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The response to natural RSV infection in HIV-infected children compared to HIV-uninfected children

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THE RESPONSE TO NATURAL RSV INFECTION IN HIV-INFECTED CHILDREN COMPARED TO HIV-UNINFECTED CHILDREN

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree
of
Master of Medicine in the branch of Paediatrics

Johannesburg, 2004

DECLARATION

I, Gillian Ann Sorour, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in Paediatrics in the University of the Witwatersrand, Johannesburg. It has not been submitted for any degree or examination at this or any other university.



Gillian A Sorour

11th day of October 2004

DEDICATION

Dedicated to my family,

Jonathan and Rachel

ABSTRACT

Respiratory syncytial virus (RSV) is the most important virus causing acute respiratory tract infections in children and is an important cause of morbidity and mortality. HIV-infected children are also at an increased risk of recurrent and more severe RSV infections. There are few studies looking at how HIV-infected infants respond immunologically to natural RSV infection.

A prospective study enrolling 39 infants (aged 1-12 months) with natural RSV infection was performed between March and June 2001 at Chris-Hani-Baragwanath hospital. Almost thirteen percent of the children with RSV infection who completed the study were HIV infected. There did not appear to be any difference between the two groups with respect to their humoral (both secretory and serum antibody) immune response to primary RSV infection. The possible reasons for this are elaborated on in the discussion.

ACKNOWLEDGEMENTS

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NOMENCLATURE

RSV	Respiratory syncytial virus
RNA	Ribonucleic acid
Ig	Immunoglobulin
IVIG	Intravenous immunoglobulin
HIV	Human immunodeficiency virus
LRTI	Lower respiratory tract infection
ELISA	Enzyme-linked immunosorbent assay
PCR	Polymerase chain reaction
CDC	Centres for disease control

1.0 INTRODUCTION

1.1. *Respiratory Syncytial Virus (RSV) infection in HIV-infected and HIV-uninfected children*

Respiratory syncytial virus (RSV) has long been recognized as the single most important virus causing acute respiratory tract infections in children.[1] Serologic studies in the 1960's showed that about 50% of infants are infected during the first RSV epidemic that they are exposed to and almost all children are infected by two years of age following exposure to a second RSV epidemic.[2]

RSV is an enveloped RNA virus in the family Paramyxoviridae. There are two major antigenic groups of RSV, designated A and B. [3,4] The viral envelope is studded with 10 different glycoproteins. Of these glycoproteins, two are important, especially in establishing infection. The surface fusion (F) glycoprotein in combination with the attachment (G) glycoprotein is responsible for fusion of the viral envelope with host cell membranes to induce infection and formation of the characteristic "syncytia" in cell cultures. F and G glycoproteins are the only viral components that induce RSV-neutralizing antibodies in the host and are the primary targets of vaccine development. G protein, which helps to mediate the attachment of the virus to host cells, is also primarily responsible for the antigenic diversity among RSV groups (i.e. either group A or B).[3] Due to the heterogeneity of G protein, neutralizing antibodies to G protein of one strain of RSV may not provide complete protection from future infection with a different strain of RSV. In addition to the heterogeneity of the G protein between subgroups A and B, there is also heterogeneity of G protein within each of the

subgroups. The F protein has more homogeneity and RSV neutralizing antibodies against F protein provide better protection against future RSV infection as well as providing protection against other strains of RSV. Based upon the homogeneity of the F protein, monoclonal antibody (palivizumab) has been produced and used in children who are at high risk of developing severe RSV disease.[4]

Many clinical findings and sophisticated animal experiments suggest a key role of immune response to RSV in the pathogenesis of the infection. There appears to be a delicate balance between immunopathology and immunoprotection.[2] Both humoral and cellular effectors, including serum antibody, secretory antibody and major histocompatability complex class 1-restricted cytotoxic T lymphocytes, mediate the immune response to RSV.[2] The humoral immune responses involving secretory and serum antibody protect against upper and lower respiratory infection, whereas cell-mediated immunity seems necessary to terminate RSV infection. [3]

