-line therapy and in patients failing to respond to monotherapy with either drug.

Cidofovir is a nucleotide analog active against CMV and other herpesviruses. Cidofovir diphosphate selectively inhibits the viral DNA polymerase and suppresses viral replication. The compound is administered intravenously at 5 mg/kg once per week for 2 weeks, as induction therapy, and at the same dose every second week during maintenance. The main adverse reaction is nephrotoxicity. Probenecid must be administered simultaneously to prevent renal damage (Lea and Bryson, 1996). CMV isolates with reduced susceptibility to cidofovir have been found among clinical isolates with high resistance to ganciclovir through mutations in the DNA polymerase gene (Tatarowitcz *et al*, 1992).

A study on penetration of cidofovir to the CSF has demonstrated CSF to plasma ratios of 2 and 4%, attained in the same patient on two different occasions, 1 h following intravenous administration (De Wit *et al*, 1996). In severe combined immunodeficiency mice (SCID) with murine cytomegalovirus induced encephalitis, the administration of high-dose cidofovir was found to be effective in delaying death, but no more efficient than ganciclovir in preventing the development of neurological disease (Neyts *et al*, 1993). There are currently no reports concerning experience with the use of cidofovir in patients with CMV neurological complications.

New drugs active against CMV with higher oral bioavailability, or lower toxicity, are currently undergoing phase I–III clinical trials. These include, the nucleoside analog lobucavir (cyclobutylguanosine), the ganciclovir prodrug RS79070, benzimidazole and its derivative 1263W94, and MSL-109, a monoclonal antibody directed against the gH glycoprotein of CMV (Field *et al*, 1990; Drew *et al*, 1997; Brown *et al*, 1997; Zou *et al*, 1996; Wang *et al*, 1997; Pollard, 1996). Antisense oligonucleotides complementary to the RNA of CMV such as ISIS 2922, represent further promising antivirals, but these have not yet been assessed in clinical trials (Anderson *et al*, 1996). No information is yet available regarding the penetration of these compounds into the CSF or the CNS.

Clinical management

Because of the poor efficacy of current antiviral treatments the clinical management of CMV encephalitis is difficult. However, a rapid aetiological diagnosis is essential, and patients with a diagnosis of CMV encephalitis should be considered for inclusion in clinical trials designed to evaluate alternative methods of treatment.

CMV encephalitis must be suspected in patients with HIV infection who present with a diffuse encephalopathy. The clinical history may be suggestive, revealing previous or concomitant extracerebral CMV infection. Both the neurological presentation, as well as neuroradiological findings, may be suggestive of, but not specific for CMV encephalitis. Other diffuse encephalopathies, primarily HIV encephalopathy, should be considered in the differential diagnosis of CMV encephalitis.

CSF should be drawn as soon as possible for determination of cell count, glucose and protein levels. PCR for detection of CMV-DNA should be requested, together with additional tests which might exclude other causes of nervous system disease, such as the detection of cryptococcal polysaccharide antigen, or PCR for other microbial genomes (Cinque *et al*, 1997). To ensure optimal interpretation of results, PCR positive samples should be re-investigated by quantitative CMV PCR. Because of their relatively low sensitivity in patients with CMV encephalitis cultures for CMV and antigen detection in the CSF are often not helpful at this stage. However, CMV isolation can be requested in addition to PCR, when characterisation of CMV isolates is of importance, e.g., to study CMV resistance to antivirals.

Patients with CMV encephalitis often have systemic CMV disease at the time of presentation and therapeutic and diagnostic interventions need to take this into account. In parallel with CSF examination peripheral blood should be examined using cell culture systems for virus isolation; pp65

antigenemia should be determined and DNA PCR may be performed using whole blood, blood cells, plasma or serum. To help decide upon antiviral treatments CMV levels should be determined by means of pp65 antigenemia or quantitative DNA PCR (Spector *et al*, 1992; Bowen *et al*, 1996).

