The utility of CSF PCR in central nervous system Varicella zoster infection in HIV

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SUBMITTED TO THE UNIVERSITY OF CAPE TOWN
In partial fulfilment of the requirements for the degree

Master of Medicine (M Med – mini-dissertation)

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

Date of submission: 15 May 2015

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DECLARATION

I, *Alan Michael Stanley*, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Abstract

Aims: To assess the clinical and cerebrospinal fluid characteristics, and the role of tuberculous meningitis (TBM) as a confounder, in a cohort of HIV positive individuals with positive varicella zoster virus (VZV) positive cerebrospinal fluid PCR.

Methods: Patients in the NHLS database at Groote Schuur Hospital with positive CSF VZV PCR who were also HIV co-infected and whose folders were available for clinical review were reviewed. Clinical and biochemical data were collected. Patients were divided into two groups based an accepted case definition for TBM. Differences between groups were assessed using Mann-Whitney U or Chi squared tests as appropriate.

Results: There were 437 for VZV PCR over three years. Of these 98 were positive and, after exclusions, 31 HIV positive patients were included for further analysis. Median age was 31 and median CD4 count was 146 cells/mm^3. 11 (35%) had meningitis and 8(25%) had encephalitis. 13 (42%) met the case definition for TBM. Patients with CNS varicella were frequently confused whereas those with TBM presented sub-acutely. There were no differences in CSF characteristics. Additional organisms were detected 6 (19%) patients. 4 (13%) patients died in hospital. CSF TB culture was requested in 24 (77%) patients and extra CNS samples were sent in only 4 patients.

Conclusion: The clinical and CSF presentation of CNS Varicella and TBM overlap and in this cohort patients were under investigated for TB. In settings of high TB prevalence the possibility of false positive PCR or incidental varicella reactivation should be considered.

Synopsis

Varicella zoster virus is an important human pathogen with several important neurological complications. The diagnosis of varicella has been revolutionised by the discovery of polymerase chain reaction (PCR) techniques. These tests have made rapid diagnosis possible facilitating faster treatment. The availability of these tests has led to an increasing number of case reports and case series of neurological illness caused by varicella in the absence of a rash. In addition several recent publications have highlighted the clinical presentation of neurological varicella and described in more detail the cerebrospinal fluid (CSF) characteristics of this infection. These cases and publications have largely been unaffected by the human immunodeficiency virus (HIV).

Varicella reactivation as shingles and subsequent neurological complications is a common clinical problem in HIV infected patients. However these patients are also at risk of numerous other infections, many of which appear to have similar presentations and CSF characteristics. PCR techniques seem ideal for this situation.

However, PCR techniques are not without their drawbacks. A number of studies have shown PCR detection of viral DNA in the CSF where clinical infection with that virus is judged unlikely. Again this has not previously been studied in HIV.

In this thesis I discuss the pathogenesis of varicella infection, latency and reactivation and review the potential pitfalls of PCR diagnostic techniques in the setting of HIV. I then undertook a retrospective analysis the clinical and CSF characteristics of patients with HIV infections and a positive varicella CSF PCR. In addition I sought to identify potential co-infections, especially tuberculosis, potentially confounding the diagnosis.

List of abbreviations

CD – Cluster of differentiation

CMV – Cytomegalovirus

CSF – Cerebrospinal fluid

DNA – Deoxyribonucleic acid

DRG – Dorsal root ganglion

EBV – Epstein Barr virus

HHV – Human herpes viruses

HIV – Human immunodeficiency virus

MHC – Major histocompatibility complex

MS – Multiple sclerosis

ORF – Open reading frame

PCR – Polymerase chain reaction

SVV – Simian varicella virus

TB – Tuberculosis

TBM – Tuberculous meningitis

VZV- Varicella zoster virus

Chapter 1: Literature review

Varicella Zoster virus (VZV) is a common human pathogen. It is grouped together with other human herpes viruses (as Human herpes virus 3) and is one of the most frequently encountered viruses in clinical practice. It is best known for causing chicken pox and shingles but has been associated with several other clinical syndromes including post herpetic neuralgia, stroke, encephalitis and myelitis[1]. This chapter will provide an overview of the biology of VZV including mechanisms of immune evasion, techniques for laboratory diagnosis, and their pitfalls, as well as the evidence for VZV causing certain clinical syndromes and the important clinical differential diagnoses.

1. Epidemiology

Infection with VZV is common and is characterised by primary infection causing chicken pox (varicella) and reactivation in later life causing shingles (zoster). It occurs worldwide. Primary infection (chicken pox) usually occurs in children and reactivation with shingles usually occurs in later adult life[2]. In temperate climates primary VZV infection occurs in seasonal epidemics in late winter and spring. This is most likely a result of respiratory spread of the virus[2]. This seasonal pattern is less marked in tropical climates where infection tends to occur throughout the year and in adolescence or early adulthood[2]. This age-related pattern is evident in sero-prevalence studies. In European countries sero-prevalence in 10-15 year olds tends to be high. For example in Sweden the seroprevalence of VZV in 12 year olds is 98%[3]. In contrast in Singapore it is only 60.5% amongst 7-12 year olds and increases to 84% among young adults (20-29)[4]. Data from Africa shows a similar pattern. In a study from Eritrea children over 5 had a sero-prevalence of 34% compared to 90-95% for adults[5].

Sero-prevalence in patients living with HIV is also high. In a recent study from South Africa the sero-prevalence of VZV was 89% in patients with HIV[6]. The mean age was 39 year old and the mean CD4+ count was 386 cell/mm^3.

Interestingly only 20% of these cases reported a history of chicken pox and 17% gave a history of a vesicular rash.

Such high rates of seropositivity imply that most adults have VZV latent in sensory and autonomic ganglia. This has implications for diagnostic testing which will be discussed further in section 4.2.