Local secretory IgA is the primary humoral mediator of resistance in the upper respiratory tract; protection of the lower respiratory tract is mediated partly by serum IgG, concentrations of which are boosted with each reinfection.[1] The protective effect of serum neutralizing antibody on the lower respiratory tract is further substantiated by data on the monthly use of RSV intravenous immune globulin (IVIG) or monoclonal antibody in high-risk infants. Palivizumab is a humanized, mouse monoclonal antibody directed against the F protein of RSV and is highly effective against type A and B RSV isolates. Mean trough serum concentrations of antibody that remained above 40μg/ml resulted in a 99% reduction of pulmonary RSV in an animal model.[5] The frequency and severity of RSV infection among children with

RSV-neutralizing antibody titers > 100μg/ml were significantly lower than those among children with lower antibody titers.[4]

Most infants possess maternal anti-RSV antibodies at birth. RSV-specific antibodies usually become undetectable by 4 months of age, but can be found in some infants up until a year. Infants born with higher levels of maternal RSV neutralizing antibodies develop milder illness and at an older age than infants with lower antibody levels. [6]

The ability to respond to viral surface glycoproteins with a protective antibody response appears to be gradually acquired over the first 6-9 months of life. Both serum and secretory antibodies are produced in response to RSV infection, even in very young infants. In young infants, however, the antibody titers that develop are usually low.[7] Infants less than 9 months of age produce less antibody to both the F and the G protein than do older infants and children.[8] In one study done by Murphy et al, one of eight younger infants failed to develop an F antibody response, while three of the eight failed to develop a G antibody response. [8] Furthermore the titer in convalescent sera of G, F and neutralizing antibodies was 8-10 fold lower in younger individuals. [8]

The observation that RSV infections are protracted in children and adults who lack competent cellular immune systems underscores the critical role of cellular immune responses in RSV disease. In mice, CD8+ cytotoxic T lymphocytes clear virus from the lungs but paradoxically may augment lung disease. CD4+ T helper cells also appear to have protective and disease-enhancing roles in RSV-infected mice. [4] Immunity elicited after infection with RSV is of short duration and unlikely to prevent re-infection; however, it may be important in reducing the severity of disease, at least

in the immunocompetent host. [9] In contrast to the high rates of re-infection in the upper respiratory tract with respiratory viruses, the incidence of severe disease in the lower respiratory tract decreases significantly with age or history of previous infection. This observation suggests that the mediators of resistance to infection and disease in the upper and lower respiratory tracts may differ, with more transient local immunity predominating in the upper tract and more durable serum antibodies playing a primary role in the lower tract.[6]

HIV infection results in a number of immunological abnormalities that could affect the frequency, severity or persistence of respiratory virus infection.

HIV-infected children are more likely to present with pneumonia rather than with bronchiolitis following primary natural infection with RSV, when compared to HIV-uninfected children.[10, 11] Ninety-two percent of HIV-infected children presented with pneumonia compared to sixty-eight percent of HIV-uninfected children in one study. [10] Exaggerated cell-mediated responses to RSV antigen and the overproduction of virus-specific IgE seem to be important to the development of wheezing or bronchiolitis in normal children.[12] Children with cell-mediated deficiencies are therefore more likely to present with pneumonia rather than wheezing.

HIV-infected children remain predisposed to developing RSV-associated severe lower respiratory tract infection (LRTI) beyond the first six months of life.[10, 13] RSV re-infections are common causes of hospitalisation of older children and adults who are seriously immunocompromised.[9] It is widely recognized that persons infected with HIV have deficiencies in humoral immunity.[9] Despite the frequent presence of

increased levels of serum immunoglobulin, the ability to produce specific serum antibody to antigens is impaired. The proposed reason for older HIV infected children developing severe LRTI may be due to them not mounting a good initial serum neutralizing antibody response to primary RSV infection compared to HIV-uninfected children.

