Journal of Medical Virology 89:1080–1087 (2017)

Clinical Utility of Bronchoalveolar Lavage Cytomegalovirus Viral Loads in the Diagnosis of Cytomegalovirus Pneumonitis in Infants

Kerusha Govender,[1,2]* Prakash Jeena,[3] and Raveen Parboosing[1,2]

[1]*Department of Virology, National Health Laboratory Service, Durban, KwaZulu Natal, South Africa*
[2]*Virology, College of Health Sciences, University of KwaZulu-Natal, Durban, KwaZulu Natal, South Africa*
[3]*Paediatrics and Child Health, College of Health Sciences, University of KwaZulu-Natal, Durban, KwaZulu Natal, South Africa*

Cytomegalovirus (CMV) pneumonitis is a significant cause of morbidity and mortality of children in Africa. The current practice for diagnosing CMV pneumonitis in this setting is based on interpretation of clinical, laboratory, and radiological findings. There is a need for a sensitive and specific laboratory test to objectively distinguish between patients with CMV pneumonitis and those with CMV infection, and non-CMV pneumonia. In this study, we compared plasma and non-bronchoscopic bronchoalveolar lavage (NBBAL) CMV viral loads in patients with CMV pneumonitis and those with CMV infection and non-CMV pneumonia. Receiver operator characteristic curve analysis was used to establish a threshold and assess utility of viral loads in the diagnosis of CMV pneumonitis. We assessed the urea dilution method, and expression of viral loads relative to the total amount of extracted nucleic acids in correcting for NBBAL dilution. CMV quantification in NBBAL specimens was more predictive of CMV pneumonitis than blood CMV quantification. The threshold of 4.03 log IU/ml in NBBAL specimens has good predictive value and can be used to guide management of infants with suspected CMV pneumonitis. Adjusting for dilution of NBBAL specimens by using the urea dilution method or by expressing the viral load relative to the total nucleic acids extracted did not provide additional analytical benefits. ***J. Med. Virol. 89:1080–1087, 2017***.

KEY WORDS: CMV pneumonia; CMV quantification; CMV diagnosis; urea dilution method; paediatric CMV

INTRODUCTION

Cytomegalovirus is a cause of pneumonia in immunocompromised hosts including transplant recipients, patients with malignancy, and HIV-infected patients [Richman et al., 2009]. The clinical problem of cytomegalovirus (CMV) infection in HIV-exposed and -infected infants with severe pneumonia has been described in Africa since the emergence of HIV, and is still a cause of morbidity and mortality in these patients [Frenkel et al., 1990; Jeena et al., 1996; Chintu et al., 2002; Zampoli et al., 2011; Kitchin et al., 2012; Hsiao et al., 2013]. In patients presenting with severe pneumonia in this setting, the diagnosis of cytomegalovirus pneumonia is made by a combination of clinical and laboratory features [Kitchin et al., 2012; Hsiao et al., 2013]. This may require an experienced or expert opinion which is not always available, especially in peripheral areas. The diagnosis may be delayed while clinicians exclude other causes of pneumonia and evaluate the patient's response to empiric treatment. Treatment decisions remain difficult because there is no highly predictive

Grant sponsor: Discovery Foundation; Grant sponsor: National Health Laboratory Service Research Trust

Conflict of interest: None.

*Correspondence to: Kerusha Govender, Department of Virology, National Health Laboratory Service, Private Bag X03, Mayville 4058, KwaZulu Natal, South Africa. E-mail: govenderk7@ukzn.ac.za

Accepted 30 October 2016

DOI 10.1002/jmv.24730
Published online 5 December 2016 in Wiley Online Library (wileyonlinelibrary.com).

and clinically useful investigative tool for diagnosing CMV pneumonitis [Zampoli et al., 2011].

A part of the challenge is determining the significance of a positive CMV laboratory result in confirming CMV disease as opposed to clinically insignificant latent infection or viral shedding [Compston et al., 2009]. The existence of CMV as a co-pathogen compounds this problem [Bates et al., 2013]. The proportion of the pulmonary disease that is actually due to CMV in this situation remains an unanswered question [Boeckh and Geballe, 2011].

Serology and the newer cellular immunology assays have limited clinical value because of the derangement of immunological functions in HIV-infected people. Merely detecting the presence of the CMV genome by blood polymerase chain reaction (PCR) alone has poor predictive value for CMV disease, as it may represent latent virus [Wiselka et al., 1999]. A test that identifies the amount of virus that is actively replicating by detecting viral antigens, such as pp65, may be useful. However, the sensitivity of the assay is affected by low peripheral blood leukocyte counts and requires timeous transport of the specimens to the laboratory with skilled personnel to perform the test. Histology, often considered a highly specific gold standard, has a limited role in routine diagnostics due to the risks in sampling lung tissue of critically ill patients [Goussard et al., 2010; Ross et al., 2011].