The algorithm (Figure 1) provides a diagnostic pathway and strategy for clinical management of patients with CMV associated neurological disease. The presence of CMV-DNA in the CSF is consistent with virus replication in the central nervous system, therefore a PCR positive result from CSF is diagnostic of a nervous system infection with CMV. This finding is an indication for treatment with drugs active against CMV. If disease severity is found to correlate with CSF viral load quantitative DNA PCR may in the future prove useful in distinguishing severe from mild forms of disease. Multiple infections are frequent in the nervous system of patients with AIDS, thus, even when a diagnosis of CMV infection of the CNS is established, other nervous system diseases cannot be excluded. Of interest, HSV-1 or HSV-2 have been shown to co-exist with CMV in 15% of cases with CMV ventriculo-encephalitis (Vago *et al*, 1996). Although both HSV-1 and 2 are susceptible to ganciclovir and foscamet, the identification of dual HSV and CMV infection might allow improved management of therapy. In contrast, because of the high sensitivity and negative predictive value of CSF PCR, a negative PCR result is likely to exclude a CMV infection of the nervous system. In such cases, other CNS disorders must be considered.

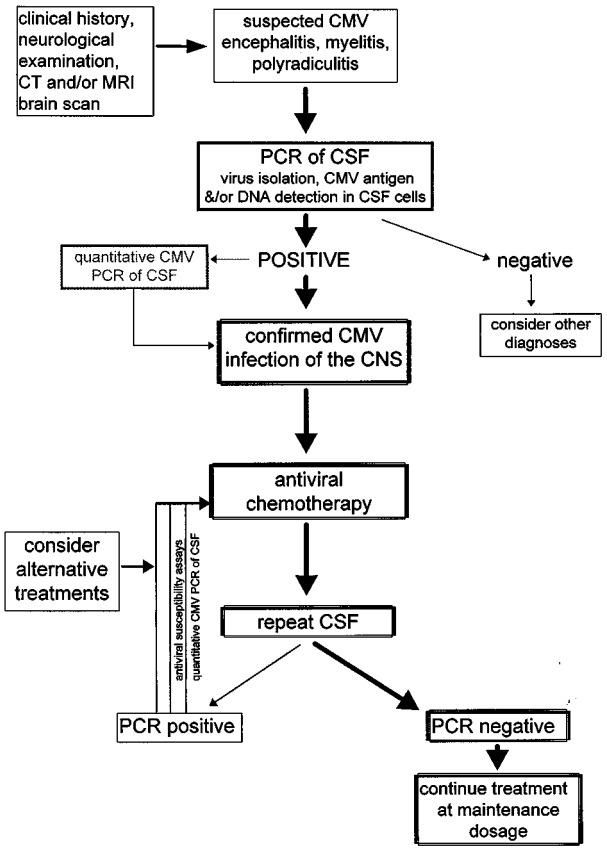

Figure 1.

No definite recommendation can presently be made regarding the optimal treatment of the complications of CMV infection of the CNS. Current evidence suggests that monotherapy with ganciclovir or foscarnet is of limited efficacy in the treatment of CMV encephalitis and clinical trials are necessary to assess the efficacy of alternative treatment strategies. In establishing new treatments, a crucial factor is the relative drug penetration into the central nervous system. The possible emergence of drug resistant mutants in patients already treated with anti-CMV drugs, suggests the need to use drug combinations. At present, aggressive combination therapy with the currently available drugs appear the most realistic option, although the often severe clinical condition of patients with CMV encephalitis, related to a poor life-expectancy and an increased vulnerability to the side effects of therapies, must be taken into account.

Following 2–3 weeks of treatment, clinical, neuroradiological, and CSF investigations should be repeated. Treatment can be considered to have been successful if a negative CSF PCR result is obtained. In these cases continuation of therapy at maintenance dosage should be considered. There is evidence that maintenance of monotherapy regimens is not effective in providing a long term inhibition of CMV replication in the CNS. Therefore, combination therapy is worthy of consideration as maintenance treatment. However, neither a 2–3 week course of either ganciclovir or foscamet as induction therapy, nor the two drugs in combination, seem to be effective in clearing CMV DNA from the CSF in the majority of cases (Cinque *et al*, 1995, 1996b; Cohen, 1996). Theoretically, in cases where the CSF remains PCR positive, full dose administration of antiviral drugs should be continued, as it is probable that the infection will not have been eradicated from the CNS. In these patients it is important to assess the susceptibility of CMV to the drugs used, especially in those cases with a previous history of anti-CMV treatment.

