SUBACUTE MEASLES ENCEPHALITIS: THE NEUROLOGICAL SEQUELAE OF THE MEASLES OUTBREAK IN SOUTH AFRICA

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DECLARATION

I, Dr Christine Herculine Albertyn, declare that the research reported here is based on independent work performed by myself (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 to any other university. This work had not been reported or published prior to registration for the MMed degree.

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ABSTRACT

Subacute Measles Encephalitis: The neurological sequelae of the measles outbreak in South Africa

Introduction

A measles outbreak occurred in South Africa between 2009 and 2011 with 18 699 confirmed cases. This highly contagious virus can affect the central nervous system in many ways. Early in the disease course there may be direct viral involvement as a primary measles encephalitis or indirectly as an inflammatory immune mediated demyelinating meningoencephalitis. Latent infections are rare and may manifest in two ways: years later as subacute sclerosing panencephalitis (SSPE) caused by viral persistence in a seemingly immunocompetent host or months later as subacute measles encephalitis (SME) in an immunocompromised host. SME is characterised by seizures, typically epilepsia partialis continua, and altered mental status and carries a high mortality. It is an elusive diagnosis and usually confirmed on brain biopsy.

Patients and results

Eight patients were diagnosed with SME between July and October 2010 at our tertiary referral hospital. All patients were HIV positive, with a median CD4 lymphocyte count of 37 cells/µl (range 1 to 268). All patients had epilepsia partialis continua during the course of the illness and other common features included encephalopathy, visual loss, hearing loss, and generalised seizures. Strikingly, cerebrospinal fluid (CSF) examination was normal in all patients and computed

tomography (CT) Brain imaging was normal in all but one patient. Magnetic resonance imaging (MRI) Brain demonstrated superficial and deep grey matter abnormalities in the majority of patients with contiguous cortical spread over weeks documented in one patient. Electroencephalograms (EEGs) showed periodic epileptiform discharges in seven patients. Diagnosis was confirmed by brain biopsy in one patient, by post-mortem examination in three patients and by supportive laboratory findings (positive measles PCR and/or measles antibodies in urine or CSF) in the remainder. The outcome was fatal in seven of the cases with a median time to death of 3 weeks.

Conclusion

South Africa has the greatest number of people living with HIV: 12.6% of the population (6·4 million people) are infected. This is the largest SME case series to date and is seen in the aftermath of a measles outbreak in South Africa. Immunocompromised patients are clearly susceptible and typically present with epilepsia partialis continua and rapid decline in neurological functioning and death ensuing within a month in the majority of cases. MRI T2-weighted signal changes in the cortical grey matter, are typical. In the absence of a brain biopsy, we propose the use of measles virus PCR in urine and CSF. The importance of herd immunity, by enforcing the national vaccination programme, is reiterated.

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DEDICATION

I would like to dedicate this dissertation to all the patients and their families who suffered the consequences of the measles outbreak.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Professor Jeannine Heckmann for her kind support in writing this thesis and for her enthusiasm and guidance in identifying and caring for the patients affected by measles encephalitis. I would also like to thank the doctors from the Department of Medicine working in the teaching hospitals in the Western Cape for referring and identifying cases. Thank you to the Division of Neurology academic staff for critically reviewing our data and for generously supporting my travel to Newcastle in the UK to present our findings at the annual Association of British Neurologists' meeting in 2011. I am grateful to Dr Diana Hardie from the Department of Virology, University of Cape Town and National Health Laboratory Service for her assistance with laboratory confirmation of cases and her inspiring work subsequently on the molecular characterisation of the measles virus. My gratitude goes out to the nursing and ancillary staff of Ward E7/E8 for their gentle care of the patients affected by measles encephalitis. Finally, I would like to thank the patients and their families for agreeing to take part in the study amidst great hardship.

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ABBREVIATIONS

ADEM	Acute disseminated encephalomyelitis
APME	Acute postinfectious measles encephalomyelitis
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed Tomography
EEG	Electroencephalogram
HIV	Human immunodeficiency virus
MIBE	Measles inclusion body encephalitis
MRI	Magnetic resonance imaging
MV	Measles virus
PCR	Polymerase chain reaction
PME	Primary measles encephalitis
SLAM	Signalling lymphocyte activation molecule
SME	Subacute measles encephalitis
SSPE	Subacute sclerosing panencephalitis

PART A: PROTOCOL

Study protocol

1. Background

South Africa has 6·4 million South Africans, or 12·6% of the total population, living with HIV (1), making our population particularly vulnerable to opportunistic infections. South Africa experienced a measles outbreak that started in late 2009 and lasted until early 2011. There was a marked increase in cases of measles, with the highest incidence reported in March 2010. Between January 2009 and September 2011 a total of 18 699 new measles cases had been reported to the National Institute of Communicable Diseases (2). A mass vaccination campaign was instituted in mid-April to early May 2010, resulting in a significant decline in new measles cases after this period.

The measles virus is highly contagious, and outbreaks are fuelled by overcrowding and poor vaccine coverage, making elimination status in South Africa difficult to attain. Measles may infect the central nervous system (CNS) as primary measles encephalitis (an acute viral encephalitis), or result after 2 - 4 weeks in a post-infectious immune-mediated inflammatory disorder or acute disseminated encephalomyelitis (ADEM). There are two further rare and latent CNS complications resulting from a preceding measles infection: subacute sclerosing panencephalitis (SSPE) caused by years of viral persistence in a seemingly immunocompetent host, and subacute measles encephalitis (SME), also referred to as measles inclusion body encephalitis (MIBE), occurring in an immunocompromised host (3). SME manifests a

few months after the acute measles infection. Patients present with seizures, often epilepsia partialis continua, and altered mental status. It carries a mortality rate of 85% and survivors often have significant neurological impairment. SME has hitherto only been described in single case reports as a rare complication of measles in the context of organ transplantation, immunosuppressive therapy, primary immunodeficiencies, and HIV/AIDS (4–12). We diagnosed eight HIV-infected patients with SME as they presented to a tertiary referral hospital between July and October 2010.

2. Research justification

South Africa has the greatest number of people infected with HIV and the highest number of new cases annually (13). In this context, the outbreak of a highly contagious virus is likely to have significant consequences for its residents. The devastating nature of SME and the difficulty in diagnosing the condition were important factors that prompted us to disseminate knowledge regarding this otherwise rare disease.

3. Objectives

By publishing our findings in the form of a case series in a nationally relevant journal, we aimed to create awareness and recognition of SME; improve time to diagnosis; assist physicians in managing patients and counselling families; and emphasise the importance of immunisation programmes.

4. Methods

All patients referred to our Neurology Division with suspected SME were assessed and special investigations performed on a case by case basis. However, baseline demographic details, HIV status, CD4 count, anti-retroviral therapy status, cerebrospinal fluid (CSF) biochemistry and cell count, EEG and neuroimaging (computed tomography (CT) or magnetic resonance imaging [MRI]) were documented in all patients with patient identifiers anonymised on a data collection form. Additional tests included serum, urine and CSF measles PCR; serum and CSF measles antibody titres, brain biopsy or post-mortem brain histology. See ***data collection form*** in ***Part D: Appendix 1*****.** We classified patients as having 'definite SME' in the presence of a suggestive clinical picture (focal seizures and/or encephalopathy) and a positive measles PCR in either tissue from a brain biopsy, CSF or urine. 'Probable SME' was diagnosed in the context of a suggestive clinical picture, normal CSF findings in the absence of other identifiable pathogens, and with supportive features such as MRI demonstrating multifocal grey matter signal abnormalities and/or positive CSF measles serology (IgG). Consent was obtained from the family of our index patient. ***See Consent form*** in ***Part D: Appendix 2***.

5. Ethics committee approval

Human Research Ethics committee approval was obtained, HREC REF 487/2010. See ***Part D: appendix 3.***

6. Dissemination of findings

Our case series was published in the South African Medical Journal (SAMJ) in May 2011. Our findings were also presented at the Groote Schuur Hospital/UCT Department of Medicine Thursday Forum in the same year. I presented our patients at the annual Neurological Association of South Africa (NASA) meeting in Durban in March 2011. In October 2011, I delivered a platform presentation at the annual Association of British Neurologists (ABN) meeting in Newcastle in the UK.

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PART B: STRUCTURED LITERATURE REVIEW

Objectives of literature review.

A. To obtain background information regarding the pathophysiology of measles and how the measles virus affects the Central Nervous System (CNS).

B. To perform a detailed review of subacute measles encephalitis (SME), reporting published information regarding risk factors, clinical presentation, special investigations, treatment and outcome.

C. To understand the pathogenesis of SME in HIV infected patients following the measles outbreak in South Africa in 2009-2010.

Literature search strategy.

For general background information regarding a review of measles and its CNS complications, Pubmed, Medline and Google Scholar were searched using keywords "measles review", "measles encephalitis", "measles central nervous system", "subacute measles encephalitis", "measles inclusion body encephalitis".

For the detailed literature review regarding SME, I searched Pubmed and Medline databases, using the following keywords, MeSH terms and Boolean operators: "Subacute measles encephalitis", "immunosuppressive measles encephalitis", "measles inclusion body encephalitis", "measles encephalitis AND (subacute OR inclusion OR immunosuppressive) NOT sclerosing".

A hand search of selected articles' bibliography was also performed.

For information regarding the measles outbreak in South Africa, the National Institute of Communicable Diseases website (www.nicd.ac.za) was accessed.

Inclusion criteria: Relevant papers available in English were reviewed. Full text articles were sourced via the University of Cape Town library server.

Exclusion criteria: the Boolean operator "NOT sclerosing" was used to limit articles referencing subacute sclerosing panencephalitis.

Quality and relevance criteria

Studies were reviewed relating to human subjects with subacute measles encephalitis with heavier weighting given to case reports with histological confirmation of diagnosis on brain biopsy or post-mortem specimens. Articles relating to primary or post-infectious measles encephalitis and SSPE were excluded.

Summary and interpretation of literature review

1. **Overview of the pathophysiology of measles and how the measles virus affects the Central Nervous System**

The measles virus (MV) is a spherical, enveloped, non-segmented, single-stranded, negative-sense RNA virus and a member of the Morbillivirus genus in the Paramyxoviridae family (14). Six proteins are found in the virion (**see Fig. 1**). The envelope has surface projections composed of the viral hemagglutinin (H) and fusion (F) glycoproteins with the matrix (M) protein lining the interior. Within this envelope, the nucleocapsid is found, formed by the genomic RNA wrapped in the nucleocapsid (N) protein and packed in the form of a symmetrical coil with the phosphoprotein (P) and large polymerase (L) proteins attached (15).