2. Varicella Zoster Virus – structure and function

All herpes viridae are double stranded DNA viruses. Eight human herpes viruses (HHV) are known and these include herpes simplex (HHV1 and 2), Varicella Zoster virus (HHV3), Epstein Barr Virus, Cytomegalovirus, Human herpes virus 6 and 7 (causing Roseola) and Kaposi's sarcoma associated herpes virus (HHV8)[7]. This group is further divided in to α, β and γ sub-families. HSV 1 and 2 and VZV constitute the α-Herpes virus group and are characterised by rapid replication and neuronal latency[8].

The VZV genome is 125 kb and is the smallest of the Human herpes viruses[9]. It encodes 70 genes 64 of which are shared with herpes simplex virus[9]. Five viral clades have been identified but 99.8% of the genome is preserved between clades[10]. The genome contains 5 repeat regions and differences in repeat length are used to distinguish different clades[9,10].

Glycoproteins are important for viral infectivity and intracellular assembly and function. This topic is extensively covered by Arvin and is summarised below[9]. At least seven glycoproteins are encoded for in the VZV genome. These are gB, gC, gE, gH, gI, gK and gL[9]. Glycoprotein B is important in viral fusion to cell membranes and for transport within the endoplasmic reticulum. Glycoproteins E and I are involved in viral replication[9]. Glycoprotein I serves as a chaperone protein regulating gE function and transport[9], and is important in the formation of the cell membrane envelope and in golgi apparatus processing[9]. Glycoprotein I and E complexes also appear to increase tight junctions between host cell thus facilitating viral spread with the host. Glycoprotein E is also important in neurovirulence[11].In particular formation of gE/gI heterodimers is essential for invasion of the dorsal root ganglion (DRG) in a knockout mouse model but the mechanism of this is

unclear[11]. Glycoproteins H and L form complexes within the infected cell and facilitate viral egress[9]. Like gE/gI complexes, they also appear to enhance cell to cell fusion[9]. The function of gK is less clear. Experiments where the gK open reading frame (ORF) is deleted renders the virus non-infectious [9].

VZV also has several regulatory proteins encoded on ORFs 4, 10, 61, 62, 63, 70, 71[9]. ORFs 62/71 and 63/70 are duplicated. These proteins perform several regulatory functions during viral replication. Protein 62 is a transactivator of transcription and also functions to upregulate several cellular proteins involved in promotion of transcription[9]. Proteins 4 and 66 are both involved in regulation of protein 62 activation and regulation[9]. In a simian model of latency ORF 63 protein is one of the early proteins expressed during replication and appears to be the only protein expressed during latency[12]. Its presence in the DRG during latency may indicate low grade viral replication constrained by an effective immune system.

3. Pathogenesis

VZV is highly species-specific, a fact which has made studying its pathogenesis in animal models difficult. It is also notoriously difficult to grow in culture. Several approaches have been developed to overcome these obstacles. A related virus simian varicella virus (SVV) can infect primates and is genetically and immunologically similar to human VZV[13]. Several researchers have studied SVV in primate models and extrapolated this information to human VZV. Another approach uses the SCID-hu mouse which is a knockout mouse model of severe combined immunodeficiency[14]. This allows human cells to be introduced and studied. This model allows human skin, ganglia and other cells to be grafted onto the mouse to examine the immune response and infective spread of VZV[14,15].

3.1 Primary infection

VZV is acquired via aerosolised viral particles or direct contact with vesicle fluid where it rapidly invades the lymphoid tissue of the oro and nasopharynx[2]. From these lymphoid tissues the virus spreads rapidly to

$CD4^+$ and $CD8^+$T lymphocytes[16]. The capacity for VZV to invade lymphocytes shows that VZV is lymphotrophic, in addition to its well-known neurotropism, a feature that may have relevance when interpreting diagnostic tests. Infected lymphocytes spread to the peripheral circulation and contribute to the first of two viraemic stages[17,18]. From here the virus seeds into the reticuloendothelial system[2]. It has been more recently recognised, from studies in the SCID-hu mouse model, that following primary viraemia VZV also spreads to the skin where it remains during the latent phase[14]. This is driven by the preferential infection of activated memory $CD4^+$ cells as well as lymphocytes expressing cutaneous lymphocyte antigen and chemokine receptor 4[14,16]. These are skin homing markers facilitating viral infection of the skin. The predilection of VZV for memory T cells is underemphasised in research on latency and reactivation. A population of VZV specific memory T cells will contribute to VZV immunity but whether these cells carry VZV DNA is not clear. However one study has shown VZV DNA in peripheral blood mononuclear cells in older adults[19]. No studies have examined this in HIV positive individuals.

Initially the infection appears to be contained by the innate immune system. This is driven predominantly by interferon-α (IFN-α). VZV inhibits Stat 1 which is a key phosphorylating enzyme in cellular production of IFN-α[14]. Without interferon-α natural killer cells cannot effectively target infected cells which then provide reservoirs for replication. Neighbouring cells can still produce IFN-α and this balance initially contains the infection[16]. This accounts for the latent phase of the infection which lasts from 7 to 21 days[14]. The innate immune response is eventually overwhelmed allowing further viral replication and the second viraemia following which the typical skin rash emerges[14]. The relatively long incubation period is partly accounted for by VZV's ability to evade the innate response but it also effectively evades the cellular components of the adaptive immune response. Perhaps most importantly VZV disrupts major histocompatibility compatibility (MHC) signalling in infected cells[9,16]. It blocks interferon γ driven MHC-2 expression in infected cells, which interferes with the CD4+ T lymphocyte recognition of infected cells[9]. At the same time MHC-1 expression is also down regulated

thus evading the CD8 T lymphocyte cytotoxic response[9,14,20]. Although during initial skin infection the innate immune system contains the infection, VZV is able to inhibit apoptosis in infected cells thus sustaining intracellular replication[14].

