The clinical presentation and outcome of CMV disease in paediatric patients at Chris Hani Baragwanath Academic Hospital- a retrospective, descriptive study.

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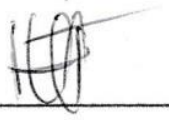
Declaration

I Hermina Mmabatho Tsholanang Nke declare that this dissertation, except where otherwise indicated is my original work. It is being submitted for the Degree of Mmed in Paediatrics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

Signed  Date 17/12/2021

Dedication

I dedicate this work to my mother and my late dad.

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Abbreviations

Abbreviation	Term
ALT	Alanine transaminase
AST	Aspartate transaminase
ART	Antiretroviral therapy
CHBAH	Chris Hani Baragwanath Academic Hospital
cCMV	Congenital cytomegalovirus
cUS	Cranial ultrasound
Hb	Haemoglobin
HIV	Human immunodeficiency virus
HIV VL	Human immunodeficiency virus viral load
ICU	Intensive care unit
NHLS	National Health Laboratory Service
RMPRU:	Respiratory and Meningeal Pathogen Research Unit

CHAPTER 1: Submissable Paper

1.1 Title page

The clinical presentation and outcome of CMV disease in paediatric patients at Chris Hani Baragwanath Academic Hospital- a retrospective, descriptive study

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Keywords: Cytomegalovirus, Cytomegalovirus viral load, congenital cytomegalovirus, HIV infection

1.2 Abstract

Background

The clinical profile and outcomes of patients with cytomegalovirus viraemia in South Africa are unknown.

Methods

This was a retrospective record review of patients admitted at Chris Hani Baragwanath Academic Hospital (CHBAH), Soweto, South Africa, from January 2016 to December 2017 with suspected CMV disease. Patients younger than 14 years of age with CMV viral load (CMV VL) >137 IU/mL (151copies/mL) were included in the study. Patients were identified through the National Health Laboratory Service (NHLS) CMV VL database.

Results

A total of 144 patients were identified during the study period. Records were not available for 19 patients. Data were analysed for 125 patients.

Most of the children who had a recorded CMV viral load were under one year of age. The median age of presentation was 90 days (IQR 48 to 150; range 1 to 4680). The most common clinical presentations were anaemia in 83/125 (66.4%), pneumonia in 59/125 (47.2%) and thrombocytopenia in 58/125 (46.4%). Of the patients with CMV VL < 10 000IU/mL, the most common clinical presentation was anaemia in 51 (60.7%) and pneumonia in 39 (46.4%). Three (3.6%) had histology confirmed biliary atresia and six (7.1%) had cranial sonar features of CMV disease. Four (28.6%) patients with cranial sonar(cUS) features of CMV disease had CMV VL > 10 000IU/mL. The in- hospital case fatality rate was 19.2 % (n=24).

Conclusion

Most patients in this group were <1year old. Clinical features compatible with CMV disease are also seen in children with a low CMV VL.

1.3 Introduction

The diagnosis of cytomegalovirus (CMV) disease is fraught with controversy. Differentiating between CMV infection versus disease remains difficult in children [1], and the decision to initiate treatment is not simple. Although the cytomegalovirus viral load (CMV VL) is used to decide whether to initiate antiviral therapy and to monitor response to therapy [2], the value is based on extrapolation from stem cell and solid organ transplant patients [3]. The viral load value associated with CMV disease is different in different immunosuppressive conditions [4,5]. CMV end-organ disease has been reported in the absence of detectable viraemia therefore undetectable CMV VL does not always exclude CMV disease [5-7].

According to the standard treatment guidelines and Essential Medicines List (EML) of South Africa the diagnosis of CMV disease in immunocompromised children is considered when the quantitative CMV VL is more than 10 000 copies/ml (9100 IU/mL), or if intranuclear inclusion bodies are found in biopsy material of a patient with clinical features suggestive of CMV disease [8]. However, some studies report patients with CMV disease having lower VL levels [3,9].

Ganciclovir is the drug of choice for the treatment of CMV and effective in the treatment of severe CMV pneumonia in children [3,10]. Jeena et. al reported improved outcomes in HIV infected children with CMV associated pneumonia treated with a combination of antiretroviral therapy (ART) and ganciclovir [11]. However, the neutropaenic side effects of ganciclovir may complicate patient management [3].

This study reports on the clinical presentation of children with any detectable CMV VL and their outcomes.

1.4 Methods

Patients admitted to the Chris Hani Baragwanath Academic Hospital (CHBAH) paediatric wards who had blood specimens submitted for CMV VL by the attending doctors and who had any detectable CMV VL (CMV VL > 151 copies/mL=137 IU/mL) were included in the study. Patients were identified through the National Health Laboratory Services (NHLS), microbiology laboratory records for the two-year period 01 January 2016 to 31 December 2017. The names and hospital numbers of patients who had specimen processed by NHLS for CMV VL were obtained from CMV VL database. The NHLS used the COBAS AmpliPrep/COBAS®TaqMan ®CMV platform test to quantify CMV DNA in plasma and reports CMV VL in IU/mL. A local standard operating procedure from CHBAH NHLS was used to convert CMV VL from copies/mL to IU/mL. A conversion factor of 0.91 was used to convert International Units /mL to copies/mL. Clinical data was retrieved from archived records, Respiratory and Meningeal Pathogen Unit (RMPRU) database, NHLS and from the Paediatric Department electronic database. Data including age, gender, CMV VL, HIV status, ART history and outcome was extracted. Congenital cytomegalovirus(cCMV) was defined as any detectable CMV VL in neonates ≤ 21 days of age. Written permission was obtained from the Chief Executive Officer of CHBAH, NHLS and RMPRU. Data were analysed with Stata version 15.5. Categorical data was reported in frequencies and percentages. We assessed the normality of continuous data using Shapiro-Wilk test. Due to the non-normality observed on the continuous data, medians and inter-quartile ranges were reported. To assess the factors associated with mortality, we used univariate and multivariate logistic regression. We report unadjusted odds ratio and adjusted odds ratio and 95% confidence intervals (95% CI). Variables were considered as significant at p-values <0.05.

Ethics

This study was completed in partial fulfilment of MMed (Paediatrics) at the University of the Witwatersrand, Ethical clearance was granted by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (clearance no. M180355).

1.5 Results

A total of 144 patients with CMV VL above 137 IU/mL were identified in the two-year duration of the study; 19 patients were excluded from the analysis due to incomplete clinical records. Data were analysed for 125 (86.8%) patients. During the 2-year study period, a total of 8948 neonates and 10750 paediatric patients were admitted to the CHBAH's neonatal unit and general paediatrics wards and 1029 blood specimens were submitted for CMV VL.

Males accounted for 74/125 (59.2%) of the study population, table1. There were 95/125 (76.0%) infants less than one year of age. Of the 95 patients, 58 (61.1%) were under three months, 24 (25.3%) were between three and six months and 13(13.7%) were between six months and one year. The median age of presentation was 90 days (IQR 48 to 150; range 1 to 4680). CMV VL was < 10 000 IU/mL in 84 (67.2%). Of the 84 patients with CMV VL <10 000 IU/mL, 53(63.1%) had viral loads between 1000 and 10 000 IU/mL and 31 (36.9%) patients had CMV VL of <1000 IU/mL.

Overall, the most common clinical presentations were anaemia in 83 (66.7%) patients, pneumonia in 59 (47.2%) and thrombocytopenia in 58(46.4%). Of the 83 patients with anaemia, 47/83 (56.6%) were HIV infected; 22 (26.5%) were wasted, 6 (7.2%) were stunted, 13 (15.7%) had microcytosis and one (1.2%) patient had pure red cell aplasia with CMV VL of 49733IU/mL. Three patients had histology confirmed CMV end-organ disease: bone marrow (n=1), oesophagus (n=1) and liver and colon (n=1).

Sixty-one (48.8%) patients were HIV infected. Of the 61 HIV positive children, 4(6.6%) had congenital CMV infection and 50 (82.0%) were <12 months of age. Thirty-nine (63.9%) had CD4 percentages of <25% of lymphocytes and the HIV viral load was >1000 copies/mL in 55(90.1%) therefore not virally suppressed. Of the 39 patients with CD4 percentage of < 25% one patient was >5years of age and had absolute CD4 count of 60. Thirty-three (54%) were initiated onto ART during the current admission episode.

Cranial imaging (cranial ultrasound and computerised tomography) reports were available for 24(19.2%) patients, amongst whom 13 (54.2%) had abnormal findings. Periventricular echo densities n=5 (20.8%), calcification n=3(12.5%), germinal matrix cyst n=1(4.2%), ventricular dilatation n=2 (8.3%), choroid plexus cyst n=1 (4.2%), intraventricular haemorrhage n=1(4.2%) and lenticulostriate vasculopathy n=1(4.2%) were reported.