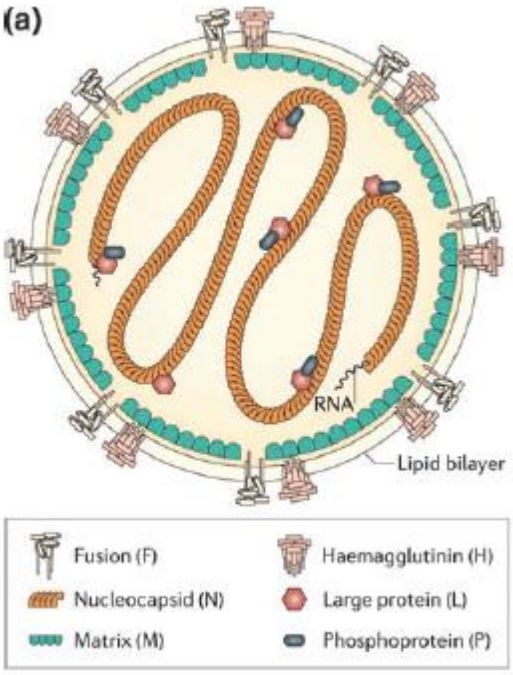

Figure 1: The enveloped measles virion demonstrating its six proteins
From: Measles virus, immune control, and persistence. Griffin DE et al. FEMS Microbiol Rev. 2012

The two originally identified cellular receptors for MV were CD46 and CD150 (signalling lymphocyte activation molecule [SLAM]). CD46 is a complement regulatory molecule that is ubiquitously expressed on all nucleated cells while SLAM is expressed on activated T and B lymphocytes and antigen-presenting cells (in concordance with the lymphotropism of measles virus), but has not been found on epithelial, endothelial, and neural cell types (16). More recently, poliovirus receptor-related 4 (PVRL4) or nectin-4 was described and functions as an adherence junction protein of the immunoglobulin superfamily and is expressed on airway epithelial cells (17). SLAM was identified as receptor for both vaccine and wild-type MV strains whereas CD46 acts as cellular receptor only for vaccine strains (18). It has therefore been suggested that other yet unknown molecules act as receptors for MV in the CNS or that the MV may gain entry into the CNS through cerebral endothelial cells or

infected monocytes (3). In a fibroblast environment, MV results in a highly productive infection that causes cytolysis, giant cell formation, and high titers of extracellular virus. In the neuron, in contrast, despite the spread of virus, there is little evidence of MV-induced cell death, syncytium formation, or infectious virus production. Electron microscopy (EM) analysis has shown that viral budding does not occur from the neuronal surface, although nucleocapsids are present in the cytoplasm and aligned at the cell membrane. See ***Figure 2***. There is no evidence of fusion between infected cell bodies, but because of the presynaptic nucleocapsid localisation, it is likely that MV can spread between neurons via synaptic connections in the absence of cell fusion and may not require a MV receptor to maintain a persistent neuronal infection (19).

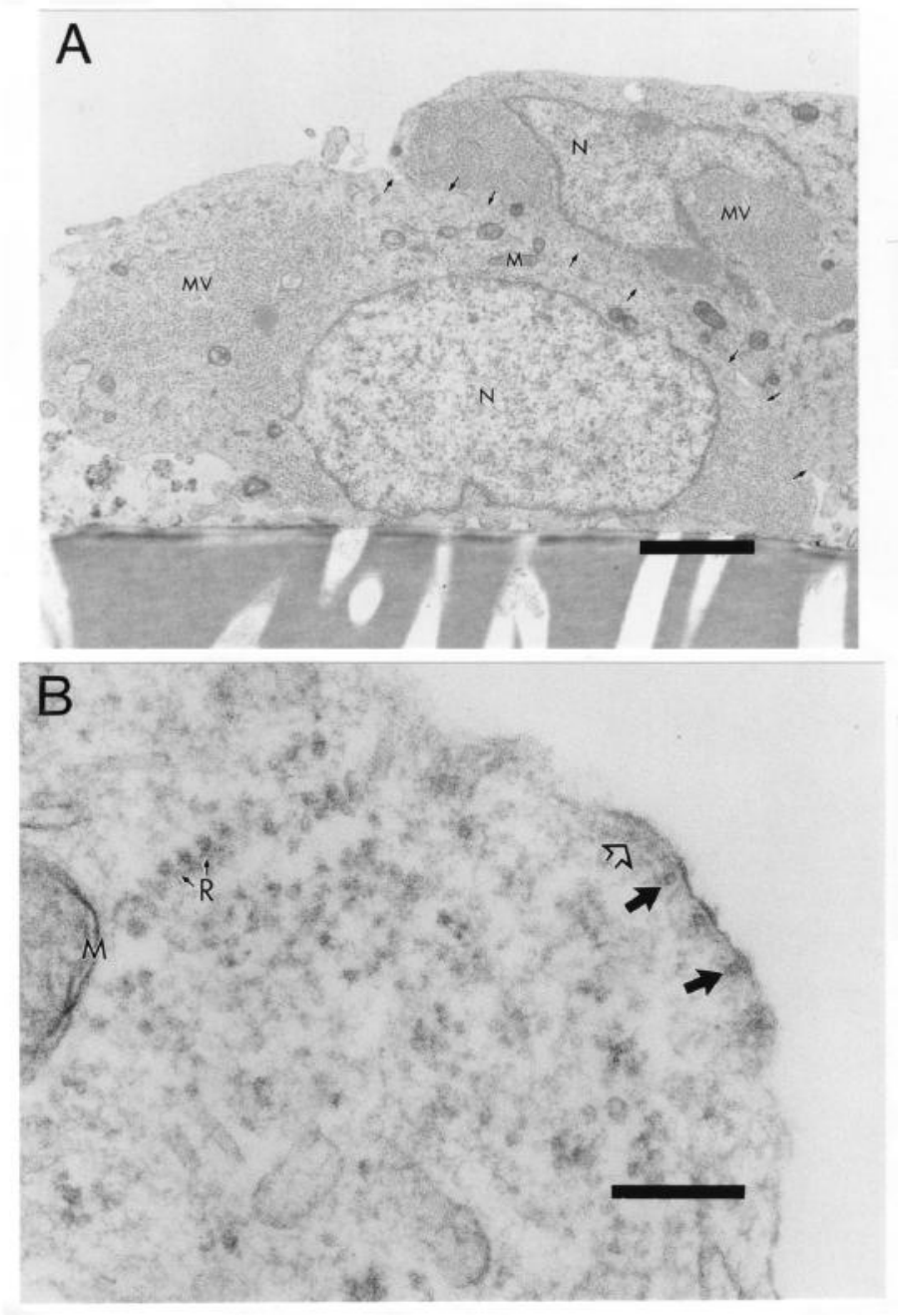

Figure 2: Incomplete budding at surface of MV-infected neurons. Primary CD46+ neuron cultures were infected with MV Edmonston (MOI = 1) or mock infected, fixed at 3 d.p.i. with glutaraldehyde, and processed for EM. (A) Two adjacent neuronal cell bodies containing cytoplasmic fuzzy nucleocapsids (MV) but few buds at the cell surface. Arrows indicate intact cell membranes separating the two cells. Magnification x 7,560. Bar = 2 um. (B) Higher magnification of infected neuron shows smooth nucleocapsid alignment at the cell surface, but only at the immature stage of budding. Magnification x 96,600. Bar = 200 nm. Closed large arrows, cross-sectional view of nucleocapsid; open large arrow, longitudinal view of nucleocapsid; N, nucleus; M, mitochondria; R with small arrows, ribosomes

From: Measles Virus Spread between Neurons Requires Cell Contact but Not CD46 Expression, Syncytium Formation, or Extracellular Virus Production. DMP Lawrence et al. J Virol 2000

This dramatic change in the mechanism of viral spread in the brain as opposed to a fibroblast environment may be due to a property of the neurons rather than to the accumulation of viral mutations. The paucity of neuronal cell death following MV infection suggests that this may be a protective strategy by the neuron to safeguard itself against cytolytic MV replication (19).

Although RNA viruses typically have high mutation rates, the MV is thought to be an antigenically monotypic virus. Neutralising epitopes on the haemagglutinin protein are highly conserved and this fortuitously means that attenuated measles vaccines that were developed decades ago from a single MV genotype remain protective worldwide today (14).

Measles is a highly contagious, directly transmitted pathogen and spreads via contaminated respiratory droplets. Outbreaks can occur in populations in which fewer than 10% of people are susceptible (20). Eradication is possible though as latent measles virus infections do not result in prolonged contagiousness and there are no animal reservoirs that maintain virus transmission; MV infection can therefore only be maintained by an unbroken chain of acute infections (14).

Adaptive cellular immunity is generally regarded as most important for the clearance of MV as patients with agammaglobulinemia recover from infection, while those with defects in cellular immunity (e.g. HIV infection, congenital immune deficiency, transplant immune suppression) are prone to develop severe disease (15). The antiviral immune response is effective in clearing virus and in establishing long-term

resistance to reinfection but is associated with paradoxical immune suppression and increased susceptibility to other (non-measles) antigens for weeks after the rash (21).

Following an incubation period of about 10 days, clinically apparent measles begins with a prodromal illness characterised by fever, cough, coryza, and conjunctivitis. Characteristic Koplik's spots—small white lesions on the buccal mucosa—might be visible during the prodrome. A few days later, the erythematous and maculopapular rash appears, starting on the face and behind the ears and then spreading to the trunk and extremities (14). Complications occur most frequently in the respiratory tract, commonly pneumonia and laryngotracheobronchitis. Diarrhoea and keratoconjunctivitis are other known complications (14).

Although CNS involvement is rare, it is the most common cause of longterm sequelae and is a relatively common cause of death, second only to lower respiratory tract complications (22). Encephalitis was diagnosed in 0.1% of patients reported with measles in the United States of America from 1987–2000 (22). A series from Germany consisting of 96 children hospitalised with measles, found measles encephalitis in two children, both with a fatal outcome (23). An Iraqi hospital based review of measles complications reported encephalitis in 2% of patients, accounting for 16.6% of deaths (24).

The measles virus can affect the CNS in one of four ways: primary measles encephalitis (PME), acute postinfectious measles encephalomyelitis (APME),

subacute measles encephalitis (SME), also termed measles inclusion body encephalitis (MIBE) and subacute sclerosing panencephalitis (SSPE). For the purpose of this dissertation, the term SME is favoured over MIBE as I find it more useful as a clinical descriptor of the illness rather than the histopathological term MIBE.

PME is a direct viral infection of the brain which occurs at the exanthem phase of the disease and affects 1-3 per 1000 patients with measles infection. MV RNA can be isolated from the brain and CSF during this stage. Ten to 15 percent of patients will die, and an additional 25% will be left with permanent neurologic deficits (3).

APME is an immune mediated disorder which does not involve direct viral infection of the brain and occurs in 1 per 1000 measles infections. It is also termed measles-induced acute disseminated encephalomyelitis (ADEM) and is primarily a post-infectious demyelinating disease which occurs during the resolving phase of the exanthem or even a few weeks following the rash (3). The goal of treatment is to dampen the immune response and corticosteroids, intravenous immunoglobulin and/or plasmapheresis have been used with success (3). It should be noted that PME and APME may be part of an overlapping spectrum, as the clinical picture and time to onset of symptoms may be similar. A series of 12 children who presented with neurological symptoms 2-7 days following the measles rash were reported (25). Eight of these patients had abnormal MRI neuroimaging: four demonstrated white matter changes consistent with a demyelinating process while four had predominantly gray matter involvement. Interestingly, the patients with gray matter

changes had a poorer outcome and it is postulated that in these patients direct viral invasion was playing a role (25).

