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

Dr Christine Herculine Albertyn, MBChB, FC Neurol (SA)
ALBCHR003

Thesis presented for the degree of Master of Medicine (MMed) in Neurology
June 2014

Supervisor: Associate Professor Jeannine Heckmann, Division of Neurology,
Department of Medicine, University of Cape Town
The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.
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.

I empower the University of Cape Town to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature: __________________________

Date: _______________________________
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 epilepsy 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.
# TABLE OF CONTENTS

DECLARATION ........................................................................................................... 2

ABSTRACT .................................................................................................................. 3

   Introduction ........................................................................................................... 3

   Patients and results .............................................................................................. 3

   Conclusion ............................................................................................................ 4

DEDICATION ............................................................................................................. 7

ACKNOWLEDGEMENTS ............................................................................................ 8

LIST OF FIGURES .................................................................................................... 9

ABBREVIATIONS ..................................................................................................... 10

PART A: PROTOCOL ............................................................................................... 11

   Study protocol ..................................................................................................... 11

      1. Background .................................................................................................. 11

      2. Research justification .................................................................................. 12

      3. Objectives .................................................................................................... 12

      4. Methodology ................................................................................................ 13

      5. Ethics committee approval .......................................................................... 13

      6. Dissemination of findings ............................................................................ 14

   References ............................................................................................................ 14

PART B: STRUCTURED LITERATURE REVIEW ...................................................... 16

   Objectives of literature review .......................................................................... 16

   Literature search strategy ................................................................................... 16

   Quality and relevance criteria ............................................................................ 17

   Summary and interpretation of literature review ............................................. 17
1. Overview of the pathophysiology of measles and how the measles virus affects the Central Nervous System ................................................................. 17
2. Subacute measles encephalitis ................................................................ 25
3. The pathogenesis of SME in the context of the measles outbreak in South Africa in 2009/2010 .................................................................................. 30

Identification of gaps and needs for further research .................................. 34

References ................................................................................................... 34

PART C: PUBLICATION READY FORMAT .................................................. 39

1. Silent casualties from the measles outbreak in South Africa .............. 39
2. Molecular characterisation of virus in the brains of patients with measles inclusion body encephalitis (MIBE) ......................................................... 43

PART D: APPENDICES .................................................................................. 53

1. Data collection form .................................................................................. 53
2. Patient consent form ................................................................................. 55
3. Human Research Ethics Committee approval ........................................ 56
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.
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>The enveloped measles virion demonstrating its six proteins</td>
<td>18</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Incomplete budding at surface of MV-infected neurons</td>
<td>20</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Measles IgM positive results per province: South Africa, January 2009-February 2011</td>
<td>31</td>
</tr>
<tr>
<td>Figure 4</td>
<td>Confirmed cases of measles reported to WHO from countries that participated in measles surveillance in Africa in 2008, 2009, and 2010.</td>
<td>32</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>ADEM</td>
<td>Acute disseminated encephalomyelitis</td>
<td></td>
</tr>
<tr>
<td>APME</td>
<td>Acute postinfectious measles encephalomyelitis</td>
<td></td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
<td></td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
<td></td>
</tr>
<tr>
<td>MIBE</td>
<td>Measles inclusion body encephalitis</td>
<td></td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
<td></td>
</tr>
<tr>
<td>MV</td>
<td>Measles virus</td>
<td></td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
<td></td>
</tr>
<tr>
<td>PME</td>
<td>Primary measles encephalitis</td>
<td></td>
</tr>
<tr>
<td>SLAM</td>
<td>Signalling lymphocyte activation molecule</td>
<td></td>
</tr>
<tr>
<td>SME</td>
<td>Subacute measles encephalitis</td>
<td></td>
</tr>
<tr>
<td>SSPE</td>
<td>Subacute sclerosing panencephalitis</td>
<td></td>
</tr>
</tbody>
</table>
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 epilepsy 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.

References


with AIDS. Neurology. 1996 Feb 1; 46(2):586-7. [http://dx.doi.org/10.1212/WNL.46.2.586], [PMID 8614546]


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

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 epilepsy 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 temporo-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 Communiqué, 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.


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.