Five hearing test reports were available; none of the patients had sensoneural hearing loss. Ophthalmology reports were available for 17 patients, for two (11.7%) retinal haemorrhages were reported.

Congenital CMV (cCMV) infection was diagnosed in 13.6% (17/125). Three (17.6%) patients with cCMV had birth weight below 1500g and 7/17 (41.2%) had birth weight >1500g and < 2500g, two (33.4%) had birth weight above 2500g and the birth weight was unknown in five patients. Nine (52.9%) patients had thrombocytopaenia, two (11.8%) patients had pneumonia and three (17.6%) had conjugated hyperbilirubinaemia. Of the 17 patients nine (52.9%) were HIV exposed and four (23.5%) were HIV infected.

Of the 84 patients with CMV VL < 10 000IU/mL, 51(60.7%) had anaemia on full blood count, 39 (46.4%) had pneumonia, 13 (15.5%) hepatitis including six with cholestasis and three of the six had biliary atresia, one (1.2%) had colitis. Six (7.1%) patients had abnormal cranial ultrasound(cUS) reports, calcifications (n=2), germinal matrix cyst(n=1), choroid plexus cyst (n=1), periventricular echo densities (n=1), periventricular echo densities and lenticulostriae vasculopathy (n=1). Nineteen (22.6%) patients were malnourished. However, two patients had clinical signs not typical of CMV: upper airway obstruction in one with CMV VL of 319 IU/mL and newly diagnosed nephroblastoma not on chemotherapy with CMV VL of 457 IU/mL in another.

Of the 125 patients, 44 (35.2%) patients were treated with ganciclovir, 44 (35.2 %) did not receive ganciclovir and ganciclovir treatment status was unknown in 37 (29.6%) patients (Table 2). Of the 44 patients treated with ganciclovir, 12 (27.3%) patients had CMV VL < 10 000 IU/mL, four (9.1%) had congenital CMV and 29 (65.9%) severe pneumonia. Ten of the 44 (22.7%) patients who were treated with ganciclovir died while seven of the 44 (15.9%) patients who did not receive ganciclovir died. The median duration of ganciclovir treatment was 14 days (IQR 10 to 14 days). Patients with high viral load were more likely to receive ganciclovir, p-value <0.001; although six (14.6%) patients with CMV VL >10 000 IU/mL were not treated with ganciclovir. Patients who were treated with ganciclovir had prolonged hospitalisation.

Outcome

Table 3 shows the outcome and factors associated with mortality. The median duration of hospital stay was 20 days (IQR 10 to 41; range 1 to 171). One hundred and one (81%) patients were discharged home or transferred back to the referring facility. Twenty-four (19.2%) patients died in hospital. Of the 24 patients who died, three (12.5%) had CMV VL ≤ 1000 IU/mL, and 21 (87.5%) patients had CMV VL >10 000 IU/mL. Fifteen (62.5%) patients who died had pneumonia; seven (46.7%) of whom received both ART and ganciclovir. Sixteen (66.7%) were HIV infected of whom 11 (68.8%) had CD4 counts < 20%. Clinical parameters that were associated with death included anaemia [OR 5.08(CI, 1.12; 23.09], colitis [OR 20.31(CI, 1.96; 210.17], HIV infection [OR 3.2(CI, 1.08; 9.48] and CMV VL> 10 000IU/mL [OR 4.33(CI, 1.11; 16.88]. On multivariable analysis, only colitis was associated with death (adjusted (aOR) 19.81; 95%CI, 1.29; 305.00) (Table3).

Table 1: Clinical characteristics

Variable	Frequency Total =125 n (%)	CMV VL >137-999IU/mL Total =31 (24.8) n (%)	CMV VL 1000-9999 IU/mL Total=53 (42.4) n (%)	CMVVL ≥10 000 IU/mL Total 41 (32.8) n (%)	p-value
Age					0.064
1 to 21days	17(13.6)	5(29.4)	10(58.8)	2(11.8)	
>21days to ≤ 1year	95(76.0)	22(23.2)	35(36.8)	38(40.0)	
>1year to ≤ 5years	11(8.8)	4(36.4)	7(63.6)	0(0.00)	
>5years to ≤ 14years	2(1.6)	0(0.0)	1(50.0)	1(50.0)	
Sex					0.507
Male	73(58.4)	17(23.3)	34(46.6)	22(30.1)	
Female	48(38.4))	14(29.2)	17(35.4)	17(35.4)	
Unknown	4(3.2)	0(0.0)	1(25.0)	3(75.0)	
HIV status					
HIV infected	61(48.8)	14(23)	23(37.7)	24(39.3)	0.327
CD4 >25%	12(19.7)	4(33.3)	4(33.3)	4(33.3)	0.870
CD4 <25%	39(63.9)	10(25.6)	14(35.9)	15(38.5)	
Unknown CD4	10(16.4)	0(0.0)	5(50.0)	5(50.0)	
HIV VL ≤1000 copies/mL	1(1.64)	0(0.0)	1(100)	0(0.0)	0.455
HIV VL >1000 copies/mL	55(90.2)	14(25.5)	21(38.1)	20(36.4)	
Unknown HIV VL	5(8.2)	0(0.0)	1(25.0)	4(75.0)	
HIV negative	50(40.0)	14(28.0)	22(44.0)	14(28.0)	
HIV unknown	14(11.2)	3(21.4.)	9(64.3)	2(14.3)	
More than one condition may be present in a child					
Anaemia	83(66.4)	18(21.7)	33(39.7)	32(38.6)	0.153
Pneumonia	59(47.2)	14(23.7)	25(42.4)	20(33.9)	0.875
Thrombocytopenia	58(46.4)	8(13.8)	26(44.8)	24(41.4)	0.013
Malnutrition	27(21.6)	8(29.6)	11(40.7)	8(29.6)	0.346
Hepatitis	22(17.6.)	6(27.3)	7(31.8)	9(40.9)	0.519
Neutropaenia	13(10.4)	1(7.7)	7(53.8)	5(38.5)	0.268
Acute gastroenteritis	11(8.8)	1(9.1)	3(27.3)	7(63.6)	
Colitis	4(3.2)	1(25.0)	0(0.00)	3(75.0)	0.159

Table 2: Ganciclovir treatment

Variable	**Ganciclovir N =44(35.2) n (%)**	**No ganciclovir N= 44(35.2) n (%)**	**Unknown N =37(29.6) n (%)**	***P*-value ***
Age				0.292
1 to 21days	4(23.5)	5(29.4.)	8(47.1)	
> 21 days to 1 year	38(40.0)	33(34.7)	24(25.3)	
>1 year to 14years	2(15.4)	6(46.1)	5(38.5)	
Clinical presentation				
Anaemia	31(37.4)	25(30.1)	27(32.5)	0.090
Pneumonia	29(49.1)	22(37.3)	8(13.6)	0.124
Thrombocytopenia	21(36.2)	18(31.0)	19(32.8)	0.511
Hepatitis	7(31.8)	6(27.3)	9(40.9)	0.650
Malnutrition	9(33.3)	14(51.9)	4(14.8)	0.591
Neutropenia	3(23.1)	3(23.1)	7(53.8)	0.395
Colitis	1(25.0)	2(50.0)	1(25.0)	0.586
HIV infected	27(44.3)	20(32.8)	14(22.9)	0.282
Median CMV VL(IQR) (IU/mL)	11300.5(3541.5-59357.5)	1453.5(564-4759)	2555(804-13683)	<0.001
In hospital death	10(22.7)	7(15.9)	7(15.9)	0.418

***p*- value compares ganciclovir use vs. no ganciclovir use**

Table 3: Outcomes and factors associated with mortality

Variable						
			Unadjusted		**Adjusted**	
	Discharged n=101 n (%)	**Death** n=24 n (%)	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
Age						
1 to 21 days	14(82.4)	3(17.6)				
22days to 1year	76(80.0)	19(20.0)	1.17(0.30;4,48)	0.822		
> 1year to14 years	11(84.6)	2(15.4)	0.85(0.12; 5.99)	0.869		
Clinical presentation						
Anaemia	61(75.3)	22(24.7)	5.08(1.12; 23.09)	0.021	2.56(0.42; 15.95)	0.309
Pneumonia	44(74.6)	15(25.4)	2.78(0.99; 7.8)	0.051		
Thrombocytopenia	44(75.9)	14(24.1)	2.6(0.92; 7.35)	0.072		
Hepatitis	16(80.0)	4(20.0)	1.33(0.38; 4.63)	0.650		
Malnutrition	22(81.5)	5(18.5)	0.93(0.28; 3.1)	0.904		
Neutropenia	12(92.3)	1(7.7)	0.41(0.05; 3.36)	0.403		
Colitis	1(25.0)	3(75.0)	20.31(1.96; 210.17)	0.012	19.81(1.29; 305.00)	0.032
HIV infected	45(73.8)	16(26.2)	3.2(1.08; 9.48)	0.036	2.38(0.56; 10.13)	0.241
Ganciclovir	34(77.3)	10(22.7)	1.56(0.53; 4.54)	0.420		
CMVVL						
CMV VL <1000 IU/mL	28(90.3)	3(8.7)				
CMVVL 1001-10 000IU/mL	45(84.9)	8(15.1)	1.66(0.41; 6.79)	0.481		
CMV VL >10 000 IU/mL	28(68.3)	13(31.7)	4.33(1.11; 16.88)	0.035	4.66(0.81; 26.94)	0.086

Multivariable analysis was done for variables with statistical significance

1.6 Discussion

This study reports on the clinical presentation and outcomes of hospitalised children who had detectable viraemia, over a 2-year period at an academic hospital in Johannesburg, South Africa.