SME is an opportunistic CNS infection in immunocompromised hosts where the MV cannot be contained and presents within months following measles infection. A detailed review of SME follows in **section 2**.

SSPE is another devastating complication of measles infection occurring in approximately one in 10 000 children (14). It primarily affects immunocompetent children with symptoms developing 6-15 years after measles infection (3). The incidence of SSPE inversely correlates with rates of measles vaccination (26). Children who contract measles before the age of 1 year are at greatest risk of later developing the disease. The antibody response to MV is accentuated with significant production of MV-specific antibody in the CSF. Thus, although present, the immune response is ineffective in clearing virus from the CNS. Frequent U to C changes suggest that mutation of viral RNA by adenosine deaminase (biased or A/I hypermutation) is occurring in persistently infected cells. There is a lack of budding of virus and therefore infectious virus cannot usually be recovered from the CNS. This in turn may be caused by mutations that accumulate in the genes for the M, F, and H envelope proteins which interfere with assembly and budding (15). It is not known whether these mutations facilitate spread within the CNS and are necessary for viral persistence or whether they accumulate because of the lack of selective pressure to maintain envelope functions during replication in the CNS, seeing as the virus can spread transsynaptically without production of infectious virus (15).

Initial symptoms of SSPE include behavioural changes and cognitive decline. Within weeks or months these symptoms become more obvious and motor dysfunction develops with typically myoclonic seizures. About half of affected patients will also develop ocular complications in the form of necrotising retinitis (3). There are high titres of measles antibody in the CSF and characteristic long latency periodic complexes on EEG (27). Death usually follows the onset of symptoms within 3 years (28). Treatment with isoprinosine and ribavirin, immunoglobulin therapy and interferons have been tried without sustained benefit (3). The rationale for using alpha-interferon therapy is that the cerebrospinal fluid interferon levels are low in patients with SSPE and exogenous administration of interferon possibly suppresses viral replication and enhances the body's immune system (29). However, an international multicentre study performed by the International Consortium on Subacute Sclerosing Panencephalitis, found no significant differences in the survival rates or morbidity between groups randomized to treatment with either oral isoprinosine (Inosiplex) alone versus combined treatment of isoprinosine and intraventricular interferon-alpha (30).

2. Subacute measles encephalitis

SME is an opportunistic CNS infection in immunocompromised patients infected with measles. It typically presents a few months following the initial infection, although it may be difficult to date accurately as many of these patients do not mount a significant T-cell response and therefore the skin rash may be absent. By 1993, there were 31 patients reported in 16 case reports and summarised in the literature (4). In

short, the findings were: mean age of 6.1 years (range 2-21 years); 70% of patients had a history of clinical measles with a latent period ranging from one to seven months; the most common presentation was seizures and altered mental status with one third of patients fulfilling criteria for epilepsia partialis continua; the majority of CSF analyses were normal; only a few patients had elevated serum and/or CSF measles antibody titres at presentation; EEG was abnormal in all patients tested; CT Brain was normal at presentation; neuropathology demonstrated varying combinations of inflammatory and necrotic changes with intranuclear viral inclusions, tubular structures consistent with paramyxovirus nucleocapsid and PCR positivity; treatment consisted of supportive measures in all and specific antiviral therapy in four patients; outcome was poor with 85% mortality and moderate to severe neurological impairment in survivors. Since this literature review of 1993, at least nine further cases have been described in eight case reports in the English literature. These more recent findings are summarised below (note that this review excludes our subsequent publication about SME in South Africa (36), see Part C: Publication format).

2.1. At risk populations

SME has been described in immunocompromised patients secondary to solid organ transplant (11,12), stem cell transplant (9), haematological malignancy (particularly acute lymphoblastic leukaemia)(4,6), auto-immune disease (ankylosing spondylitis)(10), primary immune deficiencies (5) and HIV infection (4,7).

In one case, SME followed on vaccination in a young boy with a primary immune deficiency where the vaccine strain was sequenced from brain tissue (5). There has been some concern regarding the use of live attenuated vaccines in HIV infected individuals and the American Academy of Paediatrics issued guidelines to withhold administration of the measles vaccine to severely immunosuppressed HIV infected children (as defined by CD4+ lymphocyte counts for different age groups)(31). In South Africa, HIV infected children can be immunised against measles only if they otherwise healthy without symptoms of an opportunistic infection. (32).

There has been a report of SME in an apparently immunocompetent 43 year old man who died a month after neurological presentation and had positive measles histology and PCR on brain biopsy (8). He presented with cognitive slowing, visual loss and headache. He subsequently became obtunded with hemiplegia and myoclonic jerks and demised 36 days after presentation. His CSF showed a lymphocytosis with oligoclonal bands and high CSF and serum measles antibody titres. MRI T2 weighted images showed increased signal in the subcortical and deep white matter of the right occipital and left tempero-occipital lobes. Histological examination confirmed a meningoencephalitis and paramyxovirus was seen on EM with measles RNA present. Analysis of matrix gene sequences did not demonstrate the typical U to C hypermutations and did not resemble the known SSPE virus strains. This is the only reported SME case occurring in an immunocompetent patient and demonstrates the overlapping area between SME and a fulminant form of SSPE. The myoclonic jerks, high titres of measles antibodies in the CSF, white matter changes on MRI and his immunocompetent state, would favour fulminant

SSPE. However, the rapid clinical decline, older age at presentation and histological findings are more typical of SME.

2.2. Clinical presentation

The most common presentation is with focal motor seizures, often continuous (5,6,9–12). Other presentations include hemiparaesthesiae, hemiballismus (7), cortical visual loss (8) and cognitive slowing (6). Myoclonus, choreiform movement (6), hemianopia (10) and hemiplegia (10,12) is described during the course of the illness. Over days to weeks there is an associated decrease in the level of consciousness with seizures responding poorly to anti-epileptic drugs (5,6,9–12). The latent period to presentation could only be ascertained in three patients: two who had a rash one (10) and four (11) months, respectively, prior to presentation and one who was vaccinated eight months prior (and was confirmed to be infected with vaccine strain)(5). The remaining patients had no rash or clear measles ictus.

2.3. Special investigations

2.3.1. Cerebrospinal fluid

Two patients had lymphocytic responses in their CSF (8,9), while all other patients had normal CSF biochemistry and cell counts (5–7,10–12). Measles CSF IgG is often positive, but CSF IgM was weakly positive in only one case (10) and there is only documentation of CSF measles PCR being performed in one patient: this patient tested negative on CSF PCR despite a positive brain PCR result (8).

2.3.2. Neuroimaging

All patients had neuroimaging performed in the form of MRI and although findings are not reported in a standardised way, all MRIs were abnormal at some stage of the disease, albeit not always in the early stages of the disease. Common findings include high signal lesions on T2-weighted images or fluid-attenuated inversion recovery (FLAIR) sequences involving the cortical ribbon (5,6,8–11) with frequent involvement of the thalamus (7,12) and basal ganglia (6,9). In patients where serial imaging was performed, lesions became more widespread over time (9). Contrast enhancement was reported in one patient (10).

2.3.3. EEG

EEG is abnormal in the majority of patients with findings ranging from periodic lateralised epileptiform discharges to diffuse slowing to focal status epilepticus (5,9–11).

2.3.4. Histological investigations

In six of the patients, brain biopsy and/or post-mortem studies of the brain were performed. Common findings were inflammatory changes consistent with a meningoencephalitis, the presence of intranuclear viral inclusions and visualisation of the paramyxovirus nucleocapsid on EM (5–10). Measles specific antibodies reacted with antigen in one case (6), whereas MV RNA was found using PCR technique in two patients' brain biopsy. (5,8).

2.4. Treatment

Patients were treated with varying combinations of anti-epileptic drugs, corticosteroids, Aciclovir, intravenous Immunoglobulin and Ribavirin. Two cases were reported of children with acute lymphoblastic leukaemia, aged three and four years respectively, who were given intrathecal interferon therapy (33,34). In both cases the interferon therapy halted progression, but the first patient died a month later from a leukaemia relapse while the second child was left with severe disability.

2.5. Outcome

The outcome was documented for seven of the nine patients. The outcome was fatal in six patients (5,7–10) with the remaining patient being stable but severely impaired in a vegetative state (6).

3. The pathogenesis of SME in the context of the measles outbreak in South Africa in 2009/2010

The most detailed molecular virological description of SME in HIV infected patients comes from the Department of Virology at Groote Schuur Hospital, University of Cape Town (35). This description stemmed from our series of patients through collaboration with Dr Diana Hardie at the Department of Virology.

The most recent measles outbreak in South Africa was from 2009-2011 with 18 699 deaths reported (36). A mass vaccination campaign from April to May 2010 resulted in a significant decline in new measles cases (37). See ***Figure 3*****.** This is part of a worrying trend of measles outbreaks in Africa. In 2008, only two countries had more than 1000 reported measles cases, but in 2010 a total of 16 countries had in excess

of this number. See ***Figure 4***. South Africa was second only to Malawi in terms of numbers of reported cases with 24 393 affected patients (14).

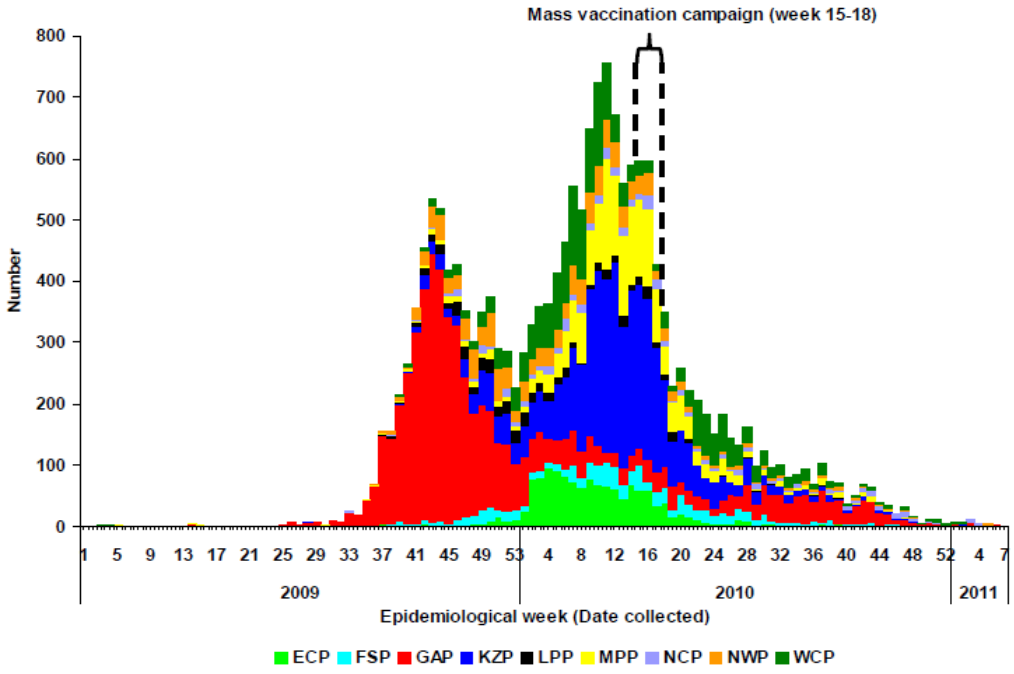

Figure 3: Measles IgM positive results per province: South Africa, January 2009 - 8 February 2011