**References**


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, Tamivoe 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. There was a steep increase in cases of measles, with the highest incidence reported in March 2010. 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 further rare but lethal 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), 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. It carries a mortality rate of 85% and survivors often have significant psychomotor retardation.6 SME has hitherto only been described in single case reports as a rare complication of measles on the context of organ transplantation,7 immunosuppressive therapy or immunodeficiencies,8,9 and HIV and AIDS.10,11 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 dizziness of the right hand and secondary generalised seizures on 3 occasions. Three months earlier, she contracted measles complicated by pneumonia (Table 1). She was unable to recall ever receiving measles vaccination. She recovered and commenced antiretroviral treatment (ART) 4 weeks later as well as cotrimoxazole prophylaxis. While CD4 count was 67 cells/μL, she had never travelled and did not abuse illicit substances.

Other than the ophthalmal palsies continua (focal motor status epilepticus) of the right hand with rhythmic protrusion of the left foot and intermittent leg twitching, she was initially relatively well. She reported no headaches, and on examination was debilitated, 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 neurosyphilis and cryptococcal antigens. 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, herpesviruses 6, fungi, toxoplasmosis, enteroviruses and measles. The HIV viral load in the CSF was undetectable. The electromyelogram (EMG) showed left peripheral lateralised epificlone discharges (PLDs). Brain imaging showed no abnormalities, but there was subtle non-enhancing left frontal cortical hypointensity on the T1 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 cerebral or retinal involvement and no papilloedema. A repeat brain MRI showed circumferential 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-resecting 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, levetiracetam 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 1 shows the clinical characteristics of the 8 patients with definite and probable SME (N=8). The median age was 28 years (range 14 - 38): all were HIV-positive (nearly 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 - 280); 4 had a history of a multi-drug-resistant, 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 epilepsy 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

Corresponding author: J Heckmann (Jeannine.heckmann@uct.ac.za)

Christine Albertyn, Edward B LeePan and Jeannine M Heckmann are affiliated to the Division of Neurology, Department of Medicine, University of Cape Town; Helen van der Plas to the Division of Infectious Disease and HIV Medicine, Department of Medicine, University of Cape Town; Diana Hardie to the Division of Virology, Department of Clinical Laboratory Sciences, University of Cape Town and the National Health Laboratory Service; Sally Candy to the Department of Radiology, University of Cape Town; and Tamivoe Tomoka to the Division of Anatomical Pathology, Department of Clinical Laboratory Sciences, University of Cape Town and the National Health Laboratory Service.

May 2011, Vol. 101, No. 5 SAMJ
Table I. Characteristics of subjects at presentation of subacute measles encephalitis (SME)

<table>
<thead>
<tr>
<th></th>
<th>Pt 1</th>
<th>Pt 2</th>
<th>Pt 3</th>
<th>Pt 4</th>
<th>Pt 5*</th>
<th>Pt 6</th>
<th>Pt 7*</th>
<th>Pt 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27</td>
<td>28</td>
<td>25</td>
<td>32</td>
<td>54</td>
<td>24</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>HIV status</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>CD4 count (abs/ul)</td>
<td>288</td>
<td>66</td>
<td>11</td>
<td>47</td>
<td>26</td>
<td>11</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>Measles infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of rash</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>History of pneumonitis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>On ART</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Period of clinical latency (weeks)</td>
<td>15</td>
<td>10</td>
<td>UK</td>
<td>UK</td>
<td>UK</td>
<td>3</td>
<td>16</td>
<td>UK</td>
</tr>
<tr>
<td>Presenting SME symptom</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focal motor seizure</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hearing loss</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual loss</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generalized motor seizure</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SME symptomatology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPC/PVARP</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Blindness</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Outcome</td>
<td>Follow-up time (weeks)</td>
<td>12</td>
<td>4</td>
<td>2.5</td>
<td>2</td>
<td>6</td>
<td>3.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Death</td>
<td>NA</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Data are means ± standard deviation.
†Data are counts of patients with symptom.
‡Data are counts of patients with symptom.
§Data are counts of patients with symptom.

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/ul at the time of measles infection, consistent with SME in an immunocompromised host. The measles virus enters the CNS with the initial viremia, but it is thought that a poor cell-mediated immune response allows virus to persist in this compartment with the potential for SME. Interestingly, the 2 survivors were those with the least compromised CD4 counts; one had reconstituted and the other was the least affected clinically. In immunocompromised animals infected with the measles virus, the repopulation of lymphocytes was associated with the diminution of viremia, suggesting that immunomodulation in HIV-infected patients may lead to 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.