CMV viraemia was more common in children under the age of one year in this study. This supports reports of early CMV infection in African children [12,13]. A study from Kenya reports on CMV viraemia and outcomes of 163 children aged between two months and twelve years diagnosed with HIV in hospital [14]. CMV VL was measured using quantitative polymerase chain reaction.[14]. Similar to Wamalwa et al. the prevalence of CMV viraemia was low in children above 5 years[14]. Of children who had a detectable CMV viral load, there were few who were older than 5 years.

The commonest clinical presentations were anaemia, pneumonia and thrombocytopenia. Pneumonia and gastroenteritis were common admission diagnosis in the report by Wamalwa et. al.[14]. Anaemia in this study group may be related to young age, HIV infection, and/ or malnutrition and iron deficiency. Two thirds of our study population presented with both anaemia and pneumonia, similar to other studies[10].

The frequency of cCMV was lower than 16% reported by Diar et. al. [15]. This may be because Diar et. al. used more than one laboratory test to define cCMV. Similar to previous reports the commonest laboratory finding in patients with cCMV was thrombocytopaenia [15,16]. A study from Zambia reports on the prevalence of cCMV, the clinical presentation and risk factors associated with cCMV. In contrast to our study, they reported jaundice as the most common clinical presentation of cCMV [17]. The difference may be because our study included neonates who were admitted in the general paediatrics ward who were admitted from home.

In other studies, cCMV was reported to be more common in patients who were exposed to HIV or HIV infected, supporting the role of maternal HIV in the transmission of CMV [10,15]. However, the frequency of cCMV in HIV infected patients in our study is lower than previously reported by Diar et. al where they looked at the characteristic and survival to discharge of neonates with cCMV at CHBAH from 2004-2008. [16,18]. This difference may be because of multiple changes in the HIV program and advances in the management of HIV

since 2008 including the implementation by South Africa of optimised World Health Organization (WHO) Prevention of Mother to Child transmission (PMTCT) programme in 2015.

The frequency of malnutrition in our study was high (21.6%) compared to that reported by the South African Demographic and Health Survey report of 2016 which shows a malnutrition rate of 3% [18]. This difference may be because our study population included hospitalized patients in a tertiary referral centre. CMV viraemia adversely affects growth and development in paediatric patients [13,19]. In addition, malnutrition causes immunosuppression in infants, children and adolescents and makes them vulnerable to infections, including CMV [20]. This cycle of malnutrition and infection has been well described [20].

We documented clinical manifestations of CMV at low-level viraemia (137 -999IU/mL). Of the patients with CMV VL < 10 000IU/mL the most common clinical presentation was anaemia in 51 (60.7.1%) and pneumonia in 39 (46.4%). Cytomegalovirus is involved in the pathogenesis of biliary atresia [21]. Three patients with CMV VL <10 000 IU/mL had biopsy confirmed biliary atresia. Neuroimaging plays an important role in the diagnosis of cCMV [22]. Typical CMV neuroimaging findings were reported in patients with CMV VL < 10 000 IU/ml. Only two patients in this group of children with low -level CMV viraemia had clinical findings not typically described in CMV disease, they were a three-month-old female with a vallecular cyst and 32-month-old female with nephroblastoma.

CMV VL is expressed differently in different laboratories. Awareness of different units and conversion factors is needed to assist in the clinical management of patients. The WHO reports an International Standards for Human cytomegalovirus (HCMV) Nucleic Acid Amplification technique. The WHO International Standards for HCMV's main aim is to calibrate secondary references used in different laboratory assays in terms of IU/mL and allow unvarying result reporting system [23].

There was no difference in mortality in patients who received ganciclovir versus those who did not. Patients with high CMV VL were more likely to receive ganciclovir, while those with lower CMV VL and milder disease were less likely to receive ganciclovir. It is possible that without ganciclovir treatment in patients with high CMV VL, the mortality in this group would have been higher than in those with low CMV VL. However, the results should be

used with caution as 30% were excluded from the assessment. Patients who received ganciclovir had prolonged hospital stay. This probably represents the severity of illness prompting ganciclovir use.

The in-hospital mortality rate in our cohort was 19.2%. There are no studies to compare our in-hospital mortality rate with. The 15% mortality rate reported by Wamalwa et.al was a six-month mortality rate and they excluded neonates from their cohort [14]. In contrast to Wamalwa et.al. CMV VL > 1000IU was not associated with mortality [14]. This may be because Wamalwa et al only looked at CMV viraemia in children with newly diagnosed HIV infection [14].

A major limitation of this study is its retrospective nature, and reliance on the use of archived data. Missing records impact on the analysis of outcomes. In addition, the use of ganciclovir and the HIV status was not recorded in 11% patients. Despite the limitations, this study documents the disease profile and outcomes of children who had any positive result on CMV VL testing. The findings suggest that CMV disease is not limited to patients with high viral loads. Prospective studies are necessary to confirm this finding.

1.7 Conclusion

This study demonstrated that cytomegalovirus infection even at low CMV VL is associated with the spectrum of clinical presentations compatible with CMV disease. Malnutrition and HIV coinfection were important comorbidities in this cohort and may contribute to the susceptibility to CMV disease. Further studies are needed to formally assess the utility of CMV VL in the diagnosis of CMV disease and the significance of which level of VL to assist starting antiviral treatment in this population.

Recommendations

Prospective studies are required to look at ganciclovir and outcomes.

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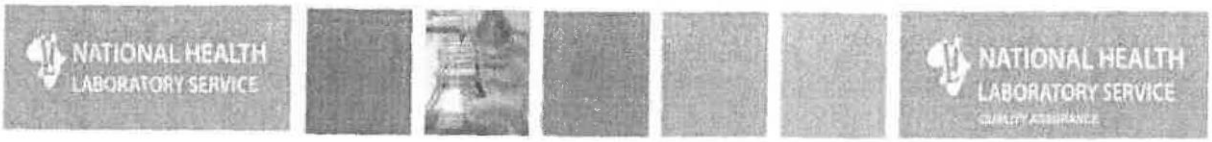
Appendix 1

Definitions

- Acute Gastroenteritis: diarrhoea for less than 2 weeks.
- Acute inflammatory demyelinating polyradiculopathy: ascending paralysis.
- Acute Malnutrition: weight for height <-2SD.
- Anaemia: Haemoglobin concentration ≤2SD below the mean for normal population. The National Health Laboratory Service refence ranges for Haemoglobin for age were used
- Antiretroviral naïve: Patient not on antiretroviral therapy (ART) or patients initiated on ART on current admission.
- Colitis: chronic diarrhoea (diarrhoea more than 2 weeks) or dysentery.
- Congenital cytomegalovirus: any detectable CMV VL within the first 21 days of life.
- Disseminated disease: Multisystem involvement.
- Hepatitis: elevation of liver enzymes by more than 2fold.
- Isolated disease: one organ or system.
- Neutropaenia: Absolute neutrophil count <1500.
- Pneumonia: Admission diagnosis of pneumonia
- Thrombocytopaenia: Platelet count< 140 $x10^9/L$



STANDARD OPERATING PROCEDURE

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Appendix 2

1. PURPOSE

This SOP should be used by all the Virology laboratory staff who perform the CMV viral load testing on CAP/CTM instrument, and those who interpret and verify results.

2. SOURCES

- COBAS Ampliprep/COBAS TaqMan CMV test user manual 2012; document revision 4

3. PRINCIPLE OF THE TEST METHOD

- The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is a nucleic acid amplification test for the quantitation of cytomegalovirus (CMV) DNA in human plasma.
- The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is based on two major processes: (1) specimen preparation to isolate CMV DNA and (2) simultaneous PCR amplification of target DNA and detection of cleaved dual-labeled oligonucleotide detection probe specific to the target.
- The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test permits automated specimen preparation followed by PCR amplification and detection of CMV target DNA and CMV Quantitation Standard (QS) DNA.
- The quantitation of CMV viral DNA is performed using the CMV QS. The CMV QS is added to each specimen at a known copy number and is carried through the subsequent steps of specimen preparation and simultaneous PCR amplification and detection of cleaved dual-labeled oligonucleotide detection probes.
- The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer calculates the CMV DNA concentration in the test specimens by comparing the CMV signal to the CMV QS signal for each specimen and control.