From: Communicable Disease Communique, February 2011

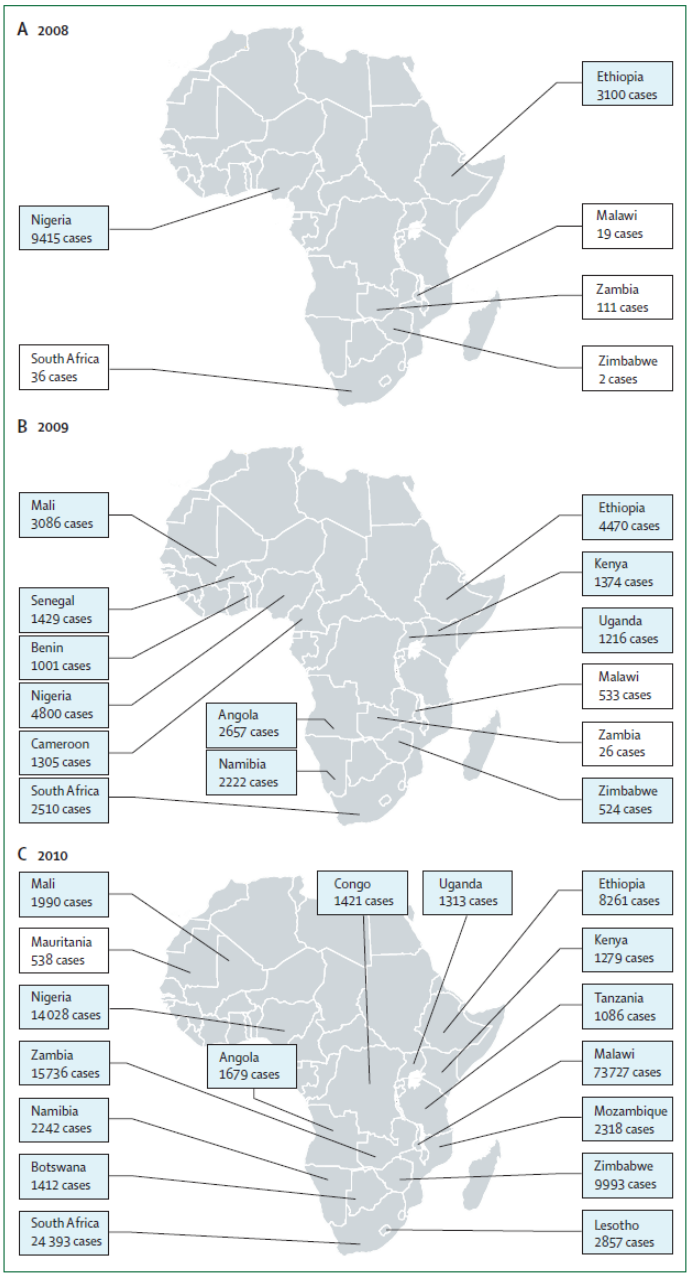

Figure 4: Confirmed cases of measles reported to WHO from countries that participated in measles surveillance in Africa in 2008, 2009, and 2010.
Countries with more than 1000 cases of measles reported to the WHO from 46 countries that participated in measles surveillance in Africa in 2008, 2009, and 2010 are shown in blue. Reported measles cases include cases confirmed clinically, by laboratory testing, or by epidemiological linkage. Countries with fewer than 1000 confirmed cases of measles reported to the WHO in 2008 and 2009 that had more than 5000 confirmed cases of measles in 2010 are shown in white.

From: Measles. Moss WJ et al. Lancet. 2012 Jan 14; 379(9811):153–64

Measles virus does not usually replicate extensively in brain tissue and it is thought that key mutations are required to confer a neurovirulent phenotype (38). The frequent mutations found in SSPE virus makes it difficult to discern whether these

mutations are a consequence of mutations accumulating in genes which are no longer essential for virus replication in the brain or whether they are in fact responsible for neurotropism. By studying the fewer mutations in virus from SME brains it may be possible to determine which were responsible for the gain in neurotropism and learn more about the pathogenesis of the disorder. Therefore, to characterize the brain virus, nucleoprotein, matrix, fusion and haemagglutinin genes from 4 cases of MIBE were compared with virus from acutely infected patients. The brain virus was very similar to the acute epidemic virus (genotype B3) with mutation rates in brain of 0.87 per 1000 bases compared to the epidemic virus of 0.56 per 1000 bases. Most of the mutations in the brain virus were different for each patient. Interestingly, one point mutation in the fusion protein (L454W) was present in 2 patients. This region is thought to interact with an as yet unknown measles virus receptor in the brain and play a role in the fusion process (35).

In measles virus from persistently infected brain the nucleoprotein gene typically retains its function as this protein is required to form intact ribonuclear protein complexes to enable the virus to move from cell to cell in the brain. The matrix protein, on the other hand, is usually highly mutated as this protein is not needed for replication in the brain (39). This was not seen in the four SME patients and may be because sufficient time had not elapsed for mutations to accumulate in this gene before the clinical presentation of MIBE in our highly immunocompromised patients.

It is probable that host factors were largely responsible for driving the disease

process. Clearly, as not all severely immunocompromised HIV patients infected with measles developed MIBE, viral factors must have played a role (35).

Identification of gaps and needs for further research.

There is agreement regarding the need for urgency relating to vaccination programmes, which is by far the most effective way of preventing measles related complications. This is of particular importance in South Africa with the large burden of HIV infection with many vulnerable patients. There remains some uncertainty regarding the overlap between "fulminant SSPE" and SME and how this might be differentiated clinically and histologically. Treatment of SME is an unmet need. Although there are case reports of Ribavirin being used, outcomes remain poor with death or severe neurological impairment in survivors. In light of the novel findings from the MIBE molecular viral study relating to our patients (35), further research needs to be done to identify host and viral factors that predispose to SME in this highly contagious virus.

Our case series involving eight patients with SME (40) was published in the South African Medical Journal in May 2011 and this manuscript follows in Part C.

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PART C: PUBLICATION READY FORMAT

1. Silent casualties from the measles outbreak in South Africa

ISSUES IN PUBLIC HEALTH

Silent casualties from the measles outbreak in South Africa

Christine Albertyn, Helen van der Plas, Diana Hardie, Sally Candy, Tamiwe Tomoka, Edward B LeePan, Jeannine M Heckmann

South Africa, home to the world's largest population of people living with HIV (5.7 million), experienced a measles outbreak that started in late 2009.[1] There was a stepped increase in cases of measles, with the highest incidence reported in March 2010.[2] By September 2010, more than 17 000 new measles cases had been reported to the National Institute of Communicable Diseases since January 2009. A mass vaccination campaign from mid-April to early May 2010 resulted in a significant decline in new measles cases.

The measles virus is highly contagious, and outbreaks are fuelled by overcrowding and poor vaccine coverage, making elimination status in South Africa difficult to attain. Measles may infect the central nervous system (CNS) as acute viral encephalitis, or result after 2 - 4 weeks in a post-infectious immune-mediated inflammatory disorder or acute disseminated encephalomyelitis (ADEM). There are 2 further rare and latent CNS complications resulting from a preceding measles infection: subacute sclerosing panencephalitis (SSPE) caused by years of viral persistence in a seemingly immunocompetent host,[3] and subacute measles encephalitis (SME), occurring in an immunocompromised host.[4]

SME manifests 1 - 7 months after the acute measles infection.[5] Patients present with seizures, often epilepsy partialis continua, and altered mental status.[5] It carries a mortality rate of 85% and survivors often have significant psychomotor retardation.[5] SME has hitherto only been described in single case reports as a rare complication of measles in the context of organ transplantation,[6,7] immunosuppressive therapy or immunodeficiencies,[5,8] and HIV and AIDS.[5,9,10] We report 8 cases of SME in HIV-infected patients who presented to a tertiary referral hospital between July and October 2010.

Case reports

The index case (Patient 1), known to be HIV-positive, presented with a 2-week history of focal twitching and clumsiness of the right hand and secondary generalised seizures on 3 occasions. Three months earlier, she contracted measles complicated by pneumonitis (Table I). She was unable to recall ever receiving measles vaccination. She recovered and commenced antiretroviral treatment (ART) 4 weeks later as well as co-trimoxazole prophylaxis. Her nadir CD4 cell count was 67 cells/μl. She had never travelled and did not abuse illicit substances.

Other than the epilepsy partialis continua (focal motor status epilepticus) of the right hand with dystonic posturing of the left foot and intermittent leg twitching, she was initially relatively well. She reported no headache, and on examination was afebrile, co-operative and not encephalopathic. Serum biochemistry including glucose was normal. She had evidence of immune reconstitution with a CD4 count of 286 and an undetectable serum HIV viral load. Cerebrospinal fluid (CSF) was acellular with normal biochemistry and negative tests for neurosyphylis and cryptococcal antigen. CSF cultures for bacteria, fungi and tuberculosis were negative. CSF polymerase chain reaction (PCR) tests were repeatedly negative for herpes simplex virus 1 and 2, cytomegalovirus, JC virus, Epstein Barr virus, herpesvirus 6, fungi, toxoplasmosis, enteroviruses and measles. The HIV viral load in the CSF was undetectable. The electroencephalogram (EEG) showed left periodic lateralised epileptiform discharges (PLEDs). Brain imaging showed no abnormalities, but there was subtle non-enhancing left frontal cortical hyperintensity on the TR sequences of the initial MRI (week 3 of SME).

The patient deteriorated rapidly over the following weeks from being independent to requiring assistance with walking and feeding. Her vision deteriorated and she developed dilated, sluggishly reactive pupils. There was no evidence of corneal or retinal involvement and no papiloedema. A repeat brain MRI showed contiguous spread of the left frontal lesion (Fig. 1) and additional multifocal T2-hyperintensities, but again limited to the cortical ribbon. A brain biopsy revealed non-necrotising encephalitis with eosinophilic inclusions, suggestive of viral aetiology. Measles PCR on brain tissue was positive, confirming SME. The seizures were finally controlled with a combination of valproate, levitiracetam and clonazepam. Despite commencement on oral ribavirin, her level of consciousness deteriorated. She is currently mute and unresponsive.

We identified a further 7 patients with either definite or probable SME. We classified as 'definite SME' a suggestive clinical picture (focal seizures and/or encephalopathy) and a positive measles PCR in either brain biopsy, CSF or urine. 'Probable SME' was diagnosed in the context of a suggestive clinical picture, normal CSF findings in the absence of other identifiable pathogens, and with supportive features such as MRI demonstrating multifocal grey matter signal abnormalities and/or positive CSF measles serology (IgG).