HIV infected children are also more likely to have concomitant bacterial or pneumocystis jiroveci (previously known as pneumocystis carinii) infection than are HIV uninfected children. [10,11]

The mortality rate in children with RSV infection is greater in HIV infected children when compared to HIV uninfected children. [10,11] This increased mortality is possibly due to the superadded infections seen in HIV infected children, or possibly related to suboptimal immune responses to RSV following natural infection.

RSV nasal shedding is prolonged in HIV-infected children when compared to HIV-uninfected children (a median of 30 days compared to 6 days in one study). [12] Cell-mediated responses are believed to be crucial in clearing RSV from the respiratory tract, which explains the prolonged shedding in HIV-infected children.

The RSV epidemic in South Africa, in HIV-uninfected children occurs in late summer to mid-autumn (March to May), but is more perennial in HIV-infected children.[13] The reason for this may be due to the virus being shed for prolonged periods of time in HIV-infected children [10,11,12]

The development of a vaccine for RSV could reduce both RSV morbidity and mortality. It is hoped that it would be especially beneficial to HIV-infected children who are at an increased risk of more infections than are HIV-uninfected children. RSV infection in HIV-infected children also occurs more often in older children (> 2 years old) than it does in HIV-uninfected children.[13]

A phase 1 trial of a live attenuated intranasal RSV vaccine was recently published. There is preliminary evidence for protection from symptomatic disease on natural re-exposure in infants > 6 months old. The vaccine was unacceptable in younger infants because of side effects of vaccine-related upper respiratory tract congestion that was associated with peak vaccine-virus recovery. [14] However, if HIV-infected children are unable to mount an adequate immune response to natural RSV infection, theoretically the live attenuated RSV vaccine may not be immunogenic or effective among them.

1.2. Objectives

The objectives of this study were:

1.2.1 Primary objective:

1. To describe mucosal IgA antibody synthesis against the F and G-protein of RSV, following natural RSV infection in HIV-infected and HIV-uninfected children.

2. To evaluate serum neutralizing antibody production following natural RSV infection in HIV-infected children and HIV-uninfected children.

1.2.2 Secondary objectives:

Analyse for differences in the clinical presentation between HIV-infected and uninfected children.

2.0 MATERIALS AND METHODS

2.1 *Study site and sample*

The study was conducted between March and June 2001 at the Chris Hani-Baragwanath hospital. All children aged 1-12 months of age who were admitted with a LRTI due to RSV infection were included in the study unless they met the exclusion criteria. Only children resident in Soweto were included in this study.

Table 1: Inclusion and exclusion study criteria

Exclusion criteria	Inclusion criteria
1. Prematurity < 37 weeks gestation	1. Aged 1-12 months
2. Chronic lung disease	2. Documented LRTI
3. Congenital heart disease	3. Resident in Soweto
4. Prior inclusion in this study	
5. Neuromuscular disorders	
6. Primary immunodeficiency disorders	

2.2 Study methods

The escort of the child was interviewed for basic demographic information and a clinical history was obtained. The child was examined and clinical and X-ray findings were documented.

Children were then further stratified as shown in figure 1.