Focal seizures are well described in SME - although the mechanism by which the measles virus induces epileptogenesis is not clear. When neurotropic measles virus is inoculated into mouse brain, it leads to a distinctive patchy cortical distribution, but in some cases involving the deep basal ganglia consistent with a polymicrogyria. White-matter abnormalities were variable and infrequent.
<table>
<thead>
<tr>
<th>Laboratory, pathology and radiological investigations in subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pt 1</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td><strong>CSF</strong></td>
</tr>
<tr>
<td><strong>Measles antibodies</strong></td>
</tr>
<tr>
<td><strong>Serum IgG</strong></td>
</tr>
<tr>
<td><strong>IgG</strong></td>
</tr>
<tr>
<td><strong>Measles PCR</strong></td>
</tr>
<tr>
<td><strong>CSF</strong></td>
</tr>
<tr>
<td><strong>Brain biopsy</strong></td>
</tr>
<tr>
<td><strong>EEG</strong></td>
</tr>
<tr>
<td><strong>CT brain</strong></td>
</tr>
<tr>
<td><strong>Location of MRI signal abnormalities</strong></td>
</tr>
<tr>
<td><strong>Superficial grey matter</strong></td>
</tr>
<tr>
<td><strong>Deep grey matter</strong></td>
</tr>
<tr>
<td><strong>White matter</strong></td>
</tr>
</tbody>
</table>

Amniocentesis, meconium, and fetal MRI are currently being investigated for their potential role in the diagnosis of SM. However, neonatal hearing loss is the most reliable diagnostic criterion, and a detailed examination of all amniotic fluid should be performed in every case. The presence of meconium in the amniotic fluid is a strong indicator of fetal compromise and may be associated with an increased risk of congenital malformations. The role of fetal MRI in the diagnosis of SM is currently under evaluation, and further studies are needed to determine its diagnostic accuracy.

Neonatal hearing screening is crucial for early identification and management of congenital hearing loss. All infants should undergo a hearing screening by the age of 6 months, with follow-up evaluations as necessary. Early intervention with hearing aids and auditory training can significantly improve language and cognitive development in infants with hearing loss.

Neural tube defects (NTDs) are a group of birth defects that affect the central nervous system. They include conditions such as spina bifida and anencephaly. NTDs are caused by a failure of the neural tube to close properly during the early stages of pregnancy. The risk of NTDs is higher in pregnancies with a history of previous NTD, family history of NTD, or parental age over 35 years. Ultrasound examination in the first trimester is recommended to screen for NTDs.

The role of maternal serology in the management of SM is controversial. While some studies have suggested an association between maternal rubella infection and SM, other studies have failed to find a significant association. Maternal serology should be considered in the context of the individual patient and the overall risk-benefit ratio.

The diagnosis of SM in the neonatal period is challenging. The presentation can vary widely, ranging from subtle neurodevelopmental delays to severe neurological impairment. Early recognition and intervention are crucial to optimize outcomes.

Neonatal hearing screening is an essential component of the comprehensive assessment of infants with suspected SM. A combination of clinical examination, audiological testing, and imaging studies can help in making an accurate diagnosis of SM. Early detection and intervention can significantly improve outcomes for infants with SM.

Neural tube defects (NTDs) are a group of serious birth defects that affect the central nervous system. They include conditions such as spina bifida and anencephaly. NTDs are caused by a failure of the neural tube to close properly during the early stages of pregnancy. The risk of NTDs is higher in pregnancies with a history of previous NTD, family history of NTD, or parental age over 35 years. Ultrasound examination in the first trimester is recommended to screen for NTDs.

The role of maternal serology in the management of SM is controversial. While some studies have suggested an association between maternal rubella infection and SM, other studies have failed to find a significant association. Maternal serology should be considered in the context of the individual patient and the overall risk-benefit ratio.

The diagnosis of SM in the neonatal period is challenging. The presentation can vary widely, ranging from subtle neurodevelopmental delays to severe neurological impairment. Early recognition and intervention are crucial to optimize outcomes.

Neonatal hearing screening is an essential component of the comprehensive assessment of infants with suspected SM. A combination of clinical examination, audiological testing, and imaging studies can help in making an accurate diagnosis of SM. Early detection and intervention can significantly improve outcomes for infants with SM.