Quantification of CMV viraemia by real time PCR is routinely used in the transplant setting to diagnose and pre-empt disease. CMV quantification in respiratory specimens may represent local viral replication and disease within the compartment being investigated. In lung transplant recipients, the quantification of CMV in bronchoalveolar lavage fluid is used to pre-empt disease [Westall et al., 2004; Kotton et al., 2013]. However, standardization of respiratory specimens is a limitation of this type of specimen [Radhakrishnan et al., 2014].

In this study, we quantified CMV in non-bronchoscopic bronchoalveolar lavage (NBBAL) and plasma specimens in infants with severe pneumonia requiring intermittent positive pressure ventilation. We assessed the viral loads against the standard practice of diagnosing CMV pneumonitis through clinical and other laboratory features. We analysed the CMV viral loads directly from the patient NBBAL specimens as well as in the extracted nucleic acid and epithelial lining fluid. The objective of this study was to evaluate the clinical utility of quantitative real time PCR in respiratory specimens in diagnosing CMV pneumonitis in infants.

MATERIALS AND METHODS

Study Population

The University of KwaZulu-Natal Biomedical Research Ethics Committee approved the study (Reference number BF203/09). Patients were recruited in the paediatric intensive care unit of Inkosi Albert Luthuli Central Hospital in KwaZulu-Natal, South Africa. This is an academic quaternary level hospital, with high level expertise compared with other facilities in the province. The antenatal prevalence of HIV in the KwaZulu-Natal province in 2012 was 37.4% [National Department of Health, 2013] and the pneumonia incidence rate in children under five was 119 cases per 1,000 children [Massyn et al., 2013]. The patients recruited for the study were infants with suspected CMV infection or disease, presenting with severe pneumonia requiring ventilation. Those who had received ganciclovir therapy for the presenting illness were excluded from the study.

Specimen Collection

Patients being admitted for severe pneumonia received diagnostic tests at admission as per routine standard of care in the unit. These included screening for HIV infection and investigating NBBAL specimens or endotracheal aspirates (or both) for tuberculosis, *Pneumocystis jiroveci*, respiratory viruses (influenza, parainfluenza, adenovirus, and respiratory syncytial virus), cytomegalovirus as well as microscopy and culture for other pathogens of the lower respiratory tract including fungal and bacterial infections.

Informed consent was obtained from patients' legal guardians, for CMV quantification to be performed on blood and NBBAL specimens in addition to the routine tests listed above.

The NBBAL procedure was done as previously described [Singh et al., 2013]. Briefly, intubated patients were monitored by electrocardiography and pulse oximetry and pre-oxygenated with 100% inspired oxygen for appropriately 1 min before the procedure.

A suction catheter was inserted as far as possible via a closed suction circuit and the first sample discarded to exclude contamination. The catheter was then reintroduced and 1 ml/kg 0.9% saline at room temperature was injected through the side port while tilting the patient to the side. Secretions were suctioned while chest physiotherapy and postural drainage were performed. The child was then tilted to the opposite side and the procedure repeated. The procedure was stopped if the oxygen saturation dropped to <85% and temporarily stopped if there was a transient drop in saturation to between 85% and 90%.

HIV PCR

EDTA blood specimens were tested for HIV by the real-time PCR COBAS® AmpliPrep/COBAS® TaqMan HIV-1 Qualitative test (Roche® Molecular Systems, Inc., Branchburg, NJ), as per South African national guidelines.

CMV Quantification in Plasma and Respiratory Specimens

Following nucleic acid extraction of plasma and NBBAL specimens on the NucliSens easyMAG system (bioMerieux, France), quantitative real-time CMV PCR was performed using the LightCycler CMV Quant kit (Roche Diagnostics GmbH, Mannheim, Germany), on the LightCycler 2.0 instrument (Roche). This method was calibrated to international units using the 1st WHO International Standard for human cytomegalovirus for nucleic acid testing (NAT)-based assays (NIBSC code 09/162), obtained from the National Institute for Biological Standards and Controls (NIBSC; Hertfordshire, UK). Calibration was performed by testing the known quantified standard in replicate and calculating a conversion factor. Results were interpreted and recorded independently of the clinical diagnosis.

CMV Quantification in The Epithelial Lining Fluid

The urea dilution method was used to correct for the dilutional effect of instilling saline during the NBBAL procedure. Urea was quantified in NBBAL specimens using the colorimetric Quanti- Chrom™ Urea Assay Kit (Gentaur, Paris, France). A dilution factor was determined for each patient by comparing NBBAL and plasma urea levels. A simple calculation allowed expression of the NBBAL CMV viral load as epithelial lining fluid (ELF) viral load [Bauer et al., 2007].

CMV Quantification in Nucleic Acid Extracts

Total nucleic acids were quantified using the BioSpec-nano spectrophotometer (Shimadzu, Japan) as a measure of the amount of nucleic acids present in the NBBAL specimen. The NBBAL CMV viral load was then expressed according to the total amount of nucleic acid extracted.