4. IDENTIFICATION OF POTENTIALLY HAZARDOUS PROCEDURES

- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and test reagents.
- Avoid microbial and nuclease contamination of reagents when removing aliquots from control vials.
- The use of sterile disposable pipettes and DNase-free pipette tips is recommended.
- Do not pool controls from different lots or from different vials of the same lot.
- Do not mix reagent cassettes or controls from different kits.
- Do not open COBAS® AmpliPrep cassettes and exchange, mix, remove or add bottles.
- Dispose of unused reagents, waste and specimens in accordance with country, federal, state and local regulations.
- Do not use a kit after its expiration date.
- Material Safety Data Sheets (MSDS) are available on request from your local Roche office.
- Specimens and controls should be handled as if infectious using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A3. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.
- Commercial liquid household bleach typically contains sodium hypochlorite at a concentration of 5.25%. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.
- CAUTION: CMV (–) C, CMV L (+) C and CMV H (+) C contain human plasma derived from human blood. The source material has been tested and found non-reactive for the presence of Hepatitis B Surface Antigen (HBsAg), antibodies to HIV-1/2 and HCV, and HIV p24 Antigen or HIV-1 RNA. Testing of Negative Human Plasma by PCR method showed no detectable CMV DNA.

No known test methods can offer complete assurance that products derived from human blood will not transmit infectious agents.

Therefore, all human sourced material should be considered potentially infectious. CMV (–) C, CMV L(+)C and CMV H(+)C should be handled as if infectious using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A3.Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

- **MGP, EB, CMV QS, $MgCl_2$** and **CMV MMX** contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide-containing solutions down laboratory sinks, flush the drains with a large volume of water to prevent azide build up.
- Wear eye protection, laboratory coats and disposable gloves when handling any reagent. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills of these reagents occur, dilute with water before wiping dry.
- Do not allow **CMV CS2** and liquid waste from the COBAS® AmpliPrep Instrument, which contain guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. These mixtures can produce a highly toxic gas.
- When disposing of used COBAS® AmpliPrep Sample Processing Units (SPUs), which contain guanidine thiocyanate, avoid any contact with sodium hypochlorite (bleach) solution. These mixtures can produce a highly toxic gas.

5. THE NATURE AND VOLUME OF SPECIMEN REQUIRED

- The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is for use with plasma specimens.
- Blood should be collected in sterile tubes using EDTA (lavender top) as the anticoagulant and mixed adequately according to the tube manufacturer's instructions.
- EDTA/PTT specimens are centrifuged at 3000 rpm for 10mins.
- The standard input volume for the assay is 0.5ml.

6. EQUIPMENT,

- COBAS Ampliprep/COBAS TaqMan – 96 Analyzer docked
- Biosafety cabinet
- Vortex mixer
- Centrifuge
- Pipettes (P200 and P1000)
- RNAse free water
- Filter tips (200µL and 1000µL)
- PPE
- Consumables and other materials refer to GPL2192
- 4 - 8°C refrigerator
- Reagents

6.1 PREPARATION AND STORAGE OF REAGENTS REQUIRED

To prevent/minimise laboratory – acquired infections, samples can be opened either in the biological safety cabinet (BSC) or on the bench when the appropriate PPE is worn.

6.2 CREATION OF WORKSHEETS/ORDERS

- Create a sample rack order in the COBAS/AMPLILINK software as per GPL2192/TADV0040
- Worksheets are created as per Appendix A: CMV viral load worksheet.

7. MAINTENANCE

Refer to TADV0116

8. DETAILED STEP BY STEP INSTRUCTION ON HOW TO PERFORM THE TEST

Prepare sample racks as follows:

- Attach barcode label clips to each sample rack position where a specimen is to be placed
- Put Input S-tube and number them 1 -24
- Attach one of the specific barcode label clips for the controls [CMV H (+) C, CMV L (+) C and CMV (-) C to each sample rack position where the controls (S-tube) are to be placed. The barcode labels clips for controls should have the same control lot number as the lot number on the control vials in the kit. Take care in assigning the right control to the position with the appropriate control barcode clip.
- Vortex all controls vials for 3-5 seconds prior to transferring into Input S-tubes
- Transfer 500µL (200µL for micro EDTA) of each specimen and control to the appropriate barcode labelled Input S- tube using a pipette with an aerosol barrier or positive displacement DNAse- free tip. Avoid transferring particulates and/or fibrin clots from the original specimen to the Input S-tube. Specimens should be transferred to tube positions as assigned on the worksheet.

- Use the AMPLILINK software and prepare data station as follows:
  Double click the AMPLILINK software
  Log onto Amplilink software by entering the assigned User ID and password

- Select the assay **(CMV 96-IU)** and create specimen orders for each specimen and controls in the ORDERS window Sample rack folder, refer to GPL2192
- Load Input S-tubes onto rack positions **F, G** or **H** of the COBAS Ampliprep instrument
- Check consumable and reagent status – refer to GPL2192
- Start the Ampliprep – refer to GPL2192

- All reagent cassettes should be removed from 2- 8°C storage, immediately loaded onto the COBAS ® Ampliprep Taqman Instrument and allowed to equilibrate to ambient temperature on the instrument for at least 30 minutes before the first sample is to be processed. Do not let the reagent cassette come to ambient temperature outside the instrument as condensation may form on the barcode labels. Do not wipe off condensation if it appears on the barcode labels

Avoid contaminating the upper part of the Input S-tube with specimens or controls.

9. ANY NECESSARY CALCULATIONS

- For diluted samples
  - This is done for samples with a minimal volume of 0.2ml
  - This 0.2 ml sample should be mixed with 0.3 ml of sterile water to make a 0.5 ml input volume.
  - A conversion factor of 2.5 should be used to calculate results from diluted samples, and this was deduced from the following formula:
    - C1V1 = C2V2, where V1 (low volume sample that needs dilution) = 0.2 ml and V2 (normal input volume) = 0.5 ml, C1 = concentration of a diluted sample, C2 = concentration of an undiluted sample.
    - C1 = C2V2/V1
      C1 = C2 x 0.5/0.2, therefore, C1 = 2.5 x C2
- For conversion from international units per ml to copies per ml
  - One copy of CMV DNA (as defined by the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test) is equivalent to 0.91 International Unit (IU) [1.1 cp/IU] on the First WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC 09/162)

- Frequency of testing
  - CMV viral load testing will normally be performed on Mondays, Wednesday and Fridays, otherwise daily when the need arises. Runs will be batched and loaded as per worksheet (Appendix A)
  - In the event where there is a shortage of reagents due to unavailability from the supplier or delays in ordering process, specimens will be referred to another laboratory.

10. TEST ACCEPTANCE CRITERIA

- The run is deemed valid when all the controls have passed, i.e. positive controls should be positive (have amplified CMV DNA target in expected ranges) and negative control should be negative.
- Check AMPLILINK software results window or printout for flags and comments that aid in assessment of run validity.
  - The run is valid if no flags appear for any of the controls [**CMV (–) C, CMV L (+) C** and **CMV H(+)C**].
  - The run is not valid if any of the following flags appear for the CMV Controls.
  - See table for interpretation of different flags.

Control	Fla	Result	Inter retation
Negative	NC_INVALID	Invalid	An invalid result or a "valid" result that was not ne ative for CMV tar et
CMV Low Positive Control	LPCINVALID	Invalid	An invalid result or a control out of range
CMV High Positive Control	HPCINVALID	Invalid	An invalid result or a control out of range

- All the invalid runs should be discussed with registrars, pathologists and medical scientists. There should be an explanation provided for any invalid run if its results are accepted.

11. INTERPRETATION OF RESULTS

- To confirm a valid run, check controls and each individual specimen for flags or comments on the result printout.
- Interpret results in terms of cp/mL or IU/mL as follows:

Titre Results	Interpretation
Target Not Detected	Report results as "LDL".
<1.50E+02 cp/mL	Report results as "LDL".
≥1.50E+02 cp/mL and ≤1.00E+07 cp/mL	Calculated results greater than or equal to 150 CMV DNA cp/mL and less than or equal to 1.00E+07 CMV DNA cp/mL are within the Linear Range of the assay.
>1.00E+07 cp/mL	Calculated cp/mL are above the range of the assay. Report results as "greater than 1.00E+07 CMV DNA cp/mL ". If quantitative results are desired, the original specimen should be diluted with CMV-negative human EDTA-plasma and the test repeated. Multiply the reported result by the dilution factor.
<1.37E+02 IU/mL	Report results as "LDL".
≥1.37E+02 IU/mL and ≤9.10E+06 IU/mL	Calculated results greater than or equal to 137 CMV DNA IU/mL and less than or equal to 9.10E+06 CMV DNA IU/mL are within the Linear Range of the assay.
>9.10E+06 IU/mL	Calculated IU/mL are above the range of the assay. Report results as "greater than 9.10E+06 CMV DNA IU/mL ". If quantitative results are desired, the original specimen should be diluted with CMV-negative human EDTA-plasma and the test repeated. Multiply the reported result by the dilution factor.