These mechanisms of immune evasions allow the VZV infection to be more productive. It is during this stage that organ involvement, which may be sub-clinical, occurs and the rash becomes clinically apparent[2]. Immuno-competent hosts present with typical chicken pox with a centrifugally spreading vesicular rash often involving mucous membranes, and fever[2]. Immunocompromised hosts are more likely to develop severe organ involvement such as pneumonia and hepatitis[21]. Central nervous system involvement is infrequent but can present as a cerebellitis, meningitis, meningo-encephalitis, vasculitis and multi-dermatomal shingles[13].

3.2 Latency and reactivation

Studying latency and reactivation of VZV has been challenging due to the lack of a suitable animal model and knowledge of these processes is still incomplete[22]. Much of the proposed pathogenesis is based on extrapolation from the SVV primate model and the SCID-Hu mouse model. Latency in sensory ganglia is a key feature of varicella infection. Following acute infection the virus becomes latent in the DRG, the sensory ganglia of the cranial nerves and autonomic ganglia [16,22]. There are two main theories of how VZV infects these ganglia. The first is that following cutaneous infection the virus invades cutaneous sensory axons and spreads via retrograde axonal transport to the dorsal root ganglia[16]. The second theory is that during the viraemic phase infected T cells transport VZV to the dorsal root ganglia[16]. It is unclear why infected lymphocytes should show a predilection for the DRG. Evidence of infection of ganglia by lymphocytes or viraemia is mainly derived from research on SVV[13]. In this model, virus can be isolated from ganglia remote from cutaneous inoculation. DRG's can also be infected following intravenous innoculation in both the simian and the guinea pig/

SCID-Hu mouse model[13]. This phenomena has also been observed in humans following live virus vaccination[23].

Following infection of the DRG the virus establishes latency. During latency viral gene production is less restricted than has been previously thought. Recent studies have shown that as many as 10 viral proteins are expressed during latency in both humans and primates[12,24]. The role of these expressed genes and proteins in maintaining latency is unclear. The most abundantly expressed protein appears to be that of ORF 63 which is also prominently expressed in the early lytic phase of primary infection and reactivation[12,24,25]. Whether this represents low level replication transiently escaping $CD4^+$ cell suppression or plays some other role in sustaining latency is unclear. However, the abundant expression of this and other proteins raises the question of whether the virus is truly latent or merely undergoing restrained low level replication. The viral load in ganglia is low but measurable suggesting that replication is indeed persistent, in contrast to herpes simplex virus[20,26]. Viral latency is probably maintained by several mechanisms including epigenetic suppression of the viral genome and cell mediated immunity[26,27,28]. Interestingly, depletion of B cells and $CD8^+$ cells does not impair the establishment of latency[28]. Although the exact mechanism of the transition from latency to replication is unclear, it is linked to declining cell mediated immunity, particularly loss of $CD4^+$ cells[29]. This dual role of $CD4^+$ cells, being essential to both establishing latency and in preventing reactivation, is intriguing and has not been adequately studied.

In addition to being present in the DRG during latency, VZV DNA can also be isolated from peripheral blood monocytes (PBMs) in otherwise healthy, elderly humans[19]. Whether this represents latency in lymphocytes or just transient reactivation (discussed below) is unclear. This finding has not been replicated and has not been studied in HIV infected patients. Newer evidence suggests that VZV can infect and be maintained in T cells although latency in these cells has not been shown[20]. It is more likely that the finding of virus in peripheral blood lymphocytes may be indicative of sub-clinical reactivation and spread without symptoms.

Reactivation of VZV is dependent on a reduction in the effective CD4 response[30]. This can occur in the setting of immunodeficiency, such as human immunodeficiency virus (HIV) infection, with reduced effective immunity in the setting of another illness, such as malignancy or tuberculosis, or with normal aging[31,32]. Inflammatory cytokines may also be involved in triggering reactivation[16]. The initial feature of reactivation is increased transcription within the DRG[12,33]. This results in an inflammatory reaction which may be severe, haemorrhagic and result in spread of viral particles to adjacent tissues including, possibly, the cerebrospinal fluid (CSF)[12]. Following this, virus spreads via axonal transport to the skin in the innervated dermatome[12,32]. Clinical reactivation of VZV typically presents as shingles with a dermatomal or multi-dermatomal vesicular skin rash[2]. The rash is frequently preceded by pain in the dermatome, which may be severe[32]. Pain persisting for more than three months following infection is known as post-herpetic neuralgia and is a common complication[8]. When VZV reactivation occurs in the absence of rash it is known as zoster sine herpetica[34]. Reactivation as shingles is rarely recurrent in healthy individuals. Recent evidence suggests that latent VZV may undergo periodic increases in viral replication that seems to exist on a spectrum from DNA merely being present in the DRG to sub-clinical reactivation to zoster sine herpetica[30]. The idea of fluctuating immunity and reactivation was suggested as part of the pathogenesis of shingles as early as 1959[35]. Supporting this proposed argument VZV DNA has been found in otherwise healthy individuals in both their saliva and peripheral blood monocytes[13]. This is probably more frequent in immunocompromised individuals and has implications for the diagnosis of VZV as the cause of a clinical syndrome in the absence of a typical rash[36,37].

3.3 Complications of reactivation

Reactivation has been associated with a number of complications. Trigeminal nerve involvement can lead to zoster ophthalmicus with corneal ulceration and blindness[16]. Immunocompromised patients are also at risk of hepatitis and pneumonia[16]. Neurological complications are frequent and may be particularly severe in the immunocompromised individual. The most common

is post-herpetic neuralgia which may occurs in up to 40% of patients and can be very difficult to treat effectively[8]. Neurological involvement can also present as meningitis, meningo-encephalitis, myelitis, cerebellitis and vasculopathies[38].