Table I shows the clinical characteristics of the 8 patients with definite and probable SME (N=3). The median age was 28 years (range 14 - 38); all were HIV-positive (newly diagnosed in 3); one was known to be on ART at the time of measles infection. The median CD4+ count at presentation was 37 (range 1 - 268); 4 had a history of a morbiliform rash, and the median time to presentation with SME following rash was 12.5 weeks. All patients developed seizures, but 3 presented with focal motor status or epilepsia partialis continua. Blindness and deafness constituted the other modes of presentation. Patient 2 developed hearing loss a few weeks after being immunised against measles as part of the nationwide measles campaign (his HIV status unknown at the time). Subsequent genotyping of the

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Table I. Characteristics of subjects at presentation of subacute measles encephalitis (SME)

	Pt 1	Pt 2	Pt 3*	Pt 4	Pt 5*	Pt 6	Pt 7*	Pt 8
Age (years)	27	14	29	25	32	34	24	38
Gender	F	M	F	M	F	F	F	M
HIV status known	Y	N	N	N	Y	Y	Y	Y
CD4 count (cells/μl)								
Presentation	268	1	66	11	47	26	11	225
CD4 nadir†	65	-	-	-	15	26	11	148
Measles infection								
History of rash	+	+	-	-	-	+	+	-
History of pneumonitis	+	+	-	-	+	-	-	-
On ART	N	N	N	N	UK	Y	N	Y‡
Period of clinical latency (weeks)	15	10	UK	UK	UK	3	16	UK
Presenting SME symptom								
Focal motor seizure	+				+	+		
Hearing loss		+				+		
Visual loss				+				+
Generalised motor seizure			+				+	+
SME symptomatology								
EPC/generalised seizure	+/+	+/+	+/-	+/-	+/-	+/+	+/+	-/+
Blindness	+	-	-	+	+	--	-	+
Encephalopathy	+	+	+	+	+	+	+	-
Outcome								
Follow-up time (weeks)	12	4	2.5	2	6	3.5	1.5	9
Death	NA	Y	Y	Y	Y	Y	Y	NA

*Refers to probable cases, the remainder are definite cases (see text).

†If known.

‡Patient on ART for 1 year preceding presentation.
M = male; F = female; Y = yes; N = no; ART = antiretroviral therapy; UK = unknown; EPC = epilepsy partialis continua; NA = not applicable.

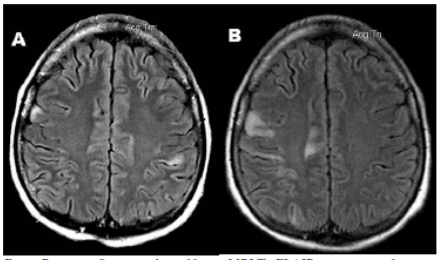

Fig. 1. Patient 1: Sequential axial brain MRI T2 FLAIR images 2 weeks apart showing contiguous extension of the left frontal hyperintense lesion.

measles virus detected in his CSF revealed wild-type measles and not the vaccine strain. SME was fatal in 6 patients, and the mean time between onset of SME and death was 21 days. The clinical and radiological picture of patient 8, who may have been on ART at the time of measles infection, appears to have stabilised prior to the initiation of ribavirin between 9 and 10 weeks. He presently has normal higher mental functions and seizures are controlled on valproate.

The laboratory and radiological investigations are shown in Table II. Routine CSF examinations were non-contributory. The MRI appearance was remarkably similar in the 7 patients scanned: T2-signal abnormality in a distinctive patchy cortical distribution, but in some also involving the deep basal ganglia consistent with a polioencephalopathy. White-matter abnormalities were variable and infrequent.

Discussion

This is the largest SME case series to date – and, alarmingly, collected over 4 months. All patients were HIV-positive and, with one exception, had CD4 counts <100 cells/μl at the time of measles infection, consistent with SME as an opportunistic infection in an immunocompromised host.[6] The measles virus enters the CNS with the initial viraemia, but it is thought that a poor cell-mediated immune response allows virus to persist in this compartment with the potential for SME.[6] Interestingly, the 2 survivors were those with the least compromised CD4 counts; one had reconstituted on ART and the other was the least affected clinically. In immunosuppressed animals infected with the measles virus, the repopulation of lymphocytes was associated with the elimination of viraemia,[6] suggesting that immune reconstitution in HIV-infected patients may enable a more robust immune response. Furthermore, human leukocyte antigen (HLA) polymorphisms influence measles antibody responses, which may be a modifying factor influencing susceptibility to this measles complication.[11]

Focal seizures are well described in SME although the mechanism by which the measles virus induces epileptogenicity is not clear. When neurotropic measles virus is inoculated into mouse brain,

Table II. Laboratory, pathology and radiological investigations in subjects

	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7	Pt 8
CSF	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Measles antibodies								
Serum IgG	Pos	Neg	Pos	Pos	Pos	ND	ND	Pos
CSF IgG	Neg	Neg	Pos	ND	Neg	ND	Neg	Pos
Measles PCR								
Urine	ND	ND	ND	Pos	Neg	Neg	ND	Pos
CSF	Neg	Pos	Neg	Pos	Neg	Neg	Neg	Neg
Brain biopsy	Pos	ND	ND*,†	ND	ND	Pos*	Neg*	ND
EEG	PLEDS	PLEDS	PLEDS	Slowing	Slowing	PLEDS	Slowing	Normal
CT brain	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Abnormal
Location of MRI signal abnormalities								
Superficial grey matter	+	+	+	+	+	+	ND	+
Deep grey matter	-	+	+	-	-	-		-
White matter	-	+	-	-	+	-		-
Histopathology	INI	ND	INI	ND	ND	INI	INI	ND

*Post-mortem brain biopsy.
†PCR failed on formalin fixed tissue.
Pos = positive; Neg = negative; ND = not done; PLEDS = periodic lateralised epileptiform discharges; INI = intranuclear and cytoplasmic inclusion bodies.

neuroglial excitotoxicity (probably via N-methyl D-aspartate (NMDA)-receptor signaling) preceded neuronal hyperexcitability resulting in intermittent and later continuous seizures, followed by neuronal cell loss.[12]

Acute onset visual loss and deafness have been infrequently reported in SME; however, sensorineural hearing loss,[13] keratitis and corneal scarring resulting in blindness, particularly in the presence of vitamin A deficiency, are known complications of acute measles.[13] Preferential involvement of occipital, thalamic and putaminal areas occur in SSPE.[14] In our patients with visual impairment, there was clinical evidence of anterior visual pathway involvement (abnormal pupillary responses) and, on MRI, posterior (occipital and/or temporal lobe) visual pathway involvement (Fig. 2). The recognition of visual and hearing loss may be important in considering a diagnosis of SME.

SME is an elusive diagnosis. The preceding measles rash may be subtle or absent in an immunocompromised host.[4] The CSF examination may be normal, and specific measles testing using antibodies and more sensitive PCR may be negative. Absence of measles virus DNA in the CSF is probably due to the intraneuronal location of the measles virus and, unlike non-neuronal cells, viral budding and shedding does not occur in the CNS.[15] Further, in the CNS, measles viral transmission occurs via trans-synaptic neuronal spread,[15] which may explain the contiguous cortical spread on sequential MRI brain images (Figs 1a, b). Therefore, in the absence of finding evidence of ongoing measles virus infection in the CSF, a definitive diagnosis would require brain biopsy.

Two outbreaks of measles have occurred in South Africa in the last decade, with one still ongoing.[16] Failure to vaccinate a critical percentage of the population is the probable cause. Vaccine effectiveness may be lowered in HIV infection, but the population vaccine effectiveness remained high,[16] underscoring the importance of immunisation programmes in an HIV-endemic population.

The fatal intersection of HIV and measles has resulted in a cluster of patients in South Africa with subacute measles encephalitis,

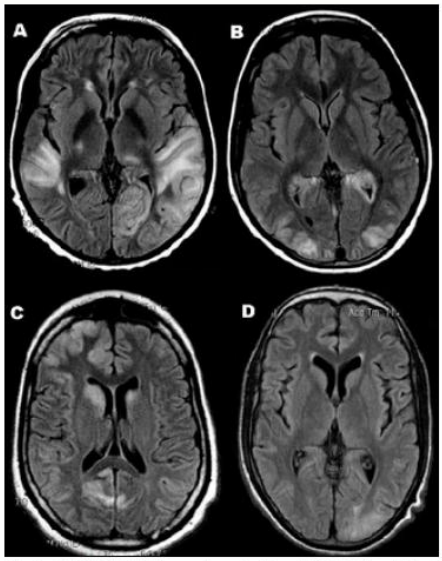

Fig. 2. Axial T2 FLAIR images demonstrating: A (Patient 6) – bilateral temporal-parietal cortical hyperintensities; B (Patient 2) – parieto-occipital cortical hyperintensities; C (Patient 3) – superficial cortical (left frontal and bilateral occipital) and deep grey matter (bilateral head of caudate) hyperintense signal abnormalities; and D (Patient 8) – hyperintense signal changes in the right occipital cortex.

many of whom might have died before reaching medical attention, which serves to remind clinicians of the importance of a vigilant and effective measles immunisation programme. By reporting these patients who present with focal seizures, often with hearing and visual loss and with evidence of a polioencephalopathy on MRI, we aim to raise awareness of this devastating complication. Although insensitive, we propose the use of measles virus PCR in CSF and/or urine as a time- and cost-effective way of confirming the diagnosis of SME in the absence of a brain biospy.

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2. Molecular characterisation of virus in the brains of patients with measles inclusion body encephalitis (MIBE)

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Molecular characterisation of virus in the brains of patients with measles inclusion body encephalitis (MIBE)

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Abstract

Background: During 2009/10 a major measles epidemic caused by genotype B3 occurred in South Africa. Measles inclusion body encephalitis (MIBE) was diagnosed in a number of highly immuno-compromised HIV patients. The diagnosis was based on typical clinical and MRI findings and positive measles virus PCR in brain or CSF.
To characterize the brain virus, nucleoprotein, matrix, fusion and haemagglutinin genes from 4 cases was compared with virus from acutely infected patients.

Methods: cDNA was synthesized using random primers and viral genes were amplified by nested RT-PCR. PCR products were sequenced in the forward and reverse direction and a contig of each gene was created. Sequences were aligned with reference sequences from GenBank and other local sequences.

Results: Brain virus was very similar to the South African epidemic virus. Features characteristic of persistent measles virus in the brain were absent. Mutation frequency in brain virus was similar to epidemic virus and had the same substitution preference (U to C and C to U). The virus of 2 patients had the same L454W mutation in the fusion protein.

Conclusion: The brain virus was very similar to the epidemic strain. The relatively few mutations probably reflect the short time from infection to brain disease in these highly immuno-compromised patients.