NB! Please note, underquantification of CMV Viral load may occur with diluted samples.

- Please note the following:
  - Specimens above the range of the assay that produce an invalid result with a flag "QS_INVALID" should not be reported as > 9.10E+06 IU/mL nor > 1.00E+07 cp/mL. The original specimen should be diluted with CMV-negative EDTA plasma and the test repeated. Multiply the reported result by the dilution factor. Nuclease free water can also be considered as it gave satisfactory results during validation. (See Validation report)
  - Titer Result "Failed". Interpretation: Specimen was not correctly processed.
  - Titer Result "Invalid". Interpretation: An Invalid Result.
- Comments that should be added to results where needed
  - "The quantitative range of CMV DNA is 137 – 910,000 IU/mL for this assay" should be added to all results.
  - The standard canned text on TRAK should be entered where there is a detectable CMV DNA
    - "Asymptomatic CMV viraemia is common in immunocompromised patients and host factors are important in determining the likelihood of disease.

The interpretation of the CMV viral load is therefore difficult as it cannot be standardized between different patient populations. As a guide: In solid organ transplant recipients, a threshold viral load of >10 000copies/ml may be used to prompt treatment, however, treatment may be initiated at a lower viral load if there is clinical evidence of CMV end-organ disease. In haematopoietic stem cell transplant recipients, a viral load cut-off of >1000 copies/ml is commonly used for pre-emptive treatment. HIV-infected patients with CD4 counts <200 cells/ul are at high risk of CMV disease. Unfortunately, there is a lack of data on the diagnostic value of CMV viral load in these patients, and transplant data is often used to guide therapy. Monitoring CMV viral load trends may be useful if indication for treatment is not clear after a single value. Please interpret these results in the context of clinical and other laboratory findings and contact the virology laboratory if you need further advice."

With all the diluted samples:

- "Please note that under quantification of CMV viral loads may occur due to dilution of samples with insufficient input volume"

12. MEASUREMENT OF UNCERTAININTY

12.1 Procedural limitations

- Only personnel trained in the techniques of PCR may use this product
- Reliable results will be influenced by how adequate specimen collection, transport, storage and processing procedures have been carried out.
- This test has been validated for use with only human plasma collected in EDTA anticoagulant. Testing other specimen types may result in inaccurate results.
- Insufficient volume samples may be diluted but this may yield under quantified CMV Viral loads. This product can only be used with the COBAS® AmpliPrep Instrument and the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer.
- Contamination from CMV positive controls and clinical specimens. This can be avoided only by good laboratory practices and careful adherence to the procedures specified in this package insert.
- Mutations within the highly conserved regions of the viral genome covered by the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test primers and/or probes may result in the under quantitation of or failure to detect the virus
- Detection of CMV DNA is dependent on the number of virus particles present in the specimen and may be affected by specimen collection methods and patient factors, (ie, age, presence of symptoms, and/or stage of the infection).
- The clinical decision thresholds for viral load values in International Units/mL (IU/mL) have not been established. Clinicians are advised to use the appropriate conversion factor from IU/mL to copies/mL (cp/mL) when making clinical decisions using results reported in IU/mL.

12.2 Interfering substances

- Elevated levels of the following were not shown to interfere with the quantitation of CMV DNA or impact the specificity of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test.

Trigl cerides	u to 3300 m /dL
Con'u ated bilirubin	u to 20 m /dL
Uncon'u ated bilirubin	u to 20 m /dL
Haemo lobin	up to 200 m /dL
Human DNA	u to 0.4 m /dL
Autoimmune diseases or respective markers	Systemic Lupus Erythematous, Rheumatoid Arthritis and Antinuclear Antibod

- Grossly haemolysed specimens must be rejetcted.
- The evaluation was performed according to CLSI Guideline EP7-A2using one lot of COBAS® AmpliPrep/COBAS® TaqMan® CMV Test reagents.

12.3 Precision and reproducibility

The CMV Viral load testing is performed in accordance with the test procedure. To evaluate the test measurement precision, this is done by using quantity values obtained by measurement of daily quality control materials. This involves precision under repeatability conditions where the results are obtained with the same method, on the same test in the same laboratory using the same equipments at the same location within intervals of time. The measurement of uncertainty performance shall be reviewed regularly at least once a month and trend analysis done.

For the test reproducibility the precision will be evaluated by reviewing the EQA results, where the test results are obtained against the same method on the same similar test item by different operators using different equipment in different locations.

> According to ISO 15189 clauses 5.5.1.4 "The laboratory shall determine the measurement uncertainty for each measurement procedure in the examination phase used to report measured quantity values on patient samples. This is easily accomplished when a normal value for measurand and standardised reference materials exists (such as serum sodium in biochemistry laboratory). However, it becomes problematic in Virology laboratory when a normal value cannot be established because viral concentrations occur over a range of values that usually corresponds to illness severity (). Also, standardised reference materials from third party suppliers are either no existent or prohibitively expensive

13. INTERNAL QUALITY CONTROL AND EXTERNAL QUALITY CONTRO

13.1 Internal quality control

- One replicate of the Negative Control, the CMV Low Positive Control and the CMV High Positive Control must be included in each run.
- The run is valid if no flags appear for any of the controls [**CMV (–) C, CMV L (+) C and CMV H(+) C**].
- Negative control
  - The CMV (–) C must yield a "Target Not Detected" result. If the CMV (–) C is flagged as invalid, then the entire run is invalid and should be repeated.

- Positive controls
  - The CMV DNA cp/mL for CMV L (+) C and CMV H (+) C should fall within their acceptable titre ranges as provided on the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test reagent cassette barcodes. If one or both of the positive controls are flagged as invalid, then the entire run is invalid and should be repeated.

13.2 External quality control

The laboratory is enrolled on an EQA programme on an ongoing basis.

Appendix 3

Research protocol

Title: The clinical presentation and outcome of CMV disease in paediatric patients at Chris Hani Baragwanath Academic Hospital- a Retrospective, descriptive study.

Investigator: Hermina Mmabatho Tsholanang Nke

MBChB, DCH, Dip HIV Man

Student no: 1729710

Degree: MMed by submissible format

Supervisors: Dr K. L. Petersen

Dr R. K. Mopeli

1. Background

Virology

Cytomegalovirus (CMV) is a double stranded DNA virus belonging to the human herpes viruses (4,5). It is an ubiquitous agent that causes infection at any stage during the course of life (4) .The natural host immune response to primary cytomegalovirus does not clear the virus completely, therefore it persists in a latent state (6) . CMV infection is usually subclinical and asymptomatic in immunocompetent individuals (7). However it can cause significant morbidity in immunocompromised states , such as human immunodeficiency virus infected patients, organ transplant recipients and new-born babies (7).

Transmission

The transmission of CMV is through body fluids and may be acquired through breastmilk, respiratory secretions or blood products.

Diagnosis: disease versus infection

Cytomegalovirus infection is defined as the presence of viral replication without symptoms while CMV disease is the presence of viral replication with evidence of disease (8,9). The challenge lies in determining the significance of positive CMV laboratory results in confirming CMV disease as opposed to latent infection or viral shedding (10). An autopsy based study in HIV infected adults reported that a CMV viral load (CMV VL) cut off of 10 000copies/ml has a specificity and positive predictive value of 100% in predicting CMV disease (11). A CMV VL of 10 000copies/ ml or more is associated with a high risk of CMV disease in solid organ transplant recipients while the value is as low as 1000copies /ml in stem cell transplant recipients (12). There are also reports of CMV disease in autopsy or biopsy samples without detectable CMV VL (9,12). A study done in the United States of America on transplant patients reported three patients with biopsy proven CMV with undetectable CMV VL and another study in South Africa reported a patient with a CMV VL of 880copies/ml with the presence of end organ disease (8,12).

Epidemiology

Primary CMV infection occurs early in life in low income settings compared to high income settings (6). In Africa CMV infection is acquired during infancy (13). High maternal seroprevelance, premastication of food and breastfeeding contribute to high seroprevalenece of CMV in African children (13).

Laboratory diagnosis

CMV infection may be diagnosed using serological tests, antigenemia assays, nucleic acid testing performed on body fluids, cell cultures and immunohistochemistry performed on tissue.