Peripheral nervous system: Lumbosacral polyradiculopathy

CMV polyradiculitis, often in combination with a myelopathy (polyradiculomyelitis), occurs in 1–2% of patients with AIDS. Encephalitis or meningoencephalitis is often present simultaneously. CMV typically involves the cauda equina and lumbosacral rootlets, with destruction of axons and myelin. Histopathological examination reveals foci of poly-

morphonuclear and mononuclear inflammation, with CMV inclusions in Schwann and endothelial cells. CMV necrotising and micronodular lesions can be found in the spinal cord. The prognosis of polyradiculitis is uncertain, the syndrome potentially being responsive to treatment with antiviral drugs (Grafe and Wiley, 1989; Miller *et al*, 1990; Cohen *et al*, 1993; Kim and Hollander, 1993; So and Olney, 1994).

Clinical diagnosis

Like CMV encephalitis, CMV polyradiculopathy generally occurs in patients with a very low CD4+ cell count and is the only manifestation of CMV disease in approximately half of the cases. The clinical syndrome of CMV polyradiculopathy is well characterised, with weakness, sensory loss and areflexia in the legs, often associated with bladder or anal sphincter dysfunction. The onset is subacute, extending over days to weeks; the disease tends to evolve into an ascending paraparesis (Grafe and Wiley, 1989; Miller *et al*, 1990; Cohen *et al*, 1993; Kim and Hollander, 1993; So and Olney, 1994).

In patients with CMV polyradiculitis or polyradiculomyelitis, gadolinium enhancement of the lumbosacral rootlets, consistent with root inflammation may be detected by MRI (Talpos *et al*, 1991). Electromyographic examination and nerve conduction studies may show a reduced amplitude of both motor and sensory nerve action potentials (Fuller, 1992).

Laboratory diagnosis

In patients with CMV polyradiculitis, as for those with CMV encephalitis, virological analysis of blood is a useful procedure to identify the presence of systemic CMV disease.

An extremely suggestive inflammatory pattern is seen in CSF. This is characterised by marked pleocytosis (hundreds to thousands of cells, mainly polymorphonuclear leukocytes), and an elevated protein content (Miller *et al*, 1990; Cohen *et al*, 1993; de Gans *et al*, 1990).

CSF PCR represents a sensitive and specific test which can establish an aetiological diagnosis of polyradiculitis. Exact data with respect to sensitivity, specificity and predictive values have not been reported, but many of the studies which assessed the diagnostic accuracy of this technique in CMV infections of the nervous system have included cases with CMV polyradiculitis or polyradiculomyelitis. The use of quantitative PCR suggests that the levels of DNA in the CSF are higher than those found in CMV encephalitis, with values up to 10^7 CMV-DNA copies per ml (Revello *et al*, 1994; Shinkai and Spector, 1995; Cinque *et al*, 1996b; Smith *et al*, 1996). Because a large number of cells and/or protein may be present in the CSF of patients with polyradiculitis, careful attention must be given to the preparation of CSF prior to DNA amplification, so that potential factors that may inhibit the PCR can be eliminated (e.g., using internal standard molecules as amplification control, and DNA purification procedures). CMV can be isolated from the CSF of about half of all cases of CMV polyradiculopathy (Cohen *et al*, 1993; So and Olney, 1994). CMV antigens can also be demonstrated by immunostaining of CSF cells (Revello *et al*, 1994; Stark *et al*, 1993). In a selected series of patients with CMV polyradiculopathy and/or CSF pleocytosis, the sensitivity of pp65 antigen detection was 91% and specificity was 100%, when compared to CSF PCR (Revello *et al*, 1994). CMV-DNA has also been identified in CSF cells by *in situ* hybridisation using radioactive probes, and by cytological examination of the CSF (de Gans *et al*, 1990).