Keywords: Measles inclusion body encephalitis, MIBE, Subacute measles encephalitis, Neuro-virulence, Mutation, Immuno-compromised, Human immunodeficiency virus

Background

During 2009 and 2010 a widespread measles virus epidemic occurred in South Africa. More than 18 000 cases were laboratory confirmed. The majority of infections were in young people. One third were infants less than one year of age and the rest were between one and 40 years [1]. This outbreak occurred in a population with a very high HIV prevalence. South Africa has an average HIV prevalence of 30.2% in women attending antenatal clinics and an estimated prevalence in all adult South Africans of 17.9% (15–49 years) [2]. During the course of this epidemic, a high rate of complications was seen in HIV-infected subjects and a number of patients developed a distinct neurological syndrome, confirmed to be measles inclusion body encephalitis (MIBE). The first 8 confirmed cases [3] were all young (under 40 years), HIV-infected and had low CD4 counts. Most gave a history of measles in the preceding weeks. MIBE was not identified in any HIV- negative individuals during this epidemic.

There are three neurological complications following acute measles infection. Within 2 weeks of the onset of the rash, an acute demyelinating encephalomyelitis (ADEM) may develop. This is an auto-immune phenomenon as measles virus is not present in the brain [4]. Measles inclusion body encephalitis, also termed subacute measles encephalitis, typically occurs one to nine months after acute measles infection in highly immuno-compromised individuals, either as a result of HIV infection or haematological malignancies [5,6]. Sub-acute sclerosing pan encephalitis (SSPE) typically occurs in apparently immuno-competent

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persons, but symptoms develop only after a prolonged latent period and the virus from brain tissue is highly defective. In both MIBE and SSPE the brain is the site of ongoing measles pathology. The virus evades immune defenses by spreading from cell to cell within the brain [7]. Key viral structural genes (matrix, fusion and haemagglutinin) are highly mutated, rendering the proteins nonfunctional [8]. We were interested to investigate the virus from brains of local MIBE cases to determine whether the virus was similarly defective and whether there were any common features which could explain the pathogenesis of the condition. Accordingly, the nucleoprotein (N), matrix (M), fusion (F) and haemagglutinin (H) genes from 4 MIBE cases (see Table 1 for clinical details) were compared with virus from patients acutely infected during this epidemic.

Results

Complete sequence data for N, M, F and H genes was obtained for brain virus of 2 patients (1 and 3) and epidemic virus from 6 patients (GenBank accession numbers KC305651-KC305689). Incomplete sequence data was obtained from brain virus of a further 2 patients (2 and 4). Partial sequence was due to limited clinical material available for PCR amplification. For patient 4, most of the H gene sequence was available (nucleotides 246–1874) and partial sequence for the F gene (nucleotide 1–1690) and for patient 2, the complete N, M and F genes were sequenced.

Phylogenetic analysis of epidemic virus

Phylogenetic analysis of the nucleoprotein and haemagglutinin genes confirmed that the South African epidemic in 2009/10 was caused by genotype B3. (Figures 1, 2) The South African sequences formed a distinct cluster within genotype B3.1 and this held true in phylogenetic trees of the F and M genes as well (Figure 3A and B). Brain and acute virus sequences clustered closely together on all phylogenetic trees (Figures 1, 2 and 3).

BLAST analysis of the South African consensus M and F genes showed that the epidemic virus was most closely related to JN635408, a 2005 isolate from New Jersey, USA. The consensus N and H genes were more closely related to 1998 measles virus isolates from Nigeria (AJ232781 and AJ 239171). A concatenated phylogenetic tree of the full N, M, F and H genes confirmed a close relationship of the South African measles viruses to B3 viruses from North Africa (Figure 4).

There was very little sequence variation in the epidemic virus. A total of 24 polymorphisms were present in the six acutely infected patients over the 6.728 Kb region sequenced (giving a mutation frequency of 0.56 per 1000 nucleotides).

Sequence analysis of brain virus

Sequences from the acute epidemic virus were aligned and used to create a South African consensus sequence to which the brain virus of patients 2, 3 and 4 were compared. The brain virus from patient 1 was compared with her own acute blood virus from 3 months earlier. In all patients the brain virus was very similar to the epidemic virus. Typical features of measles virus of SSPE/MIBE cases were not present, namely hyper mutation of the matrix and fusion proteins or truncation of C terminal end of fusion protein gene [9]. Each MIBE patient had a unique pattern of mutations in one or more of the N, M, F and H genes (Table 2). In total (for all genes sequenced) there were 18 polymorphisms present in brain virus relative to the South African consensus sequence, (Table 2).

U to C and C to U mutations were the most common mutations present in the epidemic virus and were also the most frequent in the brain virus. The calculated mutation frequency in brain virus was 0.87 per 1000 nucleotides. This was not significantly different from the mutation frequency of the epidemic virus which was 0.56 per 1000 nucleotides (p=0.19). Interestingly, of the 10 substitutions that occurred in coding regions in the brain virus, 8 were non-synonymous. The most variable region was the non coding region of the fusion gene.

Patients 1 and 3 had an identical U to G mutation at position 1944 in the F gene which gave rise to a 1454W substitution in the fusion protein. Of note, the 1454W substitution was not present in the blood virus of patient 1 collected during acute measles infection 3 months earlier.

Table 1 Details of study patients

	Patient 1	Patient 2	Patient 3	Patient 4
Age	26	14	34	24
Sex	F	M	F	F
CD4 count	66	?	26	11
Acute measles prior to disease onset	3 months	Unknown (no rash)	3 weeks	3 months
Sample type analysed	Brain	CSF	Brain	Brain
Vaccine history	Unknown	Received measles vaccine 4 months previously*	Unknown	Unknown

*Outbreak strain

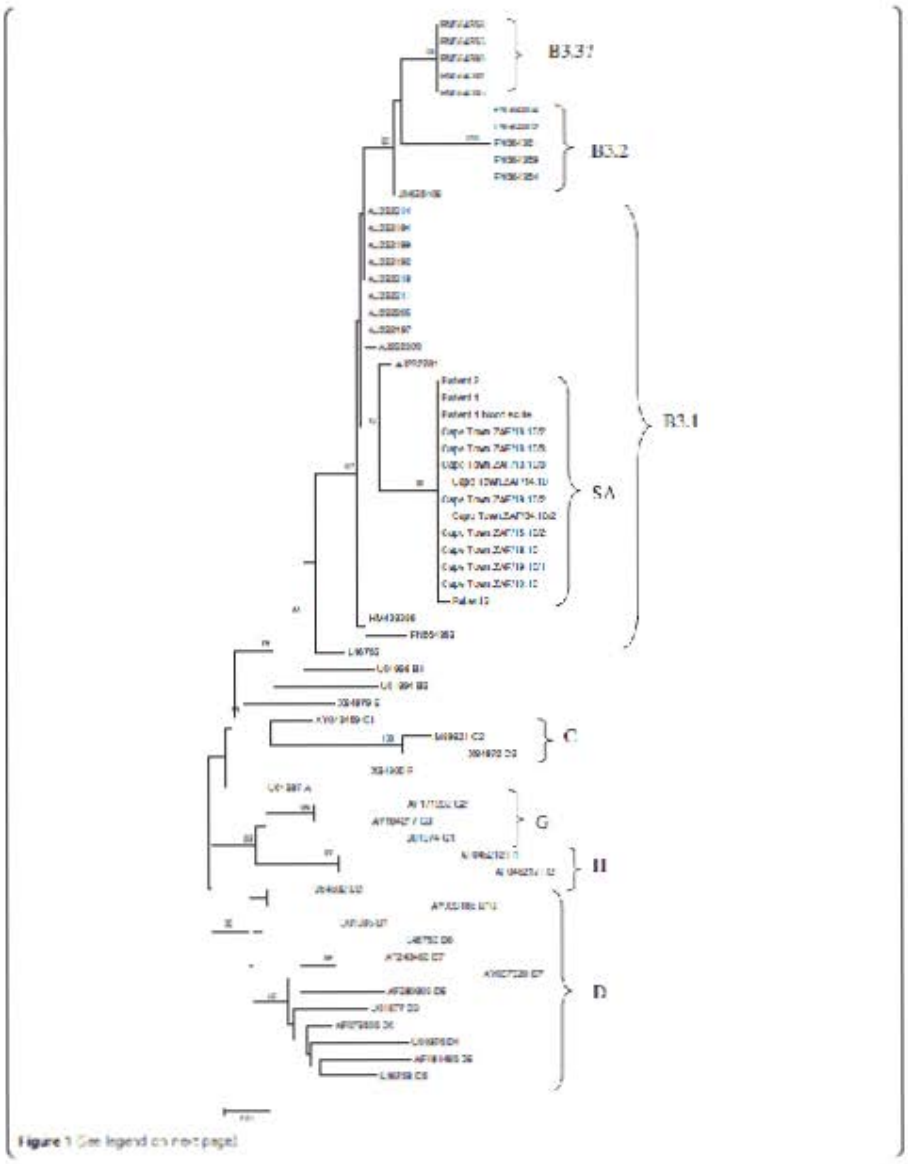

Figure 1 See legend on next page.

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Figure 1 Phylogenetic tree generated by neighbour-joining analysis of the 3' hypervariable region of the nucleoprotein gene of measles virus from patients with acute measles infection and MIBE during the measles epidemic of 2009–2010 in South Africa. Reference sequences of other measles virus genotypes were retrieved from the NCBI GenBank database and are indicated by accession numbers. Bootstrap values greater than 75% are indicated at the nodes of the tree. The branch lengths are proportional to the evolutionary distances as shown on the scale.

Discussion

The measles virus outbreak in South Africa was due to genotype B3 a known African genotype. It was probably introduced into South Africa from the north where ongoing circulation of measles virus of this genotype has been documented in various African countries for many years [10,11]. Infection spread widely in the South African population due to poor herd immunity. Analysis of measles virus sequences from acutely infected individuals showed that there was a low level of genetic variability in the epidemic virus. This is typical of a single source introduction which is followed by dissemination in a non immune population [12,13].

While invasion of the brain during acute measles may occur [14], MIBE is normally very rare. Most highly immuno-compromised patients who had acute measles in this epidemic did not develop MIBE. Clearly both viral and host factors play a role in the disease process. While much is known about the genetic characteristics of SSPE virus, this is not the case for MIBE virus. We were interested to sequence the brain virus in some of these cases to determine its similarity to the epidemic virus in order to try to gain insight into the pathogenesis of this normally very rare condition.

On the whole, the brain virus was very similar to the acute epidemic virus. This is in contrast to what is found with SSPE [9,15] and also what has previously been reported for MIBE [15]. Mutation rates were similar in brain (0.87 per 1000 bases) to the epidemic virus (0.56 per 1000 bases). However, of the 10 substitutions that occurred in the coding regions of genes from brain virus, 8 were non-synonymous (in comparison to only 4 of 22 from the epidemic virus). This could imply either that some selection pressure was acting on the virus in the brain or that a mutation generating process was operating.