Neural tube defects (NTDs) are a group of serious birth defects that affect the central nervous system. They include conditions such as spina bifida and anencephaly. NTDs are caused by a failure of the neural tube to close properly during the early stages of pregnancy. The risk of NTDs is higher in pregnancies with a history of previous NTD, family history of NTD, or parental age over 35 years. Ultrasound examination in the first trimester is recommended to screen for NTDs.

The role of maternal serology in the management of SM is controversial. While some studies have suggested an association between maternal rubella infection and SM, other studies have failed to find a significant association. Maternal serology should be considered in the context of the individual patient and the overall risk-benefit ratio.

The diagnosis of SM in the neonatal period is challenging. The presentation can vary widely, ranging from subtle neurodevelopmental delays to severe neurological impairment. Early recognition and intervention are crucial to optimize outcomes.

Neonatal hearing screening is an essential component of the comprehensive assessment of infants with suspected SM. A combination of clinical examination, audiological testing, and imaging studies can help in making an accurate diagnosis of SM. Early detection and intervention can significantly improve outcomes for infants with SM.

Neural tube defects (NTDs) are a group of serious birth defects that affect the central nervous system. They include conditions such as spina bifida and anencephaly. NTDs are caused by a failure of the neural tube to close properly during the early stages of pregnancy. The risk of NTDs is higher in pregnancies with a history of previous NTD, family history of NTD, or parental age over 35 years. Ultrasound examination in the first trimester is recommended to screen for NTDs.

The role of maternal serology in the management of SM is controversial. While some studies have suggested an association between maternal rubella infection and SM, other studies have failed to find a significant association. Maternal serology should be considered in the context of the individual patient and the overall risk-benefit ratio.

The diagnosis of SM in the neonatal period is challenging. The presentation can vary widely, ranging from subtle neurodevelopmental delays to severe neurological impairment. Early recognition and intervention are crucial to optimize outcomes.

Neonatal hearing screening is an essential component of the comprehensive assessment of infants with suspected SM. A combination of clinical examination, audiological testing, and imaging studies can help in making an accurate diagnosis of SM. Early detection and intervention can significantly improve outcomes for infants with SM.

Neural tube defects (NTDs) are a group of serious birth defects that affect the central nervous system. They include conditions such as spina bifida and anencephaly. NTDs are caused by a failure of the neural tube to close properly during the early stages of pregnancy. The risk of NTDs is higher in pregnancies with a history of previous NTD, family history of NTD, or parental age over 35 years. Ultrasound examination in the first trimester is recommended to screen for NTDs.

The role of maternal serology in the management of SM is controversial. While some studies have suggested an association between maternal rubella infection and SM, other studies have failed to find a significant association. Maternal serology should be considered in the context of the individual patient and the overall risk-benefit ratio.

The diagnosis of SM in the neonatal period is challenging. The presentation can vary widely, ranging from subtle neurodevelopmental delays to severe neurological impairment. Early recognition and intervention are crucial to optimize outcomes.

Neonatal hearing screening is an essential component of the comprehensive assessment of infants with suspected SM. A combination of clinical examination, audiological testing, and imaging studies can help in making an accurate diagnosis of SM. Early detection and intervention can significantly improve outcomes for infants with SM.

Neural tube defects (NTDs) are a group of serious birth defects that affect the central nervous system. They include conditions such as spina bifida and anencephaly. NTDs are caused by a failure of the neural tube to close properly during the early stages of pregnancy. The risk of NTDs is higher in pregnancies with a history of previous NTD, family history of NTD, or parental age over 35 years. Ultrasound examination in the first trimester is recommended to screen for NTDs.

The role of maternal serology in the management of SM is controversial. While some studies have suggested an association between maternal rubella infection and SM, other studies have failed to find a significant association. Maternal serology should be considered in the context of the individual patient and the overall risk-benefit ratio.

The diagnosis of SM in the neonatal period is challenging. The presentation can vary widely, ranging from subtle neurodevelopmental delays to severe neurological impairment. Early recognition and intervention are crucial to optimize outcomes.