Definitions

The standard practice in this paediatric intensive care unit for diagnosing CMV disease was based on a combination of clinical features at the time of presentation and the positive detection of virus in the lower respiratory tract. In this setting, a team of paediatric critical care specialists decided on the use of ganciclovir by assessing the criteria shown below on a case-by-case basis. They were blinded to the viral load results for the duration of the study, as at the time of recruitment, CMV viral loads were not standard of care.

CMV pneumonitis. CMV pneumonitis was defined according to a combination of clinical, radiological, and laboratory findings, adapted from an AIDS Clinical Trial Group definition [Wohl et al., 2009].

CMV pneumonitis was diagnosed when:

(i) there were features of CMV disease;
(ii) a chest X-ray showing predominantly reticular interstitial changes;
(iii) presence of CMV in the NBBAL by PCR testing and
(iv) a lack of clinical response to standard empiric treatment.

CMV-infected patients with non-CMV pneumonia. These were patients with presence of CMV by PCR testing, in whom the clinical presentation was due to a non-CMV cause. They were regarded as CMV-infected with non-CMV pneumonia if CMV was detected in either NBBAL or blood specimens, but the pneumonia was due to another cause, as determined by the attending paediatric critical care specialist. Radiological and clinical features informed the diagnosis such as dense opacification on chest X-ray and clinical improvement on standard empiric treatment, at the time that the CMV result was obtained.

CMV-uninfected patients. CMV-uninfected patients were those in whom blood and NBBAL specimens were CMV PCR negative.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY). The data was analysed by non-parametric analysis of variance testing and *P*-values were calculated to determine the significance of the associations. In order to correct for left-censoring of the data set, absolute values were modelled for those specimens which were below the detection or quantification limits of the assay, by assigning them midpoint values between those limits prior to log transformation [Jacqmin-Gadda et al., 2000].

Viral load results were assessed against the clinical diagnosis as defined above. Receiver operator characteristic (ROC) curve analysis was used to determine optimum cut-off points.

RESULTS

Patient Characteristics

Eighty seven infants with severe pneumonia were recruited for the study over a period of 2 years from May 2011 to May 2013. Twenty nine of these patients (33.33%) were CMV-infected and diagnosed with CMV pneumonitis. Twenty five (28.74%) were CMV-infected with non-CMV pneumonia and 33 (37.93%) were CMV-uninfected.

The characteristics of the CMV-uninfected, CMV-infected with non-CMV pneumonia, and CMV pneumonitis groups of patients are shown in Table I. A number of patients had multiple coinfections. The number and type of coinfection are listed for each patient group. Tests for significance showed that patients who have CMV pneumonitis are less likely to be respiratory syncytial virus

TABLE I. Patient Characteristics

Characteristic	All patients	CMV pneumonitis	CMV-infected with non-CMV pneumonia	CMV-uninfected patients
N (%)	87	29 (33.33%)	25 (28.74%)	33 (37.93%)
Mean age in months (95% C.I., $P < 0.05$)	3.7 (2.8–4.5)	3.5 (2.9–4.2)	3.2 (2.0–4.3)	4.1 (2.1–6.2)
Female gender (%)	44 (50.57%)	12 (41.38%)	12 (48.00%)	20 (60.61%)
HIV-infected (%)	34 (39.08%)	17 (58.62%)	8 (32.00%)	9 (27.27%)
Number of non-CMV coinfections (%)				
0	18 (20.69%)	9 (31.03%)	3 (12.00%)	6 (18.18%)
1	35 (40.23%)	9 (31.03%)	13 (52.00%)	13 (39.39%)
2	19 (21.84%)	7 (24.14%)	5 (20.00%)	7 (21.21%)
3	11 (12.64%)	4 (13.79%)	2 (8.00%)	5 (15.15%)
4	4 (4.60%)	0 (0.00%)	2 (8.00%)	2 (6.06%)
Coinfections (%)				
Viral infections				
Respiratory syncytial virus	22 (25.29%)	3 (10.34%)	11 (44.00%)	8 (24.24%)
Adenovirus	13 (14.94%)	6 (20.69%)	3 (12.00%)	4 (12.12%)
Parainfluenza	6 (6.90%)	1 (3.45%)	0 (0.00%)	5 (15.15%)
Influenza	3 (3.45%)	0 (0.00%)	1 (4.00%)	2 (6.06%)
Other viruses	6 (6.90%)	1 (3.45%)	1 (4.00%)	4 (12.12%)
Non-viral infections				
Acinetobacter baumannii	18 (20.69%)	5 (17.24%)	7 (28.00%)	6 (18.18%)
Klebsiella pneumoniae	15 (17.24%)	3 (10.34%)	4 (16.00%)	8 (24.24%)
Pneumocystis jiroveci	10 (11.49%)	3 (10.34%)	3 (12.00%)	4 (12.12%)
Candida albicans	12 (13.79%)	5 (17.24%)	1 (4.00%)	6 (18.18%)
Mycobacterium tuberculosis	6 (6.90%)	1 (3.45%)	4 (16.00%)	1 (3.03%)
Other bacteria	13 (14.94%)	5 (17.24%)	3 (12.00%)	5 (15.15%)

N, the number of the subjects of each group.