Measles virus does not usually replicate extensively in brain tissue and it is thought that key mutations may be needed to confer a neuro-virulent phenotype [16,17]. Because of the many mutations found in SSPE virus, it is difficult to determine which are responsible for neuro-tropism and which are merely the consequence of mutations accumulating in genes which are no longer essential for virus replication in the brain. By studying the fewer mutations in virus from MIBE brains it may be possible to determine which were responsible for the gain in neuro-tropism. In this study, most of the mutations in the brain virus were different for each patient and targeted different genes. However, one point mutation in the fusion protein, namely L454W was present in 2 patients (1 and 3). It is unlikely to have been a chance polymorphism in some circulating virus because it was not present in the acute measles sequence of patient 1. Both leucine and tryptophan are neutral, non-polar amino acids. However, this substitution is not favoured, especially in a membrane protein, and the substitution would be likely to change the properties of the protein [18]. This substitution falls in the extra-cellular domain of the fusion protein, adjacent to the heptad repeat B domain (HRB). Ayata *et al.* [17] showed that a single substitution (T461I) was responsible for neuro-virulence of an SSPE strain in a hamster model. This region is thought to interact with the as yet unknown measles virus receptor in the brain and plays a role in the fusion process. The independent presence of this mutation in 2 patients is interesting.

There were remarkably few mutations in all four of the genes sequenced from the brain virus. In measles virus from persistently infected brain, both in humans as well as in animal models, the nucleoprotein gene typically retains its function as this protein is required to form intact ribonuclear protein complexes to enable the virus to move from cell to cell in the brain [7]. The matrix protein, on the other hand, is usually highly mutated as this protein is not needed for replication in the brain [19,20]. Perhaps sufficient time had not elapsed for mutations to accumulate in this gene before the clinical presentation of MIBE in our highly immuno-compromised patients. In 3 MIBE patients who gave a history of acute measles, the median time from acute infection to onset of neurological disease was about 10 weeks. In all patients there was a rapid neurological deterioration after presentation, which probably reflects the poor immune control of measles virus in the brain.

In conclusion, it is probable that host factors were largely responsible for driving the disease process. However, not all severely immuno-compromised HIV patients infected with measles developed MIBE. Viral factors also must have played a role. Intriguingly the brain virus was very similar to the epidemic virus and did not show features previously reported to be characteristic of MIBE. The mutation frequency was similar to epidemic virus, but significantly these mutations were more likely to be non synonymous. A key finding was that 2 patients had the same L454W mutation in the fusion protein.

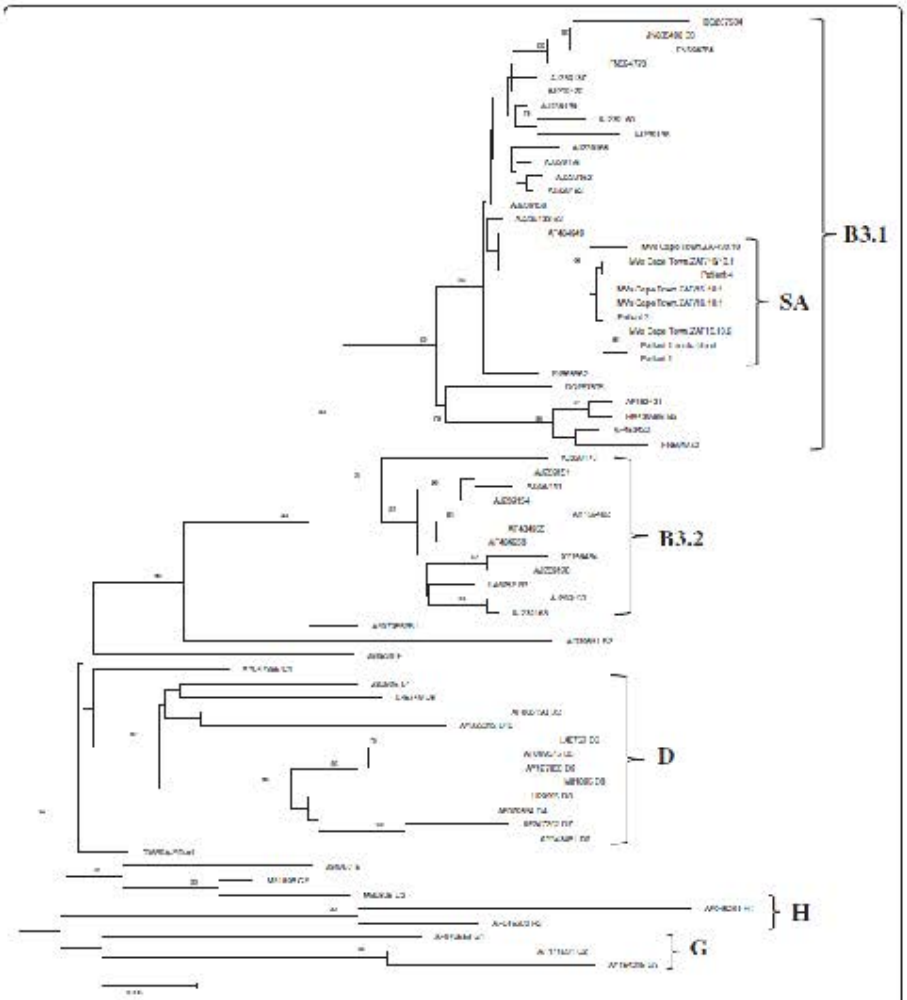

Figure 3 Phylogenetic tree generated by neighbour-joining analysis of the haem-agglutinin gene of measles virus from patients with acute measles infection and MIBE during the measles epidemic of 2009–2010 in South Africa. Reference sequences of other measles virus genotypes were retrieved from the NCBI GenBank database and are indicated by accession numbers. Bootstrap values greater than 70% are indicated at the nodes of the tree. The branch lengths are proportional to the evolutionary distances as shown on the scale.

Materials and methods

MIBE patients

Patients were identified as having MIBE based on typical clinical and magnetic resonance (MRI) findings as previously described [3] and if measles virus was detected by PCR in brain tissue or cerebrospinal fluid (CSF). An in-house diagnostic nested RT-PCR assay was used to amplify a 500 nucleotide fragment of the

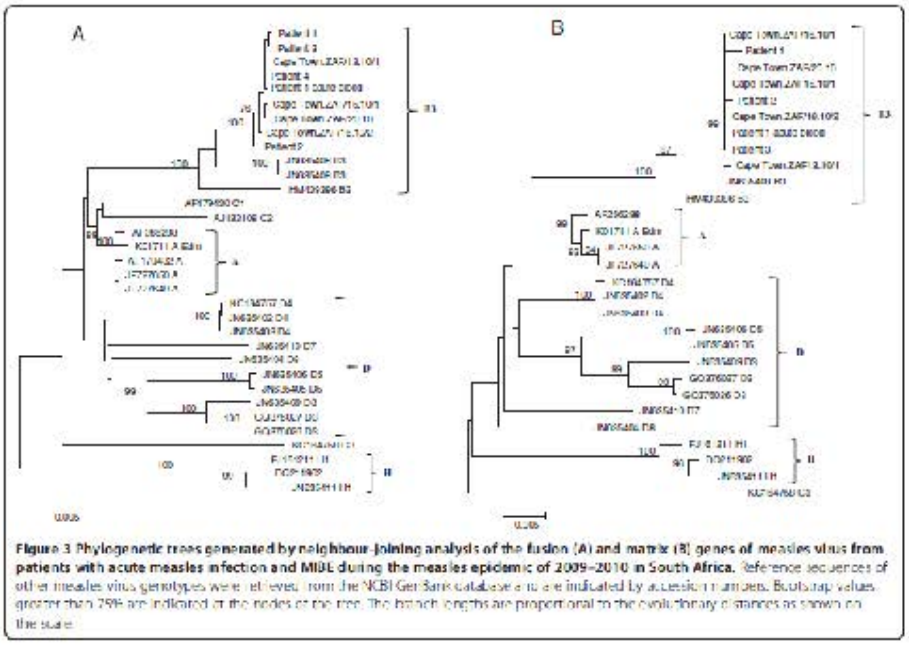

Figure 3 Phylogenetic trees generated by neighbour-joining analysis of the fusion (A) and matrix (B) genes of measles virus from patients with acute measles infection and MIBE during the measles epidemic of 2009–2010 in South Africa. Reference sequences of other measles virus genotypes were retrieved from the NCBI GenBank database and are indicated by accession numbers. Bootstrap values greater than 70% are indicated at the nodes of the tree. The branch lengths are proportional to the evolutionary distances as shown on the scale.

nucleoprotein gene [13]. Measles virus positive brain or CSF was available for study on 4 patients.

Patient 1 was a 27 year old woman who had a CD4 count of 65 at time of acute measles. MIBE onset occurred 3 months after acute measles. Measles virus PCR was positive on brain biopsy tissue. Brain virus was compared with acute virus from blood taken 3 months previously.

Patient 2 was a 14 year old boy with a CD4 count of 1 at time of neurological presentation. There was no history of a rash. This patient had received measles vaccine (Schwartz strain) during a school mass vaccination campaign some months before clinical presentation. Measles virus PCR was positive in CSF.

Patient 3 was a 34 year old woman who developed typical MIBE symptoms 3 weeks after acute measles infection. Her CD4 count at presentation was 26. Measles virus PCR was positive on post mortem brain tissue. Post mortem histology confirmed MIBE.

Patient 4 was a 24 year old woman. MIBE developed 16 weeks after acute measles when the CD4 count was 11. Measles virus PCR was positive on post mortem brain tissue.

Patients with acute measles

Measles virus amplified from a peripheral site such as urine or blood from 5 acutely infected patients was used to create a South African (SA) consensus sequence to which the brain virus was compared.

In addition, measles virus from blood collected at the time of acute measles was available from one patient who subsequently developed MIBE (patient 1). The brain virus of this patient was compared with her own acute virus rather than the consensus sequence.

Nucleic acid extraction

Total nucleic acid for measles virus screening was initially extracted using the Ensymag automated extractor (BioMerieux, Marcy l'Etoile, France) as per manufacturer's instructions. Subsequently total nucleic acid was extracted from the brain or CSF using the manual Qiagen DNA mini kit with appropriate buffers for tissue and CSF extraction as per manufacturer's instructions (Qiagen, GmbH, Germany). Nucleic acid was eluted in 50 ul elution buffer and stored at -80°C until required.