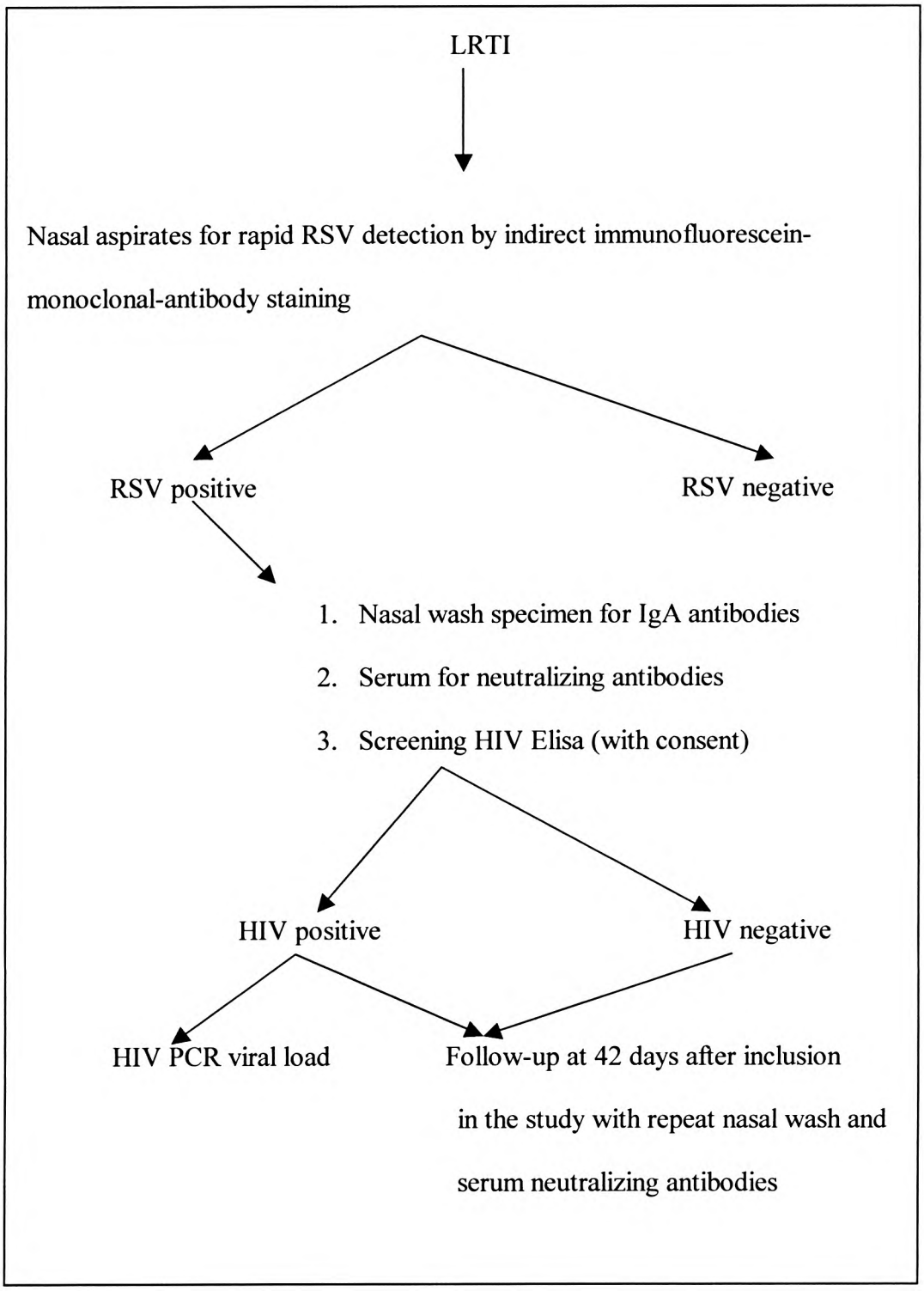

Figure 1: Study methods

An attempt was made to follow up all children included in the study at 42 days for repeat nasal wash and sera samples for detection of RSV antibodies. An appointment was given to the guardian of the child on discharge. If they failed to arrive at their appointment, they were phoned if there was a contact number, or they were visited at their home address and were brought to the hospital.

Nasal aspirates were done with a nasogastric tube (FG 8 10 cm in length, Ven Medical Products, South Africa) attached to a 5 ml syringe. Three milliliters of normal saline was injected into the nasopharynx, aspirated immediately, and placed into a viral transport medium, which was stored at 4°C and transported to the laboratory within 2 hours. Specimens were centrifuged, and the pellet was spread on a slide and fixed. Initial screening was performed by an indirect pooled-immunofluorescein test for respiratory viruses.[13] Positive samples were then tested for respiratory syncytial virus with indirect mouse anti-RSV monoclonal immunofluorescein antibody (Chemicon International Inc, Temecula, Calif). Only patients that tested positive for RSV were included in the study.