(RSV) infected ($P = 0.018$) and more likely to be HIV-infected ($P = 0.008$) than those who have non-CMV pneumonia.

Viral Loads

There was a significant difference in the mean viral loads between the CMV-infected with non-CMV pneumonia and the CMV pneumonitis groups of patients in NBBAL, plasma specimens, the dilution-adjusted, and the extraction-adjusted NBBAL measurements (Table II). The boxplots in Figure 1 shows the distribution of the viral loads between both groups of patients in the four specimen types, with differences in the median viral loads between the groups.

ROC curves for predicting CMV pneumonitis generated for CMV viral loads on each specimen type are shown in Figure 2. They illustrate the performance of the CMV viral load in each specimen type in discriminating CMV infection from CMV disease. The ROC statistics are shown in Table III. The NBBAL had a larger area under the ROC curve than other specimen types. The threshold of 4.03 log IU/ml in NBBAL was chosen based on sensitivity, specificity, and positive and negative predictive values compared with other thresholds for this specimen type. Adjusting CMV quantification for dilution and for extracted nucleic acids did not improve the area under the curve.

DISCUSSION

This study shows that quantifying CMV in NBBAL specimens is useful in diagnosing CMV pneumonitis in infants and is more predictive than plasma viral load. Other studies have shown similar results in lung transplant recipients [Chemaly et al., 2004; Westall et al., 2004], and in wheezy immunocompetent infants [Cinel et al., 2014], but to our knowledge, respiratory specimen quantification has not

TABLE II. Comparison of Mean Viral Loads in Patients With Infection Versus Disease

Type of measurement	CMV-pneumonitis	CMV-infected with non-CMV pneumonia	*P*-value
Mean NBBAL CMVVL (log IU/ml)	5.00	3.78	<0.0001
Mean Plasma CMVVL (log IU/ml)	4.17	3.50	0.0134
Mean ELF VL (log IU/ml)	6.58	5.45	0.0031
Mean NBBAL nucleic acid extract CMVVL (IU/ng nucleic acid extracted)	4.06	2.99	0.0014

NBBAL, non-bronchoscopic bronchoalveolar lavage; CMVVL, cytomegalovirus viral load; ELF, epithelial lining fluid.