4. Varicella related neurological syndromes

4.1 Diagnosis

The diagnosis of viral infections was revolutionised in the late 1980's with the development of polymerase chain reaction (PCR) techniques[39]. Prior to this the diagnosis of viral infections relied on viral culture or serological testing. VZV is particularly difficult to culture partly because it remains attached to the cell membranes during much of its replication cycle[39]. The process is also labour intensive and difficult to automate[39].

Serological testing is available but as the immunoglobulin response lags behind the clinical illness, the results are often only useful in retrospect[2]. The relevance of a positive blood serology to a central nervous system (CNS) infection is also sometimes uncertain especially in diseases, such as multiple sclerosis and HIV infection, where a polyspecific immune response is present (generalised increase in immune components directed against multiple antigens which may not be relevant to the illness) [40]. One strategy that is used in this circumstance is to determine a virus specific serum:CSF IgG index[41]. By comparing levels of virus specific antibody in the CSF and in the blood one can demonstrate higher immunoglobulin in CSF compartment. This is often used as evidence of productive infection of the CNS and can be quantified to improve diagnostic utility[40].

Molecular diagnostic testing is integral to diagnostic virology and there are several PCR techniques that are useful in the diagnosis of VZV[42]. Qualitative PCR detects the presence or absence of virus whereas quantitative PCR detects the amount of virus present as a viral load. Multiplex PCR panels, which can simultaneously screen for many pathogens, are used increasingly in the diagnosis of clinical syndromes with multiple potential infectious

aetiologies (such as encephalitis)[43]. These panels typically test for several common organisms. They are sensitive and the appeal of using one test to distinguish between several infections in a situation requiring speedy diagnosis is undeniable. However, with this ease of testing the clinical interpretation of these tests becomes an important issue.

4.2 Pitfalls in diagnostic testing

For the most part these tests are useful in diagnosing infection by VZV. However there are some caveats to be considered in the clinical interpretation of these results in certain circumstances.

4.2.1 CSF:Serum VZV IgG index

The CSF: serum VZV IgG index is regarded by many authors as the gold standard by which to diagnose VZV infection of the CNS with the caveat that testing should not be performed in the first week of the illness[38,44]. This test has never been validated against brain biopsy but the principle appears intuitive. Namely if the antibodies to a specific virus are present in a higher concentration in the CSF than in the serum this must imply CNS infection. There are however several mechanisms and circumstances in which this argument may not hold true.

Firstly a polyclonal humoral and cellular immune response is elicited in many inflammatory conditions including HIV and Multiple sclerosis (MS)[45,46,47]. Memory B cells are stimulated to produce antibodies either by specific stimulation by $CD4^+$ cells or by non-specific cytokines as part of a broad inflammatory response[47,48]. A recurring theme in the MS literature is the association with antibodies in the CSF directed against various viruses. Some authors believe that this is evidence of a viral trigger to MS but this remains unproven. The fraction of antibody in the CSF has been shown to be higher in VZV reactivation than in MS and it has been suggested that this may be useful to discriminate between non-specific activation and active infection[47]. There are, however, no reference ranges with which to make this distinction in individual patients. Furthermore patients may have positive CSF IgG indexes to more than one virus[48]. In some cases this is due to similarities

between the viruses (eg VZV and herpes simplex) but this also occurs in MS and other situations where viral infection is unlikely. Of particular importance in Sub-Saharan Africa is that the performance of these tests has not been studied in HIV infection. In the setting of HIV infection the IgG index is commonly elevated as part of the polyclonal immune activation and this is quite likely to produce false positive serological tests[49,50]. Birlea et al analysed VZV IgG index on 180 HIV positive patients with a range of neurological problems[37]. Of these 28 were positive (16%) and 24 had no clinical evidence of current or preceding VZV.

It is uncertain whether or not the elevated viral specific VZV IgG index indicates previous active, as opposed to sub-clinical, reactivation. VZV has been detected in asymptomatic HIV negative individuals and HIV positive individuals with neurological problems judged unlikely to be due to VZV[51,52,19]. This combined with the detection of protein 63 in ganglia, suggests that VZV replication is suppressed by active T cell regulation but that the course of infection is characterised by periodic viral escape and re-suppression. In the context of HIV infection reactivation may evoke a more pronounced inflammatory response. Given that the site of latency (in the DRG) is so closely related to the CSF it is not clear that such reactivations may not initiate an antibody response in the CSF compartment without necessarily indicating active infection. Further investigation of this potential mechanism seems important but technically difficult.

4.2.2 PCR

PCR is a valuable and powerful tool in the diagnosis of infectious disease. Improvements in PCR have enabled detection of minute quantities of viral DNA and with the development of real-time quantitative PCR (qPCR) the speed of processing of these results has dramatically increased[53,54]. However, the features which make it such a powerful tool also introduce a need for caution in the interpretation of PCR results. There are several potential technical problems with PCR including problems with pre-assay conditions, specimen handling, primer choice and assay design[53]. It is

important that these technical factors are addressed but a more relevant consideration for clinicians is the distinction between a laboratory true positive and a clinical true positive result. The very sensitivity of PCR means that tiny amounts of DNA are amplified giving a laboratory true positive[53]. However this may be either a clinically true or false positive. DNA from infectious organisms may present in a sample for multiple reasons; even from a site traditionally regarded as sterile, such as the CSF.

Davies et al performed an elegant study where they reviewed CSF samples over a four year period and used a multiplex PCR for a range of potential neurotropic organisms including herpes viridae[55]. They also reviewed patient's clinical records and, using strict criteria, estimated the likelihood of a CNS infection. They sought to determine factors influencing negative and positive PCR results. Interestingly the multiplex PCR was positive in 15 of 291 samples (5%) where CNS infection was judged unlikely. In 4 patients these results were in patients with an unequivocal alternative non-viral infection (meningococcal, cryptococcal and leptospirosis meningitis) and 3 were from HIV positive patients with no evidence of a CNS infection. In addition multiple pathogens were detected in 12 patients (2%). These were mostly EBV and CMV and were in the groups where clinical CNS infection was judged likely or probable. Thus in their study the problem of multiple positive results occurred in the groups where the difficulty of determining which organism was responsible was greatest and where clinical features did not appear to be helpful in determining the cause. The authors suggest that DNA may be present in the CSF as a result of carriage in lymphocytes (Trojan horse hypothesis) or due to blood brain barrier disruption as part of another infection.