Identification of RSV subgroups was by reverse transcription-polymerase chain reaction (RT-PCR). Nasopharyngeal aspirates were thawed on ice and immediately supplemented with 20 units of RNase inhibitor (Roche Molecular Biochemicals, Mannheim, Germany). Total RNA was extracted directly from 200-µl clinical specimens by use of the High Pure RNA kit (Roche Molecular Biochemicals) according to the manufacturer's instructions. In the case of very viscous specimens, the lysates were homogenized using QIAshredder homogenizer columns (Qiagen; Southern Cross Biotechnology, Johannesburg, South Africa).

Subgroups were identified by RT-PCR with group-specific 5′-primers to the G-protein RNA for differentiation between groups A and B as previously described by Sullender et al.[19] The original cDNA synthesis and PCR method described by Sullender et al. was modified in this study to a one step RT-PCR using the TitanOne Tube RT-PCR system (Roche Molecular Biochemicals). PCR products were analyzed by agarose gel electrophoresis in which the expected size for subgroup B viruses was 1.1 kb, whereas 0.9-kb bands indicated subgroup A.

Approximately 5ml of blood was taken on admission and again 6 weeks (42 days) later. The blood was then centrifuged and stored at -70°C.
Serum and nasal wash specimens collected on admission and 42 days later were sent to laboratories at Vanderbilt University School of Medicine, Nashville Tennessee, USA for measurement of RSV neutralizing antibodies and RSV-specific IgA antibody respectively.

2.3 Immunological assays

RSV serum-neutralizing antibodies were detected by a complement-enhanced, 60% plaque reduction neutralization test.[14] Convalescence titer that were four times greater than the admission titer were considered to be significant increases in antibody concentrations.
Nasal wash samples were tested for the presence of IgA antibodies to purified RSV F and G proteins by a kinetic ELISA (kELISA) originally developed for influenza.[14]

The increase in absorbance in milliOD/min of each nasal wash IgA value was expressed as a dilution of a standard serum curve ran in the assay that gave the same increase in absorbance. Initial and post- nasal wash ratios were then compared and if more than or equal to four, were considered a rise. If the initial nasal wash antibody concentration was undetectable (0) and the post antibody concentration was measurable, then this was also considered as a significant increase.
For IgA RSV-G antibody, an assay was only available against RSV subgroup A2. The other assays used were not specific for either sub-group A or B.

2.4 *HIV testing*

The parent or guardian gave informed consent for HIV testing after receiving pre-test counselling. Children were tested for HIV infection using the HIV ELISA test. If they tested positive with the ELISA, then they were further evaluated by HIV-1 RNA PCR (Roche Amplicor, version 1.5, Nutley, USA). If the PCR was <400, then the child was considered to be HIV uninfected. The CDC classification for children (<13 years of age) with HIV infection was used to stage HIV positive children.[15]

2.5 Definitions

The definitions developed by the World Health Organization for the program for the control of acute respiratory infections were used: [16]

2.5.1 Lower Respiratory Tract Infection

This was defined if at least one of the following criteria was present:
(1) Tachypnoea (≥ 60 per minute in a child < 2months and ≥ 50 per minute in a child aged 2-12 months) and the presence of lower chest wall indrawing, or (2) stridor in a calm child, or (3) wheezing, or (4) apnoea. Apnoea was defined as a witnessed episode by medical personnel of cessation of breathing for > 20secs, or cardio-respiratory arrest or bradycardia associated with cessation of breathing of any duration, or a history of the child being cyanosed having stopped breathing as volunteered by the escort.