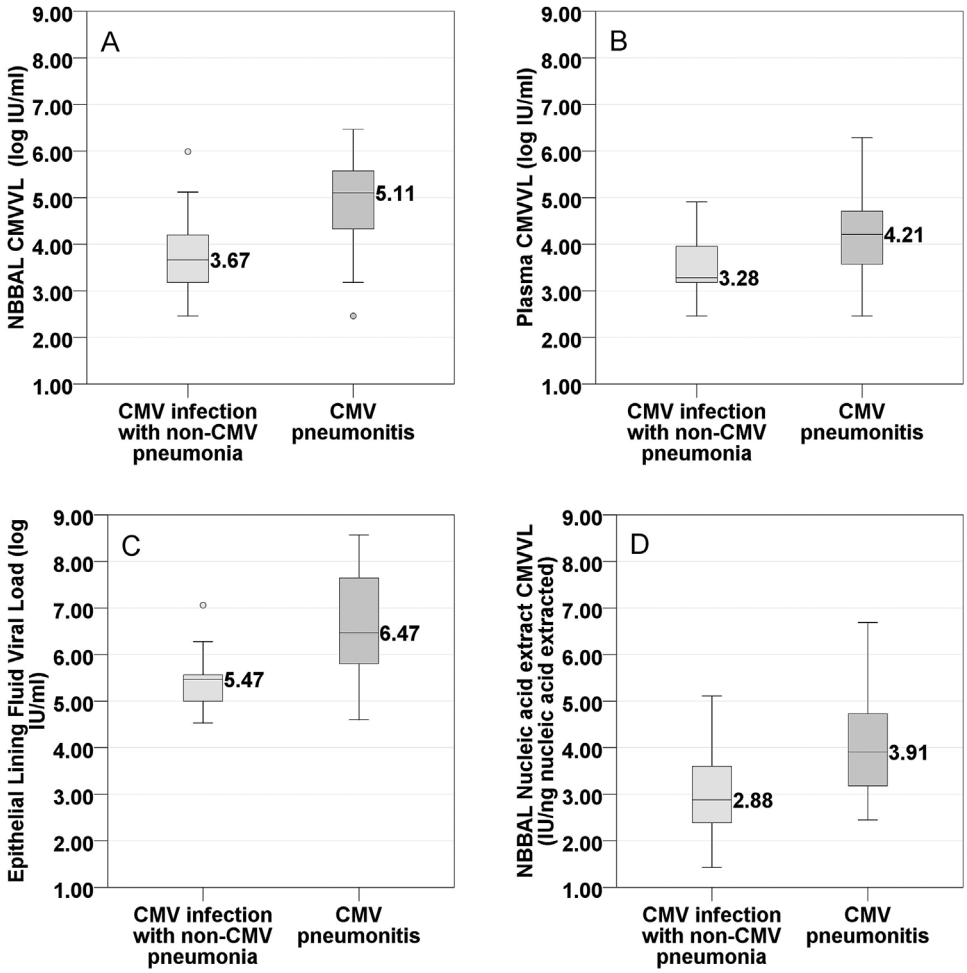

Fig. 1. Boxplot analyses of cytomegalovirus viral loads (CMVVL) showing differences in distribution and median viral load between patients with CMV infection and non-CMV pneumonia and patients with CMV pneumonia. **(A)** Boxplot showing difference in CMVVL in non-bronchoscopic bronchoalveolar lavage (NBBAL) specimens. **(B)** Boxplot showing difference in plasma CMVVL. **(C)** Boxplot showing difference in epithelial lining fluid viral load. **(D)** Boxplot showing difference in CMVVL of NBBAL normalized for nucleic acid content.

previously been evaluated in infants with severe pneumonia admitted to ICU.

Defining CMV end organ disease is a recognized problem, especially in the presence of coinfections [Ljungman et al., 2002]. The definitions used in this study reflected the standard that was being practiced in this intensive care unit. They comprised clinical and investigative evidence and response to therapy, to decide on whether the clinical picture was attributable to CMV or not. The final clinical decision was based on specialist interpretation of clinical features and investigations. The fact that the clinicians were blinded to the CMV viral load results, and viral loads correlated with disease lends credence to the definitions being used. We have effectively evaluated the CMV viral loads against a clinical decision. The CMV viral load may be useful for objectively discriminating infection from disease, especially in more peripheral areas where there may be fewer expertise. A possible benefit would be the reduction of unnecessary and unsafe transfers to specialised centers for care. Current practice requires exclusion of other causes of pneumonia, which involves waiting for other laboratory results such as microbial cultures, and may require waiting to evaluate the response to other treatment before a CMV diagnosis is made. This delay is especially relevant in settings such as ours, with a high number of coinfections. A CMV viral load may allow the clinician to evaluate the likelihood of CMV disease and start antiviral treatment earlier.

CMV viraemia can be used to predict and pre-empt CMV pneumonitis and guide ganciclovir use. The threshold of 4.03 log IU/ml in NBBAL specimens that was found in this study is comparable with that of 4.1 log genomes/ml in blood specimens which was previously proposed [Hsiao et al., 2013]. However, our sensitivity and specificity were superior (89.3% and 75.0%, respectively, compared with 76% and 69%, respectively), probably because respiratory

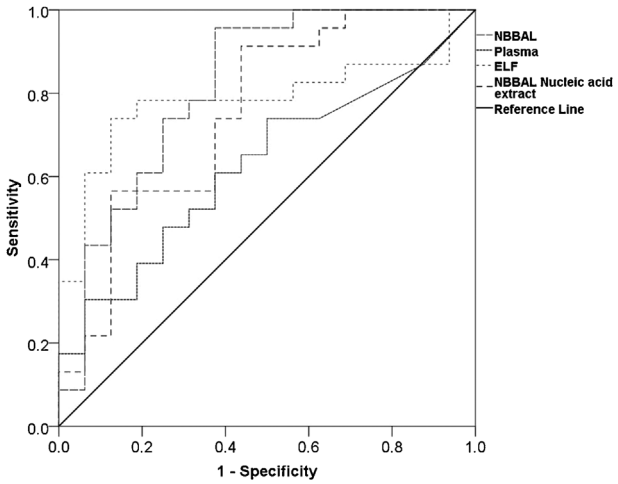

Fig. 2. Receiver operator characteristic curve analyses of the CMV viral loads measured in various specimen types for predicting CMV pneumonitis. (NBBAL, non-bronchoscopic bronchoalveolar lavage; ELF, epithelial lining fluid). Area under the curve and optimal cut-off points were determined for each specimen type.

specimens are more representative of end-organ pulmonary disease than blood specimens. The utility of blood CMV quantification is limited because blood viral loads can be confounded by the transient viraemia that accompanies the acute perinatal infection which occurs in up to 93% of HIV-exposed infants by 6 months of age [Slyker et al., 2009]. As shown in the results above and by others [Jeena et al., 1996; Chintu et al., 2002; Goussard et al., 2010; Hsiao et al., 2013], HIV infection plays a role in the development of CMV pneumonitis and a more appropriate specimen type is therefore required.