VZV DNA is also frequently present in the CSF in uncomplicated shingles, that is, with no clinical evidence of CNS involvement[56]. In the study by Haanpää et al PCR was positive in 10/42 (24%) of patients with uncomplicated shingles and in one patient this was the only CSF abnormality[56]. This study was performed using older PCR techniques and it is likely that with more sensitive PCR techniques the rate of positive PCR

may be even higher. The prevalence of HIV in this study was not reported but, as the study was conducted in Finland, it was probably low.

The issue is even more problematic in HIV infection. Shingles is common in HIV infected individuals, and frequently co-occurs with other infections due to impaired cell mediated immunity and dysregulated humoral immune system[6,57]. There are to date no studies assessing PCR positivity in uncomplicated shingles in HIV. However, following shingles, long term viral shedding has been detected in the saliva and blood of HIV positive patients even in the absence of clinical infection [58,59]. Studies of PCR in CSF of HIV positive patients with neurological disorders highlight frequent detection of VZV DNA, usually ascribed to sub-clinical reactivation of VZV[36,60]. In the study by Burke et al they concluded that VZV PCR was a useful clinical tool[60]. Nine of the 81 HIV-infected patients were VZV positive and 3 were positive for both VZV and CMV. The presentations in the former included those likely to be related to VZV (radiculomyelitis, necrotising leukoencephalopthy) as well as less definite presentations (encephalitis and aseptic meningitis). In the dual positive cases the presentation could have been due to either virus. Five of 9 positive patients had shingles at presentation. However, in another study, authors concluded that sub-clinical reactivation was more common[36]. In this study 13/ 531 samples were VZV PCR positive and an alternative diagnosis was made in 9/13. All 13 had CD4+ counts less than 50. Multiple viruses were detected in 3 cases all of whom had an alternative diagnosis unrelated to either virus. A recent study from Zambia reported similar findings. Siddiqi et al analysed CSF samples from 331 HIV infected adults with symptoms of CNS opportunistic infections[61]. Multiple infections were found in 36% of cases. Herpes viruses, most commonly EBV, were common co-pathogens. Interestingly they reported 3 cases where VZV PCR was positive with TBM.

From these studies it seems clear that the interpretation of PCR results needs a great deal of caution and careful consideration of other CSF parameters and clinical factors. CSF parameters are unfortunately also confounded by the frequent baseline abnormalities due to HIV infection itself.

5. Tuberculous meningitis and VZV CNS infection

Perhaps the most important differential diagnosis in this setting of high TB prevalence is tuberculous meningitis (TBM). TBM lacks an adequate diagnostic test and thus is frequently treated on clinical suspicion[62]. Tests traditionally used to diagnose TBM include CSF Ziel-Nielsen (ZN) and auramine staining for microscopic detection and CSF TB culture[63]. Bacilliary load is often low in TBM and thus the yield of microscopy is low. Culture performs betterwith yields of up to 60%,though this requires a large volume of CSF[63]. The organism is slow growing and thus results typically take 2-6 weeks. This period is too long to aid in immediate treatment decisions but does confirm the diagnosis. Newer techniques such as TB PCR are available. They provide a rapid result but are still volume dependent and have sensitivities ranging between 60 and 80%[64,65,66]. This test has a modest negative predictive value limiting its clinical usefulness[64,66]. To get around these diagnostic problems, research studies have defined TBM using composite scoring systems incorporating clinical case definitions andlaboratory and imaging parameters[67]. A standardised case definition was recently produced by a panel of experts[62]. While this is useful, it is cumbersome to use in clinical practice and may not be sufficiently sensitive in detecting atypical cases that can reasonably be excluded from research studies but may still require treatment.

Against the backdrop of these diagnostic difficulties care must be taken in the clinical investigation of meningitis and meningo-encephalitis which may be compatible with TBM, especially in areas of high prevalence. A positive result on another diagnostic test may distract from treatment of TBM where it is the true pathogen. Thus knowledge of the limitations of these tests is important for clinicians.

A further confounding factor is that TBM itself may predispose to VZV reactivation. TB impairs cell mediated immunity and reduces CD4+ count[28,68,69]. As discussed above these factors predispose to VZV reactivation, sub-clinical or otherwise.

6. Conclusion:

In conclusion varicella is a ubiquitous virus. It commonly causes primary infection as chicken pox and may be reactivated as shingles. In addition it can cause several important neurological complications. Following primary infection the virus establishes latency but may undergo periodic attempts at replication. Active cell mediated immunity is required to limit this. As the viruses establishes latency in the dorsal root ganglion, and may have prolonged shedding in HIV, DNA may be present in biological fluids without clinically relevant infection. Although PCR is a useful test to diagnose a range of infections, it may be too sensitive in the setting of human herpes virus infections including VZV. This is highlighted by the detection of the virus in circumstances where active infection is judged unlikely (a clinical false positive). A positive result in this circumstance may distract a treating clinician from a more relevant, but difficult to diagnose condition, such as tuberculosis. Conversely there are situations where VZV is the primary infection and where treatment is required. Multiple infections are not uncommon in HIV further complicating these decisions.

To date there are no studies in the setting of high HIV and TB prevalence examining differences in the clinical and laboratory presentations of these diseases. As a first step in addressing this problem we set out to describe the clinical and cerebrospinal fluid characteristic of HIV positive patients that were positive for VZV by PCR.