2.5.2 Bronchiolitis

This was defined if there was evidence of a LRTI (as above) with bilateral diffuse wheezing and clinical or radiological evidence of hyperinflation.

2.5.3 Pneumonia

Pneumonia was defined if there was evidence of a LRTI (as above) and the criteria for bronchiolitis were not met coupled with crepitations and bronchial breathing

on clinical examination and/or radiological evidence of pulmonary infiltrates on CXR.

2.6 Ethics and consent

Ethics approval was obtained from the Ethics Committee for Research on Human Subjects at the University of the Witwatersrand. The ethics clearance protocol number was M01-01-15. An informed consent form described what tests would be performed on the child and stated that participation in the trial was entirely voluntary. Informed consent for HIV testing was performed if the attending physician had not done this. All other investigations performed were part of the routine investigations done on children admitted with a LRTI.

2.7 Statistical analysis

The statistical analysis compared the outcome variables between HIV uninfected and HIV infected patients.

For continuous variables, the comparison was done using a two-tailed Student's t-test. For discrete variables the comparison was done using a two-tailed chi-square test or Fisher's exact test if the expected cell value was less than 5 observations. An alpha of ≤ 0.05 was considered as being significant. Since this was a descriptive study, no adjustment was made for multiple testing.

Due to wide variation in antibody titers, the data was log transformed to $\log_2$. Geometrical mean antibody titers were then calculated using the log transformed data.

Demographic and clinical data involved all children enrolled in the study, even if they didn't complete the study. For example, all 7 HIV infected children had their clinical and demographic data analysed, even though only 5 HIV infected children completed the study and had their antibody responses analysed.