Compartmentalization of CMV disease has been hypothesized, and has implications for viral pathogenesis, diagnosis, and patient treatment [Frange et al., 2013; Slyker et al., 2014]. The diagnostic implication of CMV compartmentalization has been studied in other CMV end-organ diseases, such as the quantification in cerebrospinal fluid and intestinal biopsies [Miller et al., 2006; Ganzenmueller et al., 2009]. It has been suggested that end-organ disease is a result of viraemia together with overwhelmed local immune responses, and although viraemia precedes end-organ disease, blood viral loads are not always predictable or predictive at the time of end-organ disease [Zuckerman, 2009; Kotton et al., 2013].

A high proportion of patients had co-infections, which reflects the high pathogen load in this setting. The significant relationship between HIV and CMV pneumonia has been described before [Hsiao et al., 2013]. There was a significant association between the CMV-infected with non-CMV pneumonia group and RSV infection. This may be related to the differing risk factors and patient profiles for these pathogens.

Multicenter studies have shown that there is poor inter-laboratory correlation of CMV viral loads [Pang et al., 2009; Kraft et al., 2012]. This led to an international standard, which was used in this study so that viral loads may be expressed in international units and conclusions may be portable to other centers.

However, respiratory specimens themselves have an inherent variability because of the dilutional effect of saline lavages, as well as differences in cellularity between specimens. In an attempt to standardize the specimen, we used the urea dilution method to quantify the amount of epithelial lining fluid present in the NBBAL specimen. This method has previously been shown to improve the correlation between CMV pneumonitis and CMV quantification in respiratory specimens [Bauer et al., 2007]. However, we found that quantifying CMV in epithelial lining fluid was not superior to quantifying it in NBBAL specimens directly. A moderate improvement in specificity and positive predictive value were achieved using this method, however the area under the ROC curve did not improve as there was a trade-off in the sensitivity achieved. A possible reason for this was that a manual plate-based method of urea determination was used, introducing a further variability in the final CMV quantification in ELF. An automated method should be utilized in future studies of ELF [Pocino et al., 2015].

Another method of characterizing the NBBAL specimen is determination of total cellular nucleic acids in the NBBAL extracts [Tembo et al., 2015]. This might allow expression of the CMV viral load as a function of the amount of nucleic acids present in an extract, which could be a surrogate marker of the specimen cellularity. The method has been used extensively in

TABLE III. Receiver Operator Characteristic (ROC) Curve Statistics

Type of measurement	Area under the curve	Threshold	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
NBBAL viral load	0.834	4.03 log IU/ml	89.3	75.0	80.6	85.7
Plasma viral load	0.686	3.56 log IU/ml	75.9	66.7	73.3	69.6
Epithelial lining fluid viral load	0.783	5.63 log IU/ml	78.3	81.2	85.7	72.2
NBBAL nucleic Acid extract viral load	0.787	3.18 IU/ng	76.9	64.7	76.0	61.1

NBBAL, non-bronchoscopic bronchoalveolar lavage.

mRNA expression research. However, we found this technique also did not improve the clinical utility of CMV quantification in NBBAL specimens. This is consistent with previous findings that nucleic acid determination is subject to individual patient variation as well as technical factors such as nucleic acid degradation and that these factors compound PCR errors [Bustin, 2000; Huggett et al., 2005]. However, a study of betaherpesvirus loads in Zambian children found that normalizing for the amount of DNA in a specimen did affect the findings of their research in HIV-infected children [Tembo et al., 2015]. Further studies of this method may be necessary.

Standardizing viral quantification in respiratory specimens may be achievable using housekeeping genes. Such genes have been identified for respiratory specimens [Stock et al., 2011] and may have significant clinical utility as part of a multiplex reaction in which CMV and host cell markers are simultaneously detected. Further studies looking at standardizing respiratory specimens are warranted.

A limitation of the study was that we lacked data on HIV exposure of the patients. Laboratory confirmation of maternal HIV status is not always possible in this intensive care unit as patient guardians often reside in distant areas and are not under the direct clinical care of the paediatrician. Knowledge of HIV exposure would have enhanced the data analysis [Izadnegahdar et al., 2014] but was beyond the scope of this study.

Long term follow-up of these patients is not always possible. Some patients in this province live in remote areas and their follow-up may be more economically feasible if they are followed up by the base hospital to which they have been down-referred after discharge from ICU. Specifically, information on those patients who were CMV-infected, but not treated as CMV disease would be useful to obtain clinical insight. Furthermore, a description of patients with low viral loads would inform interpretation of these results.

We have shown that CMV quantification in NBBAL specimens is more predictive of CMV pneumonitis than plasma CMV quantification. When compared with the standard method of diagnosis, the threshold of 4.03 log IU/ml in NBBAL specimens has good predictive value and can be used to guide management of infants with suspected CMV pneumonitis. Adjusting for dilution of NBBAL specimens by using the urea dilution method or by expressing the viral load relative to the total nucleic acids extracted did not provide additional analytical benefits.

ACKNOWLEDGMENTS

The authors are grateful to the patients, parents and guardians of patients, clinical staff, and laboratory staff of Inkosi Albert Luthuli Central Hospital for their part in this research.