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Chapter 2: Paper in publication ready format for submission to the South African medical journal.

Central nervous system varicella zoster virus and HIV: incidental or pathogenic?

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Abstract:

Aims: To assess the clinical and cerebrospinal fluid characteristics, and the role of tuberculous meningitis (TBM) as a confounder, in a cohort of HIV positive individuals with positive varicella zoster virus (VZV) positive cerebrospinal fluid PCR.

Methods: Patients in the NHLS database at Groote Schuur Hospital with positive CSF VZV PCR who were also HIV co-infected and whose folders were available for clinical review were reviewed. Clinical and biochemical data were collected. Patients were divided into two groups based an accepted case definition for TBM. Differences between groups were assessed using Mann-Whitney U or Chi squared tests as appropriate.

Results: There were 437 for VZV PCR over three years. Of these 98 were positive and, after exclusions, 31 HIV positive patients were included for further analysis. Median age was 31 and median CD4 count was 146 cells/mm^3. 11 (35%) had meningitis and 8(25%) had encephalitis. 13 (42%) met the case definition for TBM. Patients with CNS varicella were frequently confused whereas those with TBM presented sub-acutely. There were no differences in CSF characteristics. Additional organisms were detected 6 (19%) patients. 4 (13%) patients died in hospital. CSF TB culture was requested in 24 (77%) patients and extra CNS samples were sent in only 4 patients.

Conclusion: The clinical and CSF presentation of CNS Varicella and TBM overlap and in this cohort patients were under investigated for TB. In settings of high TB prevalence the possibility of false positive PCR or incidental varicella reactivation should be considered.

Introduction:

Varicella Zoster virus (VZV) infection has been implicated in diseases of the central nervous system (CNS) such as encephalitis, meningitis, myelitis and strokes, and may in addition trigger immune-mediated disorders like acute demyelinating encephalomyelitis (ADEM). It is well known that active VZV disease may occur without the characteristic rash making the diagnosis challenging[1]. Conversely VZV may reactivate in the setting of other illnesses (such as malignancy) where it does not directly contribute to the disease. Prior to the introduction of polymerase chain reaction (PCR) techniques, definitive diagnosis of VZV CNS infection rested on viral culture, brain biopsy, or the demonstration of a virus-specific intrathecal antibody response, methods that either lack sensitivity or fail to provide an answer within a clinically useful time frame[2]. Newer PCR methods of detecting viral DNA in the cerebrospinal fluid (CSF) have improved the diagnostic sensitivity for VZV infection in the CNS and also expanded the clinical spectrum of VZV disease[1]. An example is that VZV is now recognised as a common cause of aseptic meningitis in adults even without a rash. The clinical interpretation of CSF PCR results is however not without pitfalls. Firstly not all PCR assays of CSF pathogens have been compared against a gold standard. For example, only herpes simplex and JC virus CSF PCR tests have been validated systematically against brain biopsy. Secondly it is not clear that detection of viral DNA in the CSF necessarily represents active, clinically relevant infection. Concerns have been raised about both laboratory and clinical false positive PCR results. Quereda et al reported positive PCR in the CSF of HIV positive patients where a clinical viral infection was deemed unlikely[3]. Davies et al reported both false negative and false positives in the CSF with a multiplex PCR[4]. The accuracy of a diagnostic test is particularly important in

illnesses which share clinical and CSF features such as VZV and TB meningitis. However, adequately validated, accurate laboratory diagnostic tests are lacking in both of these conditions.

The clinical and CSF characteristics of active CNS VZV infection have recently received renewed attention[5,6,7]. Several authors have reported that VZV remains a frequent cause of CNS disease and most commonly presents as meningitis or encephalitis. Less than half of these patients have a rash or fever. The CSF usually shows an elevated white cell count (lymphocyte predominant) with raised protein[5,6,7].

Almost all studies reporting the range of clinical and CSF features of CNS varicella have been from areas with a low prevalence of HIV and TB where co-infection is an infrequent clinical concern. Consequently, it is not clear whether these features apply to HIV positive patients. One recent study from Malawi did not find a single case with positive CSF VZV in a cohort of mainly HIV positive patients with suspected meningitis[8]. We note, however, that patients in this study with confirmed bacterial, fungal or tuberculous meningitis were not tested for VZV, and so 'dual' infection was not reported.

The brunt of the HIV and TB epidemics is in Sub-Saharan Africa where resources are limited[5,6,7]. PCR diagnostics are expensive and if the diagnosis is incorrect may lead to further expensive inappropriate treatment. It is therefore important to try and identify clinical or laboratory features suggestive of CNS Varicella to allow more cost effective use and interpretation of VZV PCR and appropriately direct treatment. We aimed therefore to describe the clinical and laboratory characteristics of HIV infected patients with positive CSF VZV PCR, in order to identify features that may discriminate between infectious causes of meningitis in this population.

Methods:

We retrospectively collected data on all CSF samples sent for VZV PCR testing to the National Health Laboratory Service (NHLS) laboratory at Groote Schuur Hospital during the study period from May 2008 and August 2011. VZV PCR testing was performed on CSF according to standard

protocols at accredited laboratories. Laboratory data were extracted from the NHLS database. Cases with positive CSF VZV PCR from hospitals within our clinical service area were included for folder review. Cases were excluded if requests were submitted on fluids other than CSF or where the origin of the tested fluid was unclear. Where multiple samples were positive on the same patient, the admission CSF parameters were recorded. Detailed clinical data were obtained retrospectively from the hospital records by a clinician (AS), where hospital notes were available. The following information was recorded for the clinical episode: presence of fever(>37.5℃), headache, meningism, seizures, rash, focal neurological signs and altered level of consciousness. Clinical signs of immunocompromise, pulmonary and extrapulmonary tuberculosis or other opportunistic infection were recorded. Brain imaging by computed tomography (CT) or magnetic resonance imaging (MRI) , chest radiography and abdominal ultrasound or CT examinations performed during the hospital admission were reviewed where source images were available, or reports were recorded. CSF white cell count (WCC), total protein, glucose, culture and other PCR results were recorded for each CSF sample. In addition CD4+ cell count and white cell count were also recorded.