Neonatal hearing screening is an essential component of the comprehensive assessment of infants with suspected SM. A combination of clinical examination, audiological testing, and imaging studies can help in making an accurate diagnosis of SM. Early detection and intervention can significantly improve outcomes for infants with SM.
many of whom might have died before reaching medical attention, which serves to remind clinicians of the importance of a vigilant and effective measles immunization 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 biopsy.

The authors thank the Groote Schuur Hospital doctors who referred their cases, and Drs Hawelet and Ta who assisted with the neuropathological examinations of the postmortem specimens. This study was funded by PF7576/08/01 through the ANOVA Health Institute. The UCT research ethics committee approved the reporting of the clinical material (407/2010).
2. Molecular characterisation of virus in the brains of patients with measles inclusion body encephalitis (MIBE)
Molecular characterisation of virus in the brains of patients with measles inclusion body encephalitis (MIBE)

Diana R Hardie¹, Christine Albertyn², Jeannine M Heckmann³ and Heidi EM Smuts¹

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 were 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
persons, but symptoms develop only after a prolonged latent period and the virus from brain starts to localize in the brain [7]. Key viral structural genes (matrix, fusion and hemagglutinin) are highly mutated, rendering the protein nonfunctional [8]. We were interested to investigate whether the virus was similar to other epidemic viruses and whether there were any common features which could explain the pathogenesis of the condition. Accordingly, the nucleoprotein (N), matrix (M), capsid (C), and polymerase (L) genes from 4 MDE cases (see Table 1) for clinical details were compared with viruses 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 4 patients. Complete sequence numbers KC006021-KC006095). Incomplete sequence data was obtained from brain virus of a further 2 patients (2 and 4). Partial sequence data was also limited clinical material available for PCR amplification. For patient 1, most of the H gene sequence was available (nucleotide 246-1074) and partial sequence for the F gene (nucleotide 1-4575) and the F gene was sequenced.

Phylogenetic analysis of epidemic virus
Phylogenetic analysis of the nucleoprotein and hemagglutinin genes confirmed that the South African epidemic in 2008/09 was caused by genotype 95, (Figures 1, 2). The South African sequence formed a distinct cluster within genotype 95 and the high level in phylogenetic trees of the F and M genes as well (Figure 3A and 3B). Brain and acute virus sequences clustered closely together on all phylogenetic trees (Figures 1, 2, and 3).

MLST analysis of the South African coronavirus M and F genes showed that the epidemic virus was most closely related to JN651649, a 2006 isolate from New Jersey, USA. The consensus N and H genes were more closely related to L9660 isolates from India (L96601 and L96603). A concatenated phylogenetic tree of the TBR M, F, and H genes confirmed a close relationship of the South African epidemic strain to L96603 (Figure 4).

There was very little sequence variation in the epidemic virus. A total of 24 polymorphisms were present in the strain acutely infected patients over the 6733 kb region sequenced (giving a mutation frequency of 0.016 per 1000 nucleotides).

Sequence analysis of brain virus
Sequences from the acute epidemic coronavirus 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 nonviable viruses of SSCP/MDE were not present, namely hypermutation of the matrix and fusion proteins or truncation of C-terminal end of fusion protein gene [9]. Each MDE 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 13 polymorphisms present in brain virus relative to the South African consensus sequence (Table 2).

L to C and G to U mutations were the most common mutations present in the epidemic strain and were also the most frequent in the brain virus. The calculated mutation frequency in brain virus was 0.07 per 1000 nucleotides. This was not significantly different from the mutation frequency of the epidemic virus which was 0.86 per 1000 nucleotides (p = 0.19). Interestingly, the 10 substitutions that occurred in coding regions in the brain virus 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 C mutation at position 1946 in the F gene which gave rise to a 148-AW substitution in the fusion protein. Of note, the L148-W substitution was not present in the blood virus of patient 1 collected during acute measles infection 3 months earlier.
Discussion

The measles virus outbreak in South Africa was due to genotype B5, a known African genotype. It was probably introduced into South Africa from the north where an 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 actively infected individuals showed that there was a level of genetic variability in the epidemic virus. This is typical of a single-source introduction which is followed by dissemination via non-immune population [10,11].