Treatment

In contrast to CMV encephalitis, CMV polyradiculitis is more likely to respond to antiviral therapy. Both ganciclovir and foscarnet, and combination therapy, have been demonstrated to have clinical efficacy in this condition (Cohen *et al*, 1993; Kim and Hollander, 1993; So and Olney, 1994; Domingo *et al*, 1994; Decker *et al*, 1994). Neurological improvement is observed in approximately half of the patients who receive a standard dose of ganciclovir. In some cases, a response has been noted only several weeks following the initiation of therapy (Cohen *et al*, 1993; Kim and Hollander, 1993). Treatment failure may, however, occur, possible explanations for this include a delay in initiation of treatment, or inadequate treatment courses. Development of viral resistance to ganciclovir has been documented to occur a few to several months after initiation of therapy (Wolf *et al*, 1995; Smith *et al*, 1996; Ebright and Crane, 1991; Tokumoto and Hollander, 1993).

Clinical management

A CMV infection should be suspected in HIV-infected patients presenting with a polyradiculopathy or a polyradiculomyelitis. The differential diagnosis includes a chronic demyelinating polyradiculopathy of no established aetiology, occurring in patients with only moderate levels of immunosuppression and characterised by a benign clinical course. Other opportunistic conditions involving lumbosacral nerve roots and the spinal cord may present with symptoms and signs resembling CMV polyradiculitis.

Though the clinical presentation and standard CSF analysis may be suggestive, CMV-DNA or antigen detection, or CMV isolation from CSF are needed to establish an aetiological diagnosis.

When formulating a treatment strategy, consideration should be given to previous antiviral therapy for CMV infection. For patients who have

not received any antiviral therapy, either ganciclovir or foscarnet still represent the first choice for treatment. In those patients with a previous history of anti-CMV therapy and who subsequently develop CMV polyradiculitis, CMV resistance should be suspected, and an alternative antiviral therapy instituted (i.e. switch to the second drug or combination therapy). Detection of CMV-DNA or CMV antigen in the CSF should also be used to evaluate the efficacy of antiviral therapy, and should be repeated at regular intervals, e.g. monthly, during the treatment follow-up. Initial clinical and virological improvement may occur during the standard 3 weeks of induction therapy. However, longer treatment courses at induction doses may be required to completely suppress CMV replication. As for treatment of CMV encephalitis, controlled studies are required to assess the tolerance levels and efficacy of new treatment strategies; for example the concurrent administration of ganciclovir and foscarnet, for both induction and maintenance therapy.

Other peripheral neuropathies

Among the other peripheral neuropathies observed in HIV-infected patients, mononeuritis multiplex and painful peripheral neuropathy, have been described in association with CMV infection (Said *et al*, 1991; Fuller, 1992; Roullet *et al*, 1994; Jeantils *et al*, 1986; Fuller *et al*, 1989, 1993). CMV has also been demonstrated in nerves with no apparent clinical correlate (Roullet *et al*, 1994).

Mononeuropathy multiplex has been reported in 3% of patients with AIDS. Histologically this condition is characterised by the presence of multifocal axonal, or both axonal and demyelinative lesions, with a patchy distribution, in the cranial and peripheral nerves. Its association with CMV infection is substantiated by the frequent observation of CMV inclusions in Schwann cells, associated with polymorphonuclear cell infiltrates and necrosis (Said *et al*, 1991; Fuller, 1992; Roullet *et al*, 1994). The disease is rapidly progressive, but may respond to ganciclovir treatment (Roullet *et al*, 1994).