Serological tests are used to determine the presence of previous CMV infection by the presence of CMV IgG (14). An acute or recent infection is indicated by the presence of IgM (14). However CMV IgM lacks specificity for primary infection because IgM can persist for months after primary infection (14).

Antigenemia assays such as pp65 are used for CMV virus quantification in blood specimen. It depends on the use of monoclonal antibodies that detect the viral pp65 antigen (14). The test is limited to detection of the virus in leucocytes (14). In addition it is labour intensive , the samples have to be processed within six hours and in neutropenic patients false negative results may occur (14). The viral pp65 is not routinely performed at Chris Hani Barangwanath Academic Hospital (CHBAH).

Nucleic acid testing is used to detect CMV nucleic acid in the blood and other body fluids (15). It is performed by using polymerase chain reaction(PCR) targeting viral DNA or mRNA (15). Quantitative nucleic acid testing(QNAT) measures CMV VL in whole blood, plasma, serum, cerebrospinal fluid(CSF), aqueous and vitreous humour, respiratory samples and in urine (15). Whole blood samples usually have high viral loads compared to plasma and serum (15,16). Whole blood preparations are more sensitive than cell free plasma but their specificity may be compromised by detection of latent non replicating virus (15).

CMV VL is important in assessing risk of disease, diagnosing disease and monitoring response to therapy(16). The interpretation of CMV VL values can be difficult, and various factors like specimen type should be taken into consideration when assessing risk of CMV disease (16) .

Clinical manifestation

In older children CMV disease may be isolated or disseminated. In neonates it manifests as congenital CMV.

Congenital CMV is diagnosed when CMV is detected in body fluids within three weeks of birth. The prevalence of congenital CMV is approximately 0.6% in developed countries and 1%-5% in developing world (5). A study done at CHBAH reported an incidence of 0.26/1000 live births (17) . It may be asymptomatic in as many as 85%-90% of patients (5) . The clinical presentation of symptomatic congenital CMV is jaundice, pethechia, hepatomegaly, splenomegaly, microcephaly and sensorineural hearing loss (5,18). Sensorineural hearing loss may present at birth or may be delayed (5). Laboratory investigations may show thrombocytopenia, transaminitis and conjugated hyperbilirubinaemia (18). Periventricular calcifications may be seen on skull Xray or cranial sonar.

The diagnosis of CMV pneumonitis currently is based on clinical findings of severe pneumonia with hypoxia, laboratory findings, presence of CMV VL in bronchoalveolar lavage and evidence of interstitial lung disease on x-rays (10,19). There is a challenge in distinguishing asymptomatic CMV infection from CMV pneumonia, because of this challenge the terms used are CMV related pneumonitis or CMV associated pneumonits (defined as CMV DNA PCR positive with clinical and x-ray features of CMV) and pneumonia with CMV infection (defined as CMV DNA PCR positive without clinical and radiological features of CMV(20). The definitive diagnosis of CMV pneumonitis requires lung biopsy for histological confirmation(19). It is difficult to make an accurate diagnosis of CMV pneumonits without histological confirmation(19). A study done at the University of Kwa Zulu Natal showed that quantification of CMV in broncho alveolar lavage is more predictive of CMV pneumonitis compared to serum viral load (10). The quantification of CMV in bronchoalveolar lavage is not routinely done at CHBAH.

A study done at Stellenbosch University reported histologically confirmed CMV pneumonia in 72% of cases (21).

Gastrointestinal CMV may presents as colitis, esophagitis and hepatitis. Patients with CMV colitis have dysentery while those with eosophagitis have dysphagia, anorexia and weight loss (22). In paediatric patients CMV has a predominance of oesophageal and small bowel involvement(23)

The prevalence of CMV retinitis (CMV –R) in children with probable systemic CMV infection is unknown in South Africa (7). A study done in Cape Town showed a prevalence of probable CMV-R of 4.9% (7). It is reported that CMV-R occurs more frequently in adults (7). It presents with blurred vision, floaters, photophobia but may be asymptomatic (22). CMV retinopathy is diagnosed on fundoscopy and changes of active CMV- R are retinal vasculitis, retinal necrosis producing oedema associated with haemorrhage (7,22).

CMV of the central nervous system may present as encephalitis, transverse myelitis and acute inflammatory polyradiculopathy (22). Patients with encephalitis have headaches and personality changes. Acute inflammatory demyelinating polyradiculopathy presents with progressive ascending parasthesiae,sensory loss and arreflexia (22).

Treatment

The treatment of CMV disease includes systemic and local products, local products being for ophthalmological indications (22). The cost of drugs licenced for the treatment of CMV precludes treatment in resource limited countries. In South Africa ganciclovir and valgancilovir are registered for the systemic treatment of CMV. Current costs for ganciclovir and valganciclovir for the public sector are as follows, a vial of ganciclovir 500mg/5ml costs R395.20, valganciclor 50mg/ml suspension (100ml) costs R1762.88 and valganciclovir 450mg 60 tablets cost R10280.29. The treatment of CMV disease is controversial. Ganciclovir and valganciclovir are associated with haematological adverse events especially neutropenia, anaemia and thrombocytopenia (22).

2. Problem statement

Systemic CMV VL testing is used in the diagnosis of CMV disease. Most studies on CMV VL were done in transplant patients and adult patients. There is a need to determine the clinical presentation and outcome of patients with CMV viraemia and CMV disease in the general paediatric population.

3. Objectives

- To determine the spectrum of clinical presentation of patients with detectable CMV VL and CMV disease including tissue diagnosis.
- To determine the outcomes in patients who received treatment and those who did not.

4. Methods

Study design

A retrospective record review of children who were admitted at Chris Hani Baragwanath Academic hospital with detectable CMV VL (as per National health Laboratory Services) from 01 January 2016 to 31st December 2017. It is a retrospective descriptive and analytic study.

Sample population

Inclusion Criteria

The sample population will include all children between the ages of 0 days and 14 years identified in the following ways:

- Patients with detectable CMV VL from the National Health Laboratory services (NHLS) and, or
- Patients with CMV related deaths as assessed by Child Health and Mortality Prevention Surveillance (CHAMPS) study at Respiratory and Meningeal Pathogens Research Unit (RMPRU) with permission and, or
- Patients who have received ganciclovir as per CHBAH pharmacy records.

Definitions

The following definition will apply:

- CMV infection: presence of viral replication with no signs and symptoms,
- CMV disease: presence of viral load >1000 with evidence of disease.
- Congenital CMV: the detection of CMV in body fluids within three weeks of birth.
- Severe pneumonia: lower respiratory tract infection characterized by severe respiratory distress (grunting, severe chest indrawings) and/or oxygen saturation of <90%.

  Signs of pneumonia with general danger signs (inability to breastfeed or drink, lethargy or unconsciousness.
- Colitis: chronic diarrhoea (diarrhoea for more than two weeks) or dysentery
- Hepatitis: elevation of liver enzymes by 2 to 3 folds.
- Thrombocytopaenia: Platelet count of <140
- Anaemia: Hb concentration, Hct or RBC mass > 2 SD below the mean for normal population
- Neutropaenia: absolute neutrophil count >2SD below the mean for age and race
- Severe acute malnutrition: weight for height < -3SD or Mid upper arm Circumference <115mm or the presence of oedema of both feet.
- Moderate acute Malnutrition: weight for height <-2SD
- Acute inflammatory demyelinating polyradiculopathy: ascending paralysis

Setting

The study will be conducted at CHBAH, a public government hospital in Johannesburg, which serves the community of Soweto and is a referral centre for southern Gauteng and other provinces.

4. Data collection

Records will be retrieved from archived bed letters and data will be extracted as per Appendix A. Patients' confidentiality will be maintained by use of coded data sheets instead of names.

5. Data Analysis

Data collected will be captured using Microsoft excel 2010. Descriptive statistics such as mean, standard deviation, median and interquartile range will be used for continuous variables while frequency and percentage will be used for categorical variables. The association between categorical factors will determined using Chi- Square test. The outcome such as death and morbidity will be analysed.

6. Ethics

Ethical clearance will be requested from Human Research Ethics Committee of the University of the Witwatersrand before data is collected. As this is a retrospective record review, no consent will be needed from individual patients or parents. Permission will be obtained from hospital authorities to access patient records.

7. Cost /Funding

The cost involved is for stationery and printing and will be covered by the investigator.

8. Limitation

This retrospective record review may be limited by the availabilty and quality of hospital records.