3.0 RESULTS

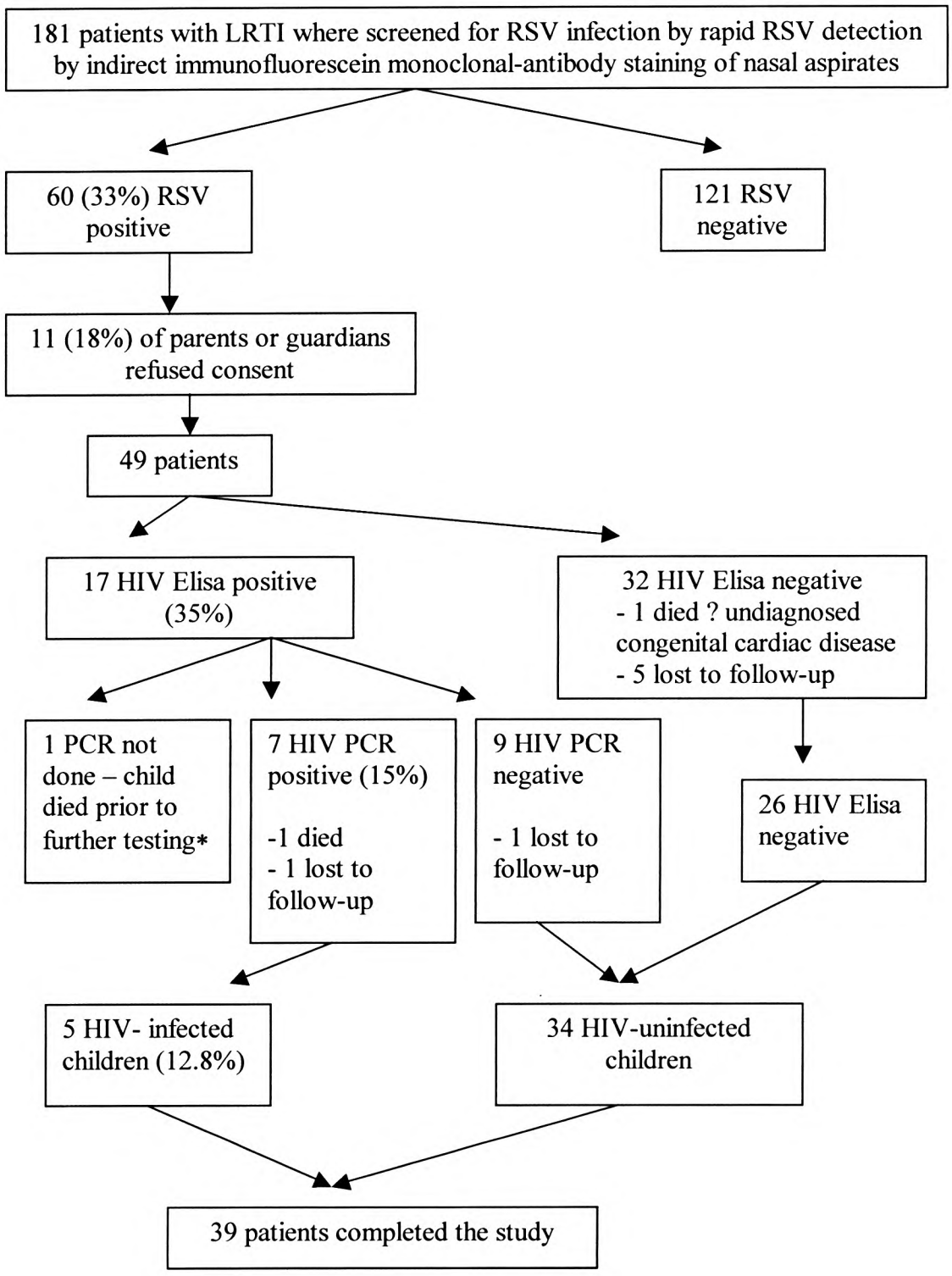

Figure 2: A schematic representation of the number of children that were screened and subsequently enrolled into the study, as well as the loss to follow-up during the course of the study. * Not included in analysis as HIV status unknown

In total, 7 (14%) of the 49 patients who gave consent to be included in the study were lost to follow-up and 3 children (6%) died before completion of the study.

When clinical characteristics were analysed, all patients (except one child who was HIV Elisa positive but PCR unknown) who had been consented and in whom data was recorded were analysed. In terms of measurement of RSV neutralizing and nasal wash antibodies, only the 39 patients who completed the study were analysed.

There were no significant differences in the age or sex of the HIV infected children compared to the HIV uninfected children (see table 2). However, HIV infected children tended to be older than HIV uninfected children.

According to the Wellcome classification all of the HIV-infected children were well nourished. In the HIV-uninfected group there were four children who were underweight and one child who had kwashiorkor. The median weight for age z scores were significantly different between the two groups and showed that the HIV-infected children that were sick enough to be hospitalised were more nutritionally compromised than the HIV-infected group.

Table 2: Demographic data of HIV infected and HIV uninfected children*

		HIV uninfected N=41 (85%)	**HIV infected N=7 (14.5%)**	***P* value**
Mean age in months (s.d.)		4.3 (3.1)	7.47 (7.8)	0.34
Sex (n)	*Male*	24 (58%)	3 (43%)	0.44
	Female	17 (41%)	4 (57 %)	
Median weight for age Z scores (range)		-1.1 (-5.3 to 0.8)	-0.38 (-4.4 to 3.4)	0.04