Patients were categorised by whether they presented clinically with encephalitis, meningitis, myelopathy, stroke or radiculopathy. Encephalitis was defined as a febrile illness with altered level of consciousness or seizure on presentation, with or without focal signs other than paraparesis. Fever was defined as temperature greater than 37.5^0 on admission. Altered level of consciousness was defined as a Glasgow Coma Score of 14 or less. Meningitis was defined as a febrile illness with headache and an abnormal CSF without altered level of consciousness, paraparesis or seizures. Myelopathy was defined as the acute onset of bilateral limb weakness which the attending clinician considered consistent with spinal cord lesion. Stroke was defined as the acute onset of focal neurological signs. Radiculopathy was defined as an acute radicular pain with or without a blistering rash. Any conflict in categorisation was resolved by consensus between authors.

Patients were classified into definite or probable TBM based on an internationally accepted case definition[9]. Briefly: patients were classified as

having ‘Definite TBM’ if CSF smear was positive for acid fast bacilli (AFBs) or CSF cultured *mycobacterium tuberculosis*. ‘Probable TBM’ was diagnosed in patients with AFBs identified or *mycobacterium tuberculosis* cultured from specimens outside the CNS, or with imaging findings highly suggestive of tuberculosis (on brain imaging, chest radiograph or abdominal ultrasound). For the purposes of analysis, because of low numbers of microbiologically confirmed cases, ‘Definite’ and ‘Probable TBM’ was analysed together as ‘Probable TBM’. All other patients were classified as CNS varicella.

Data was analysed using SPSS 22 (IBM Corporation, USA). Due to the small sample size and non-normal distribution of the data continuous variables were analysed using the Mann-Whitney U test and ordinal/ nominal data was analysed using Chi squared test. Ethical approval for the study was obtained from the University of Cape Town Human Research Ethics Committee (REC Reference no. 01/2015).

Results

Over a 3 year period there were 437 requests for VZV on CSF registered on the laboratory database of which 98 were positive for VZV PCR. 57 patients were excluded (see study profile). Of the remaining 37 cases, 31 were HIV positive and thus included in further analysis. The median age was 31 years, the median CD4+ cell count was 146 cell/mm^3 and 61% were female. 13 (42%) patients met the case definition for probable TBM. In terms of clinical presentation 11 (35%) patients presented as meningitis, 8(25%) as encephalitis, 6(19%) as myelopathy and 5(16%) with strokes. There was no difference between the probable TBM and CNS varicella groups with respect to clinical presentation (p=0.157). 26 patients (83%) presented with rash and there was no difference between the TBM and CNS Varicella groups (p=0.924). 8 patients (26%) presented with confusion and patients in the CNS varicella group were more likely to be confused (p=0.05). Patients in the TBM group were more likely to have a sub-acute presentation with a median duration of illness of 9 days compared to 3 days in the CNS varicella group (p=0.21). There were no differences between groups with respect to fever on admission, seizures, headache or focal signs.

In terms of cerebrospinal fluid (CSF) analysis the median protein concentration was 1.81g/dl and median glucose concentration 3.1mmol/l. The median lymphocyte count was 51(range 0 - 970) and median neutrophil count was 3.5 (range 0 - 300). There were no differences in any of these parameters between groups. TB culture was requested in 24 cases (77%). No cultures were positive but one case was smear positive for acid fast bacilli. Extra-CNS TB samples were sent in only 4 patients, one of whom a positive sputum culture with a negative M.TB PCR. Additional organisms were detected in the CSF in 6 patients as follows: Treponema pallidum (1), Cryptococcus (1), Epstein-barr virus (2) and cytomegalovirus (2). Twenty-one (68%) patients had chest radiograph or abdominal ultrasound to investigate for extra-CNS TB. Nine (43%) of these had features suggestive of TB. Ten (32%) patients had no record of imaging studies performed.

Twenty-three (74%) patients were treated with Acylovir and seventeen (55%) were treated with anti-tuberculous therapy. Fifteen (48%) patients were treated with both acyclovir and TB treatment. In the acyclovir group nine patients were started on intravenous therapy and switched to oral therapy, and three were treated with oral therapy alone. One patient was treated with TB treatment and antibiotics. In the Probable TB group, 8/13 (62%) were commenced on TB treatment and 11/13 (84%) on acyclovir. In the CNS varicella group, 9/18 (50%) were started on TB treatment, and 15/18 (83%) on acyclovir.

4 (13%) patients died in hospital and there was no difference in mortality between the TB and varicella groups.

Discussion

In this retrospective study 42% of patients with positive CSF VZV PCR met current case definitions for probable or definite TBM. Patients with TBM had

a longer duration of illness prior to presentation and those in the CNS varicella group were more likely to be encephalopathic. Apart from these features there were no differences in the clinical presentation or CSF characteristics to distinguish the two groups. Despite the high prevalence of TB in the communities studied the level of investigation for tuberculosis was quite low; possibly because finding the VZV rash or positive CSF PCR directed the clinical suspicion towards VZV. This is concerning given the high morbidity and mortality associated with TBM. Importantly rash was as common in the TBM group as the VZV group.