REFERENCES

The 2012 National Antenatal Sentinel HIV and Herpes Simplex type-2 prevalence Survey in South Africa, National Department of Health. 2013. https://www.health-e.org.za/wp-content/uploads/2014/05/ASHIVHerp_Report2014_22May2014.pdf

Bates M, Mudenda V, Mwaba P, Zumla A. 2013. Deaths due to respiratory tract infections in Africa: A review of autopsy studies. Curr Opin Pulm Med 19:229–237.

Bauer CC, Jaksch P, Aberle SW, Haber H, Lang G, Klepetko W, Hofmann H, Puchhammer-Stockl E. 2007. Relationship between cytomegalovirus DNA load in epithelial lining fluid and plasma of lung transplant recipients and analysis of coinfection with Epstein-Barr virus and human herpesvirus 6 in the lung compartment. J Clin Microbiol 45:324–328.

Boeckh M, Geballe AP. 2011. Cytomegalovirus: Pathogen, paradigm, and puzzle. J Clin Invest 121:1673–1680.

Bustin SA. 2000. Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. J Mol Endocrinol 25:169–193.

Chemaly RF, Yen-Lieberman B, Castilla EA, Reilly A, Arrigain S, Farver C, Avery RK, Gordon SM, Procop GW. 2004. Correlation between viral loads of cytomegalovirus in blood and bronchoalveolar lavage specimens from lung transplant recipients determined by histology and immunohistochemistry. J Clin Microbiol 42:2168–2172.

Chintu C, Mudenda V, Lucas S, Nunn A, Lishimpi K, Maswahu D, Kasolo F, Mwaba P, Bhat G, Terunuma H, Zumla A. 2002. Lung diseases at necropsy in African children dying from respiratory illnesses: A descriptive necropsy study. Lancet 360:985–990.

Cinel G, Pekcan S, Ozcelik U, Alp A, Yalcin E, Dogru Ersoz D, Kiper N. 2014. Cytomegalovirus infection in immunocompetent wheezy infants: The diagnostic value of CMV PCR in bronchoalveolar lavage fluid. J Clin Pharm Ther, 39:399–403.

Compston LI, LI C, Sarkodie F, Owusu-Ofori S, Opare-Sem O, Allain JP. 2009. Prevalence of persistent and latent viruses in untreated patients infected with HIV-1 from Ghana, West Africa. J Med Virol 81:1860–1868.

Frange P, Boutolleau D, Leruez-Ville M, Touzot F, Cros G, Heritier S, Moshous D, Neven B, Fischer A, Blanche S. 2013. Temporal and spatial compartmentalization of drug-resistant cytomegalovirus (CMV) in a child with CMV meningoencephalitis: Implications for sampling in molecular diagnosis. J Clin Microbiol 51:4266–4269.

Frenkel LD, Gaur S, Tsolia M, Scudder R, Howell R, Kesarwala H. 1990. Cytomegalovirus infection in children with AIDS. Rev Infect Dis 12:S820.

Ganzenmueller T, Henke-Gendo C, Schlue J, Wedemeyer J, Huebner S, Heim A. 2009. Quantification of cytomegalovirus DNA levels in intestinal biopsies as a diagnostic tool for CMV intestinal disease. J Clin Virol 46:254–258.

Goussard P, Kling S, Gie R P, Nel ED, Heyns L, Rossouw GJ, Janson JT. 2010. CMV pneumonia in HIV-infected ventilated infants. Pediatr Pulmonol 45:650–655.

Hsiao NY, Zampoli M, Morrow B, Zar HJ, Hardie D. 2013. Cytomegalovirus viraemia in HIV exposed and infected infants: Prevalence and clinical utility for diagnosing CMV pneumonia. J Clin Virol 58:74–78.

Huggett J, Dheda K, Bustin S, Zumla A. 2005. Real-time RT-PCR normalisation; strategies and considerations. Genes Immun 6:279–284.

Izadnegahdar R, Fox MP, Jeena P, Qazi SA, Thea DM. 2014. Revisiting pneumonia and exposure status in infants born to HIV-infected mothers. Pediatr Infect Dis J 33:70–72.

Jacqmin-Gadda H, Thiebaut R, Chene G, Commenges D. 2000. Analysis of left-censored longitudinal data with application to viral load in HIV infection. Biostatistics 1:355–368.

Jeena PM, Coovadia HM, Chrystal V. 1996. Pneumocystis carinii and cytomegalovirus infections in severely ill, HIV-infected African infants. Ann Trop Paediatr 16:361–368.

Kitchin OP, Masekela R, Becker P, Moodley T, Risenga SM, Green RJ. 2012. Outcome of human immunodeficiency virus-exposed and -infected children admitted to a pediatric intensive care unit for respiratory failure. Pediatr Crit Care Med 13:516–519.

Kotton CN, Kumar D, Caliendo AM, Asberg A, Chou S, Danziger-Isakov L, Humar A. 2013. Updated international consensus

guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation 96:333–360.