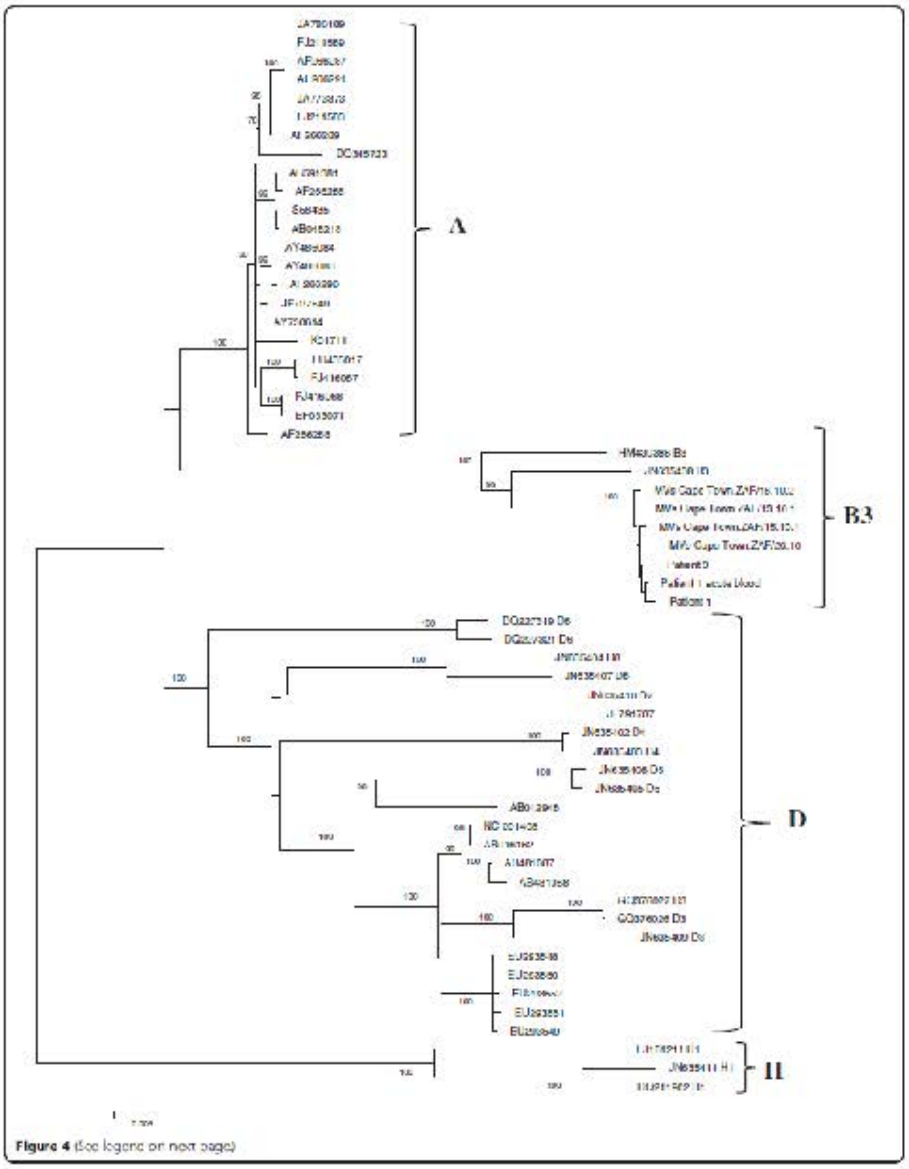

Figure 4 (See legend on next page)

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Figure 4 Phylogenetic tree generated by neighbour-joining analysis of concatenated nucleoprotein, matrix, fusion and haemagglutitin genes of measles virus from patients with acute measles infection and MIBE during the measles epidemic of 2009–2010 in South Africa. Reference sequences of other measles virus genotypes were retrieved from the NCBI GenBank database and are indicated by accession numbers. Bootstrap values greater than 70% are indicated at the nodes of the tree. The branch lengths are proportional to the evolutionary distances as shown on the scale.

cDNA synthesis and PCR

RNA was converted into cDNA using the RevertAid First Strand cDNA synthesis kit (Fermentas Life Sciences) and random hexamers. Briefly 11 μl RNA was incubated with 1 μl random hexamers supplied with kit at 80°C for 3 minutes and then cooled to 37°C before the addition of 4 μl 5× reaction buffer, 1 μl Ribolock RNase inhibitor (20 U/μl), 2 μl 10 mM dNTP mix and 1 μl RevertAid M-MuLV reverse transcriptase (200 U/μl) in a final 20 μl reaction volume. The mixture was incubated at 37°C for 90 min and the reaction terminated by heating to 70°C for 5 minutes. cDNA was stored at −20°C until required.

The complete nucleoprotein (N), matrix (M), fusion (F) and haemagglutinin (H) genes were amplified with primers described by Tillieux *et al.* [21]. In cases where there was no amplification after one round of PCR, nested primers designed for this study were used. (Additional file 1: Table S1).

Measles virus from a patient with acute measles was used as the positive control for all the PCR assays. PCR amplicons were generated using 6μl cDNA and Supertherm Taq DNA polymerase (IMR Holdings, UK) with the following PCR cycling conditions: an initial denaturation step of 3 min at 95°C, followed by 40 cycles of amplification (15 sec at 94°C, 30 s at 50°C and 45 s 72°C) followed by 7 min at 72°C. The nested PCR was performed using the same cycling conditions but with an increase in the annealing temperature to 55°C and 3 μl outer product.

PCR products were electrophoresed through 2% agarose gel and visualized by ethidium bromide staining and UV illumination.

Sequencing and phylogenetic analysis

PCR products were sequenced directly in both directions with primers used for PCR amplification. The BigDye

Table 2 Nucleotide and amino acid differences in the nucleoprotein (N), matrix (M), fusion (F) and haemaglutinin (H) genes between the South African measles consensus B3.1 sequence and brain virus from 4 patients with MIBE

Gene	Nucleotide position	Nucleotide					Amino acid position	Amino acid				
		SACon	Pat1	Pat2	Pat3	Pat4		SACon	Pat1	Pat2	Pat3	Pat4
N	572	A	A	A	**G**	n/d	171	I	I	I	V	n/d
	1622	U	U	U	**C**	n/d	544	L	L	L	P	n/d
M	348	U	C	U	U	n/d	99	I	T	I	I	n/d
	388	U	**C**	U	U	n/d	119	L	P	L	L	n/d
	391	A	A	**U**	A	n/d	123	T	T	S	T	n/d
F	105	C	C	**U**	C	C						
	177	C	C	**G**	C	C						
	162	C	C	**U**	C	C						
	303	C	C	C	C	**U**						
	333	G	G	G	**C**	G						
	331	C	C	**A**	C	C						
	461	G	G	**C**	G	G						
	766	U	U	U	**C**	U						
	1483	G	G	G	G	**A**						
	1583	A	**C**	A	A	A	537	M	I	M	M	M
	1914	U	**G**	U	**G**	n/d	414	L	W	W	W	n/d
H	1367	C	**U**	n/d	C		448	R	C	R	R	R
	1742	C	**U**	n/d	C	C						

SACon South African consensus sequence, *Pat1* patient1, *Pat2* patient2, *Pat3* patient3, *Pat4* patient4, *n/d* not done. Bold font indicates nucleotide that varies from consensus. Nucleotides 105 to 461 in the fusion gene are non coding.

terminator cycle sequencing kit was used (Applied Biosystems, Foster City CA, USA). Sequences were assembled and aligned against the measles virus genotype B3 reference sequence (accession number HM439386) using DNA Baser Sequence Assembler v3.5.0.

The 3′ hypervariable N and Hgenes were aligned with reference sequences from GenBank using ClustalW and neighbor-joining phylogenetic trees constructed using MEGA version 5 with 1000 bootstrap re-samplings [22]. Similar neighbor-joining phylogenetic trees were also constructed for the F and M genes as well as a concatenated tree of all genes sequenced.

Ethical approval

This study was approved by the Human Research Ethics Committee of the University of Cape Town. (HREC REF: 163/2011).

Availability of supporting data

The data supporting the results of this article is included within the article (and its additional file(s). The local measles virus sequences were deposited in GenBank. [Gen Bank: KC305651-KC305689].

Additional file

Additional file 1: Table S1. Primer sequences used in amplification of measles virus nucleocapsid, matrix, fusion and haemaglutinin genes.

Competing interests

None of the authors have any competing interests.

Authors' contributions

DH: conceived the study, analysed the results and wrote the manuscript. JH and CA: identified the cases of MIBE and helped to write the manuscript. HS: designed primers to amplify measles virus genes; assembled contigs of the genes; performed phylogenetic analysis and wrote part of the manuscript. All authors read and approved the final maunscript.

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PART D: APPENDICES

1. Data collection form

Data collection form

Patient number:

Medical folder number:

Age:

Gender:

Medical comorbidities:

HIV status:

Known HIV +ve at presentation: Y/N

On antiretroviral Rx at presentation: Y/N

CD4 nadir:

CD4 at presentation:

History of skin rash or documented measles infection:

Time between skin rash and presentation:

Other opportunistic infection:

Pneumonitis at presentation: Y/N

Presenting symptom:

Seizures? (if so, state if focal or generalised or both):

Visual impairment/blindness?: Y/N

Hearing impairment/deafness?: Y/N

CSF biochemistry and cell count:

CSF measles PCR +/- IgG IgM:

Urine measles PCR: +/-/nd

EEG:

CT Brain:

MRI Brain:

Brain biopsy:

Post-mortem histology:

Outcome:

Time from presentation to death:

2. Patient consent form

Consent form

For a patient's consent to publication of information about them in an International Medical Journal.

Name of person described in article Ms [redacted]

Subject matter of photograph or article: Case report

Title of article: Subacute measles encephalitis

Corresponding author: C. Alberty / J Heckmann

No. [redacted]

I [redacted] [insert full name] give my consent for this information about MYSELF/MY CHILD OR WARD/MY RELATIVE [circle correct description] relating to the subject matter above ("the information") to appear in unknown - lancet? journal.

I understand the following:

(1) The information will be published without my name attached and the author will make every attempt to ensure my anonymity. I understand, however, that complete anonymity cannot be guaranteed. It is possible that somebody somewhere – perhaps, for example, somebody who looked after me if I while was in hospital or a relative – may identify me.

(2) The text of the article will be edited for style, grammar, consistency, and length.

(3) The information may be published in an International Medical Journal which is distributed worldwide. The journal goes mainly to doctors but is seen by many non-doctors, including journalists.

(4) The information may also be placed on the journals website.

(5) I can withdrew my consent at any time before publication, but once the information has been committed to publication ("gone to press") it will not be possible to revoke the consent.

Signed: [redacted] Date: 27/8/10

sister of subject in presence of mother

Name of person taking consent: C. Alberty Date: 27/8/10

Prof J Heckmann also discussed issues with patient + her mother. mm

3. Human Research Ethics Committee approval

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Faculty of Health Sciences Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • Facsimile [021] 406 6411
e-mail: sumayah.ariefdien@uct.ac.za

15 October 2010

HREC REF: 487/2010

A/Prof J Heckmann
Division of Neurology
E8-74
NGSH

Dear A/Prof Heckmann

PROJECT TITLE: SUBACUTE MEASLES ENCEPHALITIS: AN AUDIT OF THE CAPE TOWN EXPERIENCE

Thank you for submitting your study to the Health Science Faculty Research Ethics Committee for review

It is a pleasure to inform you that the Ethics Committee has granted **approval** for a retrospective audit and publication of the original case.

Approval is granted for one year till the 15 October 2011.

Please submit a progress form, using the standardised Annual Report Form (FHS016), if the study continues beyond the approval period. Please submit a Standard Closure form (FHS010)if the study is completed within the approval period.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

sAriefdien

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

sAriefdien