While infection of the brain during acute measles may occur [14], MIEE is normally very rare. Most highly immunocompromised patients who had acute measles in this epidemic did not develop MIEE. 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 MIEE virus. We recently attempted to sequence the entire virus genome 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 extraordinarily 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 SDE [9,12] and also what has previously been reported for MIEE [15]. Mutation rates were similar in both (6.87 per 1000 bases) to the epidemic virus (6.58 per 1000 bases). However, of the 10 substitutions that occurred in the coding regions of genes from brain tissues, 8 were nonsense mutations (in comparison to only 4 of 53 from the epidemic virus). This could imply either that some selection process 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 neurotrophic phenotype [16,17]. Because of the many mutations found in SDE virus, it is difficult to determine which are responsible for neurotropism and which are merely the consequence of mutations accumulating in genes which are no longer essential for survival in the brain. By studying the fewer mutations in virus from acute brains it may be possible to determine which were responsible for the gain in neurotropism. 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 (S456W) was present in 3 patients (1 and 3). It is unlikely that there has been a chance polymorphism in some circulating virus because it was not present in the acute measles sequence of patient 1. Both mutations and suppressors are needed, and point analyses. 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 was in the extra cellular domain of the fusion protein adjacent to the heptad repeat 8 domain (HR8). Ayata et al. [17] showed that a single substitution (T86A) was responsible for neurotropism 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 play a role in the fusion process. The independent presence of this mutation in 2 patients is remarkable.

There were remarkably few mutations in all four of the genes sequenced from the brain tissues. In most brain tissues a single potential neurotrophic point mutation was present, but in humans as well as immortal cells, the neurotrophic gene typically functioned as this protein is required to form lens ribonuclear protein complexes to enable the virus to move from cell to cell in the brain [19]. The single point, on the other hand, is usually highly mutated at this protein is not needed for replication in the human [16,17]. Perhaps sufficient time had not elapsed for mutations to accumulate in this gene after the initial presentation of MIEE in our highly immunocompromised patients. In 3 MIEE patients who also had a history of acute measles, the median time from acute infection to onset of neurological disease was about 15 years. In all patients there was 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 immunocompromised HIV patients infected with measles developed MIEE. Viral factors also must have played a role. Interestingly the brain virus was very similar to the epidemic virus and did not show features previously reported to be characteristic of MIEE. The mutation frequency was similar in epidemic virus, but significantly these mutations were more likely to be nonsynonymous. A key finding was that 1 patient had the same S456W mutation in the fusion protein.
Figure 3: Phylogenetic tree generated by neighbour-joining analysis of the locus sequence of patients with Mcune–Albright syndrome and the MIF during the period 2010–2013 in South Africa. Reference strains of known genotype were removed from the HCV database and are indicated by asterisks. Genotype values greater than 90% are classified within a single cluster. The branch lengths are proportional to the evolutionary distance as measured by the relative time.

Materials and methods
Previously described [3] and if necessary virus was detected by PCR in bone marrow or cerebrospinal fluid.

Patients were identified as having MIF based on typical clinical and magnetic resonance (MRI) findings as postulated by the first author.
Figure 3 Phylogenetic trees generated by molecular phylogenetic analysis of the fusion (F) and matrix (M) genes of measles virus from patients with acute measles infection and MEE during the measles epidemic of 2009–2010 in South Africa. Reference sequences of each of measles virus genotypes were obtained from GenBank and included in the phylogenetic analysis. Branch lengths are proportional to the evolutionary distance among viruses.

Measles virus positive brains or CSF was available for study in 4 patients.

Patient 1 was a 27 year old woman who had a CD4 count of 66 at time of acute measles. MEE onset occurred 3 months after acute measles. Measles virus PCR was positive on brain tissue. Brain virus was compared with acute virus from blood from 3 months previously.

Patient 2 was a 46 year old woman who had a CD4 count of 1 at time of neurological presentation. There was no history of a rash. This patient had received measles vaccine 6 months prior to this. A measles outbreak occurred during a school vacation camp. The CSF and a nasal aspirate were negative for measles virus.

Patient 3 was a 14 year old boy with a CD4 count of 1 at time of neurological presentation. There was no history of rash. This patient developed measles 6 months prior to this presentation. Measles virus PCR was positive in CSF.

Patient 4 was a 24 year old woman who developed typical measles symptoms 3 weeks after acute measles appearance. Her CD4 count at presentation was 100. Measles virus PCR was positive on post mortem brain tissue. Post mortem histology confirmed MEE.