Kraft CS, Armstrong WS, Caliendo AM. 2012. Interpreting quantitative cytomegalovirus DNA testing: Understanding the laboratory perspective. Clin Infect Dis 54:1793–1797.

Ljungman P, Griffiths P, Paya C. 2002. Definitions of cytomegalovirus infection and disease in transplant recipients. Clin Infect Dis 34:1094–1097.

Massyn N, Day C, Dombo M, Barron P, English R, Padarath A. District health barometer 2012/13 Durban: Health Systems Trust 2013. http://www.hst.org.za/publications/district-health-barometer-201213

Miller GG, Boivin G, Dummer JS, Mcconnell T, Becher MW, Kassim A, Tang YW. 2006. Cytomegalovirus ventriculoencephalitis in a peripheral blood stem cell transplant recipient. Clin Infect Dis 42:e26–e29.

Pang XL, Fox JD, Fenton JM, Miller GG, Caliendo AM, Preiksaitis JK, American Society of Transplantation Infectious Diseases Community of, P. and Canadian Society of, T. 2009. Interlaboratory comparison of cytomegalovirus viral load assays. Am J Transplant 9:258–268.

Pocino K, Minucci A, Manieri R, Conti G, DE Luca D, Capoluongo ED, 2015. Description of an automated method for urea nitrogen determination in bronchoalveolar lavage fluid (BALF) of neonates and infants J Lab Autom 20;636–641.

Radhakrishnan D, Yamashita C, Gillio-Meina C, Fraser DD. 2014. Translational research in pediatrics III: Bronchoalveolar lavage. Pediatrics 134:135–154.

Richman DD, Whitley RJ, Hayden FG. 2009. Clinical Virology Amer Society for Microbiology. 3rd edition. Washington, DC: ASM Press.

Ross SA, Novak Z, Pati S, Boppana SB. 2011. Overview of the diagnosis of cytomegalovirus infection. Infect Disord Drug Targets 11:466–474.

Singh R, Thula SA, Jeena PM. 2013. Lung infiltrates in antiretroviral-naive HIV-infected children with chronic lung disease: Value of non-bronchoscopic bronchoalveolar lavage in the detection of Candida albicans. J Trop Pediatr 59:59–63.

Slyker J, Farquhar C, Atkinson C, Asbjornsdottir K, Roxby A, Drake A, Kiarie J, Wald A, Boeckh M, Richardson B, Odem-Davis K, John-Stewart G, Emery V. 2014. Compartmentalized cytomegalovirus replication and transmission in the setting of maternal HIV-1 infection. Clin Infect Dis 58:564–572.

Slyker JA, Lohman-Payne BL, John-Stewart GC, Maleche-Obimbo E, Emery S, Richardson B, Dong T, Iversen AK, Mbori-Ngacha D, Overbaugh J, Emery VC, Rowland-Jones SL. 2009. Acute cytomegalovirus infection in Kenyan HIV-infected infants. AIDS 23:2173–2181.

Stock C, Leoni P, Shi-Wen X, Abraham D, Nicholson A, Wells A, Renzoni E, Lindahl G. 2011. Identification of stable housekeeping genes for real-time PCR in human pulmonary fibroblasts. Eur Respir J 38 p3805.

Tembo J, Kabwe M, Chilukutu L, Chilufya M, Mwaanza N, Chabala C, Zumla A, Bates M. 2015. Prevalence and risk factors for betaherpesvirus DNAemia in children >3 weeks and <2 years of age admitted to a large referral hospital in sub-Saharan Africa. Clin Infect Dis 60:423–431.

Westall GP, Michaelides A, Williams TJ, Snell GI, Kotsimbos TC. 2004. Human cytomegalovirus load in plasma and bronchoalveolar lavage fluid: A longitudinal study of lung transplant recipients. J Infect Dis 190:1076–1083.

Wiselka MJ, Nicholson KG, Rowley S, Bibby K. 1999. Cytomegalovirus viraemia has poor predictive value for the development of cytomegalovirus disease in patients with advanced HIV-infection. J Infect 39:187–192.

Wohl DA, Kendall MA, Andersen J, Crumpacker C, Spector SA, Feinberg J, Alston-Smith B, Owens S, Chafey S, Marco M, Maxwell S, Lurain N, Jabs D, Benson C, Keiser P, Jacobson MA. 2009. Low rate of CMV end-organ disease in HIV-infected patients despite low CD4+ cell counts and CMV viremia: Results of ACTG protocol A5030. HIV Clin Trials 10:143–152.

Zampoli M, Morrow B, Hsiao NY, Whitelaw A, Zar HJ. 2011. Prevalence and outcome of cytomegalovirus-associated pneumonia in relation to human immunodeficiency virus infection. Pediatr Infect Dis J 30:413–417.

Zuckerman AJ. 2009. Principles and practice of clinical virology. Chichester, UK; Hoboken: NJ John Wiley & Sons.