9. Timeline

	Sep2017	Oct 2017	Nov 2017	Dec 2017	Jan 2018	Feb 2018	March 2018	April 2018	May 2018	June 2018	July 2018	Aug 2018	Sept 2018
Literature review													
Protocol preparation													
Ethics approval													
Post graduate approval													
Hospital approval													
Department of health approval													
Data collection													
Data analysis													
Write up report													

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Appendix A

Study no:

Date of specimen

Sex □ Male □Female

Date of Birth: Age

Ward:

CMV VL	
Congenital cytomegalovirus	□Yes □No Jaundice □Yes □ No Hepatomegaly □Yes □No Splenomegaly □Yes □ Ventriculomegaly □Yes □No Periventricular calcifications □ Yes □ No Microcephaly □Yes □No
Severe Pneumonia	□Yes □No
Colitis	□Yes □No
Hepatitis	□Yes □No
Thrombocytopenia (platelets < 140) Neutropenia Anaemia	□yes □No □ Yes □No □Yes □No
SAM (W/H <-3SD)	□Yes □No
MAM (W/H <-2SD)	□Yes □No
HIV status at presentation PMTCT NVP HAART	□ Neg □Pos □Exposed □ Unknown Maternal ART □Yes □No □Unknown □Yes □No □Unknown □ Yes □No Duration
Retinitis	□Yes □No
Acute inflammatory demyelinating polyradiculopathy	□Yes □No
Encephalitis	□ Yes □No

Other	
gancyclovir	□Yes □ No Duration
Admission in ICU Admission date	□Yes □No
Outcome	□death □discharge □transfer □unknown Date of death/discharge /transfer

Appendix 4

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Dr HMT Nke

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M180355

NAME: (Principal Investigator)	Dr HMT Nke
DEPARTMENT:	School of Clinical Medicine Department of Paediatrics and Child Health Medical School University
PROJECT TITLE:	The clinical presentation and outcome of CMV disease in paediatric patients at Chris Hani Baragwanath Hospital - a retospective, descriptive study
DATE CONSIDERED:	06/04/2018
DECISION:	Approved unconditionally
CONDITIONS:	**New title noted on 12/01/2021**
SUPERVISOR:	Drs K Petersen and Dr RK Mopeli
APPROVED BY:	Dr N Naran, Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	27/06/2018

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **«Missing mail merge field»** and therefore reports and re-certification will be due in the month of **«Missing mail merge field»** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator Date

Appendix 5

final MMED draft for submission.docx

ORIGINALITY REPORT

9%	6%	8%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	bmcinfectdis.biomedcentral.com Internet Source	1%
2	repository.up.ac.za Internet Source	1%
3	Submitted to University of Witwatersrand Student Paper	<1%
4	qmro.qmul.ac.uk Internet Source	<1%
5	"Oral Abstracts", American Journal of Transplantation, 2020 Publication	<1%
6	O.L. Caballero, M.C.S.L. Costa, A. Trevisan, R.M. Oliveira et al. "Monitoring human cytomegalovirus viral load in peripheral blood leukocytes of renal transplant recipients by a simple limiting dilution-PCR assay", Brazilian Journal of Medical and Biological Research, 1999 Publication	<1%

Appendix 6

Author Guidelines

SAJCH Author Guidelines

Please view the Author Tutorial for guidance on how to submit on Editorial Manager.

To submit a manuscript, please proceed to the *SAJCH* Editorial Manager website: Editorial Manager

There is currently a backlog of articles in production. Authors submitting new articles should note that the earliest date for publication will be in the second quarter of 2022.

To access and submit an article already in production, please see the SAJCH author guidelines here.

Please take the time to familiarise yourself with the policies and processes below. If you still have any questions, please do not hesitate to ask our editorial staff (tel.: +27 (0)21 532 1281, email: submissions@hmpg.co.za).

Article Processing Charges

All articles published in the South African Journal of Child Health are open access and freely available online upon publication. This is made possible by applying a business model to offset the costs of peer review management, copyediting, design and production, by charging an article-processing charge (APC) of R4 000 (incl Vat) for all articles published. The charge applies only to Research articles submitted after 1 Jan 2019. The APC is standard and does not vary based on length, colour, figures, or other elements.

When submitting a Research article to the SAJCH, the submitting author must agree to pay the APC should the article be accepted for publication. The APC is payable when your manuscript is editorially accepted and before production commences for publication. The submitting author will be notified that payment is due and given details on the available methods of payment. Prompt payment is advised; the article will not enter into production until payment is received.

Queries can be directed to claudian@hmpg.co.za.

Please refer to the section on 'Sponsored Supplements' regarding the publication of supplements, where a charge is applicable. Queries can be directed to dianes@hmpg.co.za or claudian@hmpg.co.za

Authorship

Named authors must consent to publication. Authorship should be based on: (*i*) substantial contribution to conceptualisation, design, analysis and interpretation of data; (*ii*) drafting or critical revision of important scientific content; or (*iii*) approval of the version to be published. These conditions must all be met for an individual to be included as an author (uniform requirements for manuscripts submitted to biomedical journals; refer to www.icmje.org)

If authors' names are added or deleted after submission of an article, or the order of the names is changed, all authors must agree to this in writing.

Please note that co-authors will be requested to verify their contribution upon submission. Non-verification may lead to delays in the processing of submissions.

Author contributions should be listed/described in the manuscript.

Conflicts of interest

Conflicts of interest can derive from any kind of relationship or association that may influence authors' or reviewers' opinions about the subject matter of a paper. The existence of a conflict – whether actual, perceived or potential – does not preclude publication of an article. However, we aim to ensure that, in such cases, readers have all the information they need to enable them to make an informed assessment about a publication's message and conclusions. We require that both authors and reviewers declare all sources of support for their research, any personal or financial relationships (including honoraria, speaking fees, gifts received, etc) with relevant individuals or organisations connected to the topic of the paper, and any association with a product or subject that may constitute a real, perceived or potential conflict of interest. If you are unsure whether a specific relationship constitutes a conflict, please contact the editorial team for advice. If a conflict remains undisclosed and is later brought to the attention of the editorial team, it will be considered a serious issue prompting an investigation with the possibility of retraction.

Research ethics committee approval

Authors must provide evidence of Research Ethics Committee approval of the research where relevant. Ensure the correct, full ethics committee name and reference number is included in the manuscript.

If the study was carried out using data from provincial healthcare facilities, or required active data collection through facility visits or staff interviews, approval should be sought from the relevant provincial authorities. For South African authors, please refer to the guidelines for submission to the National Health Research Database. Research involving human subjects must be conducted according to the principles outlined in the Declaration of Helsinki. Please refer to the National Department of Health's guideline on Ethics in Health research: principles, processes and structures to ensure that the appropriate requirements for conducting research have been met, and that the HPCSA's General Ethical Guidelines for Health Researchers have been adhered to.

Clinical trials

Since 1st December 2005, all clinical trials conducted in South Africa have been required to be registered in the South African National Clinical Trials Register. The *SAJCH* therefore requires that clinical trials be registered in the relevant public trials registry at or before the time of first patient enrollment as a condition for publication. The trial registry name and registration number must be included in the manuscript.

Protection of rights to privacy

Patient

Information that would enable identification of individual patients should not be published in written descriptions, photographs, radiographs and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) has given informed written consent for publication and distribution. We further recommend that the published article is disseminated not only to the involved researchers but also to the patients/participants from whom the data was drawn. Refer to Protection of Research Participants. The signed consent form should be submitted with the manuscript to enable verification by the editorial team.

Other individuals

Any individual who is identifiable in an image must provide written agreement that the image may be used in that context in the *SAJCH.*

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The *SAJCH* is committed to protecting the privacy of its website and submission system users. The names, personal particulars and email addresses entered in the website or submission system will not be made available to any third party without the user's permission or due process. By registering to use the website or submission system, users consent to receive communication from the *SAJCH* or its publisher HMPG on matters relating to the journal or associated publications. Queries with regard to privacy may be directed to publishing@hmpg.co.za.

Ethnic/race classification

Use of racial or ethnicity classifications in research is fraught with problems. If you choose to use a research design that involves classification of participants based on race or ethnicity, or discuss issues with reference to such classifications, please ensure that you include a detailed rationale for doing so, ensure that the categories you describe are carefully defined, and that socioeconomic, cultural and lifestyle variables that may underlie perceived racial disparities are appropriately controlled for. Please also clearly specify whether race or ethnicity is classified as reported by the patient (self-identifying) or as perceived by the investigators. Please note thatit is not appropriate to use self-reported or investigator-assigned racial or ethnic categories for genetic studies.

Continuing Professional Development (CPD)

SAJCH is an HPCSA-accredited service provider of CPD materials. Principal authors can earn up to 15 CPD continuing education units (CEUs) for publishing an article; co-authors are eligible to earn up to 5 CEUs; and reviewers of articles can earn 3 CEUs. Each month, *SAJCH* also publishes a CPD-accredited questionnaire relating to the academic content of the journal. Successful completion of the questionnaire with a pass rate of 70% will earn the reader 3 CEUs. Administration of our CPD programme is managed by Medical Practice Consulting. To complete questionnaires and obtain certificates, please visit MRP Consulting

MANUSCRIPT PREPARATION

Preparing an article for anonymous review

To ensure a fair and unbiased review process, all submissions are to include an anonymised version of the manuscript. The exceptions to this requirement are Editorials, Correspondence, Book reviews and Obituary submissions.