Painful peripheral neuropathy has been found in 7.5% of AIDS patients and is considered to be a variant of the more common distal symmetrical peripheral neuropathy, occurring in up to one third of patients with AIDS (Tokumoto and Hollander, 1993). Histologically, this neuropathy is characterised by axonal atrophy with loss of myelin. Although the aetiology and pathogenesis of this condition is not fully understood, painful peripheral neuropathy has been found with a higher frequency in AIDS patients with systemic CMV disease, and this association is significantly higher than that found for other neuropathies or AIDS conditions (Tokumoto and Hollander, 1993). Evidence which suggests that CMV may have an aetiological role in this syndrome includes the demonstration, in biopsy or autopsy tissues, of CMV inclusions within Schwann cells (Grafe and Wiley, 1989; Fuller, 1992). The prognosis of painful peripheral neuropathy is poor, although a response to ganciclovir has been reported (Fuller, 1992).

Clinical diagnosis

Both mononeuritis multiplex and painful peripheral neuropathy present subacutely, in patients with a very low CD4+ cell count, i.e. <50 per mm^3.

Mononeuritis multiplex associated with CMV is a severe sensor-motor neuropathy, which may involve only a few or several nerves. Electromyographic and neurographic studies may reveal its asymmetrical and multifocal nature (Said *et al*, 1991; Spector *et al*, 1992; Decker *et al*, 1994).

The painful peripheral neuropathy is clinically characterised by distal sensory loss and weakness, accompanied by pain, which is usually limited to the feet. Electrophysiologic examination shows a distal, symmetrical, sensor-motor or predominantly sensory neuropathy (Fuller, 1992).

Laboratory diagnosis

In both mononeuritis multiplex and painful peripheral neuropathy, analysis of the blood may reveal the presence of CMV viremia.

CSF analysis often shows pleocytosis and/or high protein content. Such findings are common, however, in many neurological disorders occurring in HIV-infected patients (Fuller *et al*, 1993). Recently, CMV-DNA has been demonstrated in the CSF, by PCR, in 90% of patients with mononeuritis multiplex (Roullet *et al*, 1994). It is likely that the positive PCR results were related to simultaneous clinical or subclinical CNS involvement.

Biopsy of peripheral nerves has long been the only means available to identify both the nature of neuropathy and the aetiological agent. It has the disadvantage that the portion of the nerve that is sampled, generally the distal part, may show no lesions of neuropathy and therefore provide false negative results (Said *et al*, 1991).

Treatment

Although controlled studies are not available, reports of individual cases or small series of patients suggest that ganciclovir may be effective for the treatment of patients with both mononeuritis multiplex and painful symmetrical polyneuropathy (Said *et al*, 1991; Fuller, 1992; Roullet *et al*, 1994).

Clinical management

When clinical and electrophysiological studies demonstrate the presence of either a mononeuropathy multiplex or a distal symmetric neuropathy, CMV infection should be included in the differ-

ential diagnosis. The mononeuritis multiplex attributable to CMV should be differentiated from the less malignant form occurring earlier in the course of HIV infection.

In the absence of spinal root or central nervous system involvement it is unlikely that CMV would be demonstrated in the CSF of patients with CMV infection of peripheral nerves. Therefore, the diagnosis of mononeuritis and distal symmetrical neuropathy, as well as the subsequent clinical management, are based upon systemic and neurological findings. An aetiological diagnosis can only be achieved by means of nerve biopsy.

Conclusions

CMV neurological complications represent an important cause of mortality and morbidity among AIDS patients. Because of the difficulties in establishing an '*in vivo*' diagnosis, CMV encephalitis has probably been under-diagnosed in the past. An aetiological diagnosis of CMV encephalitis and polyradiculitis or polyradiculomyelitis, can now be obtained by CSF PCR for detection of CMV-DNA. This technique, together with quantitative PCR tests have a fundamental role in the follow-up of treatment.

CMV encephalitis responds only poorly to current antiviral treatments, whilst polyradiculomyelitis may have a better prognosis. At present, early identification of drug resistance, and new treatment strategies (e.g. the use of drug combinations and of new antiviral compounds with higher penetration into the central nervous system), are required to provide optimal treatment. Early identification of patients at risk of developing CMV disease and chronic suppression of CMV replication during the early stages of infection must be considered a high priority, so that CMV invasion of the CNS and its dramatic consequences may be prevented.

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