Reactivation of VZV manifesting as shingles is a common occurrence in people who are living with HIV. Sub-clinical 'reactivation' has been described in the setting of HIV[10]. These cases were detected by CSF PCR or IgG index and these results were felt to indicate active CNS infection in the absence of clinical features. However, in recent years there have been positive CSF PCR results detected in patients in whom a replicative VZV infection of the CNS is thought unlikely, particularly in HIV infected patients[11]. This further complicates the interpretation of CSF PCR results and it would be helpful to know what clinical and CSF features raise the clinical suspicion of active, and clinically relevant, VZV infection. However the manner in which HIV influences the clinical and cerebrospinal fluid (CSF) features of VZV infection of the CNS is not clear. Our study suggests that in the setting of HIV the CSF features of VZV are similar to those of TB. Given the absence of a highly accurate diagnostic test for TBM, an alternative explanation may be that detection of VZV DNA occurs more readily in patients with a concurrent infection such as HIV or TBM. CD4+ lymphocytes seem pivotal in controlling both primary infection, reactivation and possibly latency[12,13]. In its own right TB reduces CD4+ and thus predisposes to VZV reactivation[14,15]. A recent study from India examined CSF and brain tissue from 55 HIV positive patient who came to post mortem with confirmed TBM, Cryptococcal meningitis or CNS toxoplasmosis[16]. In this study 82% had a positive CSF PCR for a herpes virus and 45% had more than one positive PCR. Varicella was detected in 3.6%.

Sub-clinical detection of immunological evidence (elevated IgG or IgG ratio) of VZV in the CSF has also been described in HIV[11,17]. Whether or not this indicates clinically relevant disease is unclear. It is possible that virus specific IgG may be elevated due to the polyspecific immune activation in HIV. Detection of immunoglobulins directed against various viruses has been detected in the context of other neurological diseases characterised by polyspecific immune activation such as multiple sclerosis (MS). For example IgG to several viruses have been detected in the CSF of MS patients prompting speculation that a viral infection is responsible for triggering MS[18]. However most authors suggest that these IgG are detected as epiphenomenon, possibly upregulated in the setting of more general inflammation[18]. It is of interest that viral DNA has also been recovered from the CSF of MS patients who did not have an active viral CNS infection[19]. Another explanation for the relative increase of CSF IgG is that that patients with HIV may experience more vigorous local reactivation of VZV in the dorsal root ganglion as a resulted of impaired cell mediated immunity. This would result in breakdown of the blood brain barrier resulting in exposure of the CSF compartment to antigen. This may in its own right be sufficiently inflammatory to induce a CNS immune response without necessarily progressing to active CNS disease. There is to date no evidence for this hypothesis.

There are also several reasons why viral DNA may be present in the CSF without active infection. Davies et al report up to 25% of general viral PCR results to be positive in settings judged unlikely to represent CNS infection (i.e. indicate false positive results)[4]. In that study, 13% of the cohort were known to be HIV positive; much lower than the HIV prevalence in sub Saharan Africa, which in hospital based meningitis cohorts ranges between 70-90%. They suggest that clinical false positive PCR's may occur due to a technical laboratory error. A positive PCR could also represent an epiphenomenon of reactivation of latent viruses in the setting of an immunological stressor or impaired cell mediated immunity. In either case the significance of a positive result becomes a clinical judgement. This is highlighted in their study by the relatively high incidence of EBV detection

considered unlikely to be causal. Low levels of EBV and CMV DNA in the CSF of HIV positive patients are not uncommon and a viral load may help determine clinical significance[20]. This was also observed in our study with both EBV and CMV DNA being detected in several patients. Two patients also had clear evidence of an alternative pathogen more likely to be clinically significant (neurosyphilis and cryptococcal meningitis) supporting the assertion that the reactivation of VZV was incidental. However, it is not uncommon for patients with late HIV infection to present with multiple concurrent opportunistic infections.

Given the immune dysregulation that accompanies HIV, tuberculosis (TB) and the IRIS phenomenon the interpretation of immunoglobulin and PCR based tests in the setting of high HIV/ TB co-infection needs to be interrogated; especially in light of the arguments presented above.

In our study PCR was commonly positive in patients with CNS disease, often in patients who had normal CSF parameters, no rash or clinical features of primary varicella or reactivation (zoster). These could reflect a false positive result or viral reactivation which may not be clinically relevant. The difficulty in interpretation of CSF findings is compounded by the fact that, in HIV, baseline CSF inflammation is frequently high with a background pleocytosis and elevated IgG index. Acting on a positive PCR results may result in inappropriately stopping TB treatment where TB is the actual primary infection. Our study suggests that it may be difficult to distinguish between CNS infections on clinical grounds. A rash may be present but this does not necessarily implicate VZV as the primary pathogen as has been discussed. CSF parameters are non-specific.

Lastly the role of VZV in HIV positive stroke needs careful interrogation. While there have been clear and elegantly demonstrated cases of VZV vasculitis causing stroke it is unclear how HIV influences this association. As highlighted in this study HIV positive stroke patients with no clinical suspicion of VZV and normal CSF parameters are frequently PCR positive.

Our study does have limitations. Firstly it is a retrospective study and workup of the cases varied and was incomplete in several cases. Secondly the

reference standards for the diagnosis of TBM and VZV CNS infection remain suboptimal. Lastly resources did not allow further analysis and comparison to the VZV negative CSF PCR's.

In conclusion the interpretation of CSF VZV PCR and IgG is not as clear cut as it would initially seem. While a positive PCR does show the presence of viral DNA in the CSF this may not always equate to clinically significant active infection. Similarly there are several reasons why CSF IgG may be increased without active CNS infection. These tests are particularly problematic in the setting of the immune dysregulation that accompanies HIV. We sought to identify clinical features suggestive of VZV and factors influencing the detection of VZV DNA in the CSF. Our study found that many patients with positive VZV PCR meet the clinical case definition for TBM and that these cases cannot be reliably distinguished on CSF parameters or a positive PCR. The differential diagnosis of VZV CNS infection is broad and includes other treatable infections. Care should be taken to adequately exclude these causes especially TBM in areas of high prevalence. In a setting of high TB/HIV prevalence, it remains difficult to distinguish between TBM and CNS varicella on clinical and laboratory parameters, despite the availability of VZV CSF PCR.

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