In addition, Measles virus was isolated from blood collected at the time of acute measles. Additional virus was available from one patient who subsequently developed MEE (patient 1). The brain smear of this patient was compared with the acute measles virus.

Nucleic acid extraction

Total nucleic acid for measles virus screening was initially extracted using the Qiagen automated extractor (Qiagen). DNA was then analyzed using the PCR assay for measles virus as per manufacturer’s instructions. Subsequently, total nucleic acid was extracted from the brain or CSF using the manual Qiagen DNA kit (Qiagen). Nucleic acid was eluted in 50 ul elution buffer and stored at -80°C until required.
cDNA synthesis and PCR
DNA was converted into cDNA using the ReverAid First Strand cDNA synthesis kit (Thermo Fisher Scientific) and random hexamers. Briefly, 11 μl of DNA was incubated with 1 μl random hexamers and 0.5× ReverAid Reaction buffer in a total volume of 2 μl before the reaction was incubated at 94°C for 5 min. Then, the reaction was cooled at 4°C. cDNA was stored at -20°C until required.

The cDNA was amplified using specific primers described by Pillers et al. [1]. 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 PCRs. A positive control PCR amplicon was generated using primers and HotStart Taq DNA polymerase (Roche Applied Science) with the following PCR cycling conditions: initial denaturation step of 3 min at 95°C, followed by 40 cycles of amplification (95°C for 15 sec, 59°C for 30 sec, and 72°C for 1 min) 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 60°C and 30 sec per product.

PCR products were sequenced directly with primers used for PCR amplification. The sequences

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide position</th>
<th>S.Com</th>
<th>Part1</th>
<th>Part2</th>
<th>Part3</th>
<th>Part4</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>419</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>G</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>423</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>G</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>426</td>
<td>U</td>
<td>C</td>
<td>U</td>
<td>U</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>429</td>
<td>U</td>
<td>C</td>
<td>U</td>
<td>U</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>432</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>435</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>438</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>1</td>
</tr>
</tbody>
</table>

(Continued)
terminator cycle sequencing kit was used (Applied Biosystems, Foster City, CA, USA). Sequences were assembled and aligned against the measles virus genotype B reference sequence (accession number HM439886) using DNA Baser Sequence Assembler v3.5.0.

The 3′ hypervariable N and H genes were assembled 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: 162/2011)

Availability of supporting data

The data supporting the results of this article is included within the article (and its additional files). The local measles virus sequences were deposited in GenBank. (GenBank: KC306461-KC306469).

Additional file

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

Competing Interests

None of the authors have any competing interests.

Authors’ contributions

The conceived the study, analyzed the results and wrote the manuscript. JH and CA identified the cases of MM, and helped to write the manuscript. JH designed primers to amplify measles virus genes, assembled consensus of the genes performed phylogenetic analyses and wrote part of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This study was funded, in part by the Polio Evolution Research Foundation (PERF).

Author details

1Division of Medical Virology, Department of Clinical Laboratory Sciences, University of Cape Town and National Health Laboratory Services, Cape Town, South Africa. 2Department of Neurology, Department of Medicine, University of Cape Town, Cape Town, South Africa.

Received: 18 April 2013 Accepted: 9 September 2013 Published: 12 September 2013

References


doi:10.1186/1471-2105-13-283

Cite this article as: Handel et al.: Molecular characterization of virus in the brains of patients with measles inclusion body encephalitis (MIME). Virol 2013 16:283.

52
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: [Name]

Subject matter of photograph or article: [Caption]

Title of article: Subacute measles encephalitis

Corresponding author: C. Albertson J. Hedemann

I, [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 [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 journal’s website.

5. I can withdraw 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: [Signature]

Date: 27/8/16

Name of person taking consent: [Signature]

Date: 27/8/16

Note: J. Hedemann also discussed issues with patient and her mother.
3. Human Research Ethics Committee approval

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Faculty of Health Sciences Research Ethics Committee
Room E22-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • Facsimile [021] 406 6411
e-mail: sarah.harefielsen@uct.ac.za

15 October 2010

HREC REF: 487/2010

A/Prof J Heckmann
Division of Neurology
EB-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 (HIS016), if the study continues beyond the approval period. Please submit a Standard Closure form (HIS010) 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.

sarah.harefielsen
Yours sincerely,

[Signature]

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSP 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, 36 and 312.