Submitting a manuscript that needs additional blinding can slow down your review process, so please be sure to follow these simple guidelines as much as possible:

- An anonymous version should not contain any author, affiliation or particular institutional details that will enable identification.
- Please remove title page, acknowledgements, contact details, funding grants to a named person, and any running headers of author names.
- Mask self-citations by referring to your own work in third person.

General article format/layout

Submitted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction prior to being sent for review, which will delay publication.

General:

- Manuscripts must be written in UK English (this includes spelling).
- The manuscript must be in Microsoft Word or RTF document format. Text must be 1.5 line spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes). Pages and lines should be numbered consecutively.
- Please make your article concise, even if it is below the word limit.
- Qualifications, ***full*** affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.
- Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.
- Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dL).
- Litres is denoted with an uppercase L e.g. 'mL' for millilitres).
- Units should be preceded by a space (except for % and ºC), e.g. '40 kg' and '20 cm' but '50%' and '19ºC'.
- Please be sure to insert proper symbols e.g. µ not u for micro, a not a for alpha, b not B for beta, etc.
- Numbers should be written as grouped per thousand-units, i.e. 4 000, 22 160.
- Quotes should be placed in single quotation marks: i.e. The respondent stated: '...'
- Round brackets (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.

If you wish material to be in a box, simply indicate this in the text. You may use the table format –this is the *only* exception. Please DO NOT use fill, format lines and so on.

SAJCH is a Journal on child health, therefore for articles involving genetics, it is the responsibility of authors to apply the following:

- Please ensure that all genes are in italics, and proteins/enzymes/hormones are not.
- Ensure that all genes are presented in the correct case e.g. TP53 not Tp53.

** NB: Copyeditors cannot be expected to pick up and correct errors wrt the above, although they will raise queries where concerned.

- Define all genes, proteins and related shorthand terms at first mention, e.g. '188del11' can be glossed as 'an 11 bp deletion at nucleotide 188.'

- Use the latest approved gene or protein symbol as appropriate:

- Human Gene Mapping Workshop (HGMW): genetic notations and symbols
- HUGO Gene Nomenclature Committee: approved gene symbols and nomenclature
- OMIM: Online Mendelian Inheritance in Man (MIM) nomenclature and instructions
- Bennet et al. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Counsel 2008;17:424-433: standard human pedigree nomenclature.

Preparation notes by article type

Research

Guideline word limit: 3 000 words (excluding abstract and bibliography)

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Where appropriate, sample size calculations should be included to demonstrate that the study is not underpowered. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

- May include up to 3 illustrations or tables.
- A max of 20 - 25references

Structured abstract

- This should be no more than 250words, with the following recommended headings:
  - **Background:** why the study is being done and how it relates to other published work.
  - **Objectives:** what the study intends to find out
  - **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
  - **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
  - **Conclusion:** must be supported by the data, include recommendations for further study/actions.
  - Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors. It should be able to be intelligible to the reader without referral to the main body of the article.
  - Do not include any references in the abstracts.

Here is an example of a good abstract.

***Scientific letters*/short reports**

These include case reports, side effects of drugs and brief or negative research findings.

Guideline word limit: 1500 words

- Abstract: unstructured, of about 100-150 words
- May include only one illustration or table
- A maximum of 6 references

Editorials

Guideline word limit: 1 000 words

These opinion or comment articles are usually commissioned but we are happy to consider and peer review unsolicited editorials. Editorials should be accessible and interesting to readers without specialist knowledge of the subject under discussion and should have an element of topicality (why is a comment on this issue relevant now?) There should be a clear message to the piece, supported by evidence.

Please make clear the type of evidence that supports each key statement, e.g.:

- expert opinion
- personal clinical experience
- observational studies
- trials
- systematic reviews.

Review articles

Review articlesshould always be discussed with the Editor prior to submission.

Guideline word limit: 4 000 words

These are welcome, but should be either commissioned or discussed with the Editor before submission. A review article should provide a clear, up-to-date account of the topic and be aimed at non-specialist hospital doctors and general practitioners. They should be aligned to practice in South and/or sub-Saharan Africa and not a precis of reviews published in the international literature

Please ensure that your article includes:

- Abstract: unstructured, of about 100-150 words, explaining the review and why it is important
- Methods: Outline the sources and selection methods, including search strategy and keywords used for identifying references from online bibliographic databases. Discuss the quality of evidence.
- When writing: clarify the evidence you used for key statements and the strength of the evidence. Do not present statements or opinions without such evidence, or if you have to, say that there is little or no evidence and that this is opinion. Avoid specialist jargon and abbreviations, and provide advice specific to southern Africa.
- Personal details: Please supply your qualifications, position and affiliations and MP number (used for CPD points); address, telephone number and fax number, and your e-mail address; and a short personal profile (50 words) and a few words about your current fields of interest.

Correspondence (Letters to the Editor)

Guideline word limit: 400 words

Letters to the editor should relate either to a paper or article published by the SAJCH or to a topical issue of particular relevance to the journal's readership

- May include only one illustration or table
- Must include a correspondence address.

Obituaries

Guideline word limit: 400 words

Should be offered within the first year of the practitioner's death, and may be accompanied by a photograph.

Illustrations/photos/scans

- If illustrations submitted have been published elsewhere, the author(s) should provide evidence of consent to republication obtained from the copyright holder.
- Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'.
- Each figure must have a caption/legend: Fig. 1. Description (any abbreviations in full).
- All images must be of high enough resolution/quality for print.
- All illustrations (graphs, diagrams, charts, etc.) must be in PDF form.
- Ensure all graph axes are labelled appropriately, with a heading/description and units (as necessary) indicated. Do not include decimal places if not necessary e.g. 0; 1.0; 2.0; 3.0; 4.0 etc.
- Scans/photos showing a specific feature e.g. *Intermediate magnification micrograph of a low malignant potential (LMP) mucinous ovarian tumour. (H&E stain).* –include an arrow to show the tumour.
- Each image must be attached individually as a 'supplementary file' upon submission (not solely embedded in the accompanying manuscript) and named Fig. 1, Fig. 2, etc.

Tables

- Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.
- Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author.
- Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.
- Number each table in Arabic numerals (Table 1, Table 2, etc.) consecutively as they are referred to in the text.
- Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable.
- Ensure each table has a concise title and column headings, and include units where necessary.
- Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

Do not: Use [Enter] within a row to make 'new rows':

Rather:

Each row of data must have its own proper row:

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- Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.
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- Wherever possible, references must be accompanied by a digital object identifier (DOI) link). Authors are encouraged to use the DOI lookup service offered by CrossRef:
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Some examples:

- *Journal references:* Price NC, Jacobs NN, Roberts DA, et al. Importance of asking about glaucoma. Stat Med 1998;289(1):350-355. http://dx/doi.org/10.1000/hgjr.182
- *Book references:* Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975:96-101.
- *Chapter/section in a book:* Weinstein L, Swartz MN. Pathogenic Properties of Invading Microorganisms. In: Sodeman WA, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974:457-472.
- *Internet references:* World Health Organization. The World Health Report 2002 - Reducing Risks, Promoting Healthy Life. Geneva: WHO, 2002. http://www.who.int/whr/2002 (accessed 16 January 2010).
- Legal references
- Government Gazettes:

National Department of Health, South Africa. National Policy for Health Act, 1990 (Act No. 116 of 1990). Free primary health care services. Government Gazette No. 17507:1514. 1996.

In this example, 17507 is the Gazette Number. This is followed by :1514 - this is the notice number in this Gazette.

- Provincial Gazettes:

  Gauteng Province, South Africa; Department of Agriculture, Conservation, Environment and Land Affairs. Publication of the Gauteng health care waste management draft regulations. Gauteng Provincial Gazette No. 373:3003, 2003.

- Acts:

  South Africa. National Health Act No. 61 of 2003.

- Regulations to an Act:

  South Africa. National Health Act of 2003. Regulations: Rendering of clinical forensic medicine services. Government Gazette No. 35099, 2012. (Published under Government Notice R176).

- Bills:

  South Africa. Traditional Health Practitioners Bill, No. B66B-2003, 2006.

- Green/white papers:

  South Africa. Department of Health Green Paper: National Health Insurance in South Africa. 2011.

- Case law:

  Rex v Jopp and Another 1949 (4) SA 11 (N)

  Rex v Jopp and Another: Name of the parties concerned

  1949: Date of decision (or when the case was heard)

  (4): Volume number

  SA: SA Law Reports

  11: Page or section number

  (N): In this case Natal - where the case was heard. Similarly, (C) woud indicate Cape, (G) Gauteng, and so on.

  NOTE: no . after the v

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