*Data from all patients enrolled was used if the HIV PCR was known

Table 3: Clinical data compared between HIV groups*

	HIV Uninfected (N=41)	HIV Infected (N=7)	*P* value
Mean of duration of admission (s.d)	5 days (2.09)	7.86 days (7.82)	**0.05**
Rhinitis	24 (58%)	4 (57.1%)	0.95
Diarrhoea	8 (19%)	0	0.20
Vomiting	7 (16.6%)	4 (57.1%)	**0.02**
Mean temperature (s.d.)	37.55°C (0.63)	38.04°C (1.03)	0.09
Tachypnoea	38 (90.47%)	7 (100%)	0.46
Wheezing	23 (54.7%)	5 (71.4%)	0.45
Crepitations	35 (83.3%)	7 (100%)	0.28
Bronchial breathing	3 (7.1%)	0 (0%)	0.46
Mean of oxygen saturation (s.d) [1]	86% (5.04)	88% (2.37)	0.32
Abnormal CXR [2]	24 (85%)	7 (100%)	**0.04**
Hyperinflation on CXR [2]	11 (39%)	6 (85.71%)	**0.005**
Mean of white cell count (s.d.)	11.737 (4.544)	16.314 (4.697)	**0.02**
Mean of lymphocyte count (s.d.)	38 (16.6)	50.2 (19.6)	0.10
Mean of haemoglobin (s.d.)	11.32 (1.343)	10.51 (1.292)	0.15
Mean of platelet count (s.d.)	453 (153)	350 (75)	0.06

*Data from all patients enrolled was used unless otherwise stated (see below)

[1] Data only tested in 35 HIV-uninfected and 6 HIV-infected children

[2] CXR done in 14 HIV-uninfected and all 7 HIV-infected children

HIV-infected children had a significantly longer hospital stay when compared to HIV-uninfected children. They were also more likely to present with vomiting than were the HIV-uninfected children and had a significantly higher white cell count on admission (see table 3). The mean lymphocyte count however was comparable between the two groups. HIV-infected children were also more likely to have hyperinflation and bronchopneumonic changes on their chest X-rays. There were no differences between HIV-infected and -uninfected children in the other clinical characteristics that were analysed (table 3).

The group of children who were HIV exposed but uninfected (i.e. HIV PCR negative) was compared to both the HIV infected and the HIV uninfected groups. The mean age on admission for HIV infected children was 7.47 months (s.d. = 7.89) and for the HIV exposed but uninfected group was 2.52 months (s.d.= 1.16) *P* value = 0.054. With respect to other clinical characteristics, there were no statistical differences found between the three groups.

The RSV group responsible for causing most infections was subgroup B. Twenty-seven (69%) of 39 children were infected with RSV subgroup B while four children (10%) were infected with subgroup A. Sub grouping of the RSV that was identified was not performed in the remaining 8 (20,5%).

Table 4: Nasal wash and serum neutralizing antibody responses in children undergoing primary infection with RSV

	HIV-uninfected N=34 (87%)	HIV-infected N=5 (12.8%)	*P* value (χ^2)
Number with >= four-fold rise in nasal wash IgA RSV-F antibody	30(88%)[1]	4(80%)	0.61 (0.26)
Number with >= four-fold rise in nasal wash IgA RSV-G antibody[2]	12(35%)	0(0%)	0.11 (2.55)
Geometric mean antibody concentration of RSV serum IgG antibody on admission (95% C.I.)	4.81 (4.3-5.4)	3.813 (2.6-5.7)	P=0.20
Geometric mean antibody concentration of RSV serum IgG antibody at six weeks (95% C.I.)	7.22 (6.6-8.0)	7.03 (4.0-12.2)	P=0.67
Number with >= four-fold rise in RSV serum neutralizing antibody	19(55%)	3(60%)	0.92 (0.01)

[1] Number in parenthesis is percentage

[2] Only IgA against RSV G protein of subgroup A2 was measured

HIV-infected children had as good a humoral (both secretory and serum antibody) response to natural RSV infection as HIV-uninfected children did. There was no difference in the proportion of HIV-uninfected (88%) and HIV-infected (80%) children that had a significant rise in nasal wash IgA RSV F-protein antibody to natural RSV infection (see table 4). The response with nasal wash IgA RSV G-protein antibody (35% in HIV-uninfected and 0% in HIV-infected children) was similar between the two groups. Serum neutralizing IgG antibody response was also similar between the two

groups with 55% of the HIV-uninfected and 60% of the HIV-infected children showing a response (see table 4).

Three out of the five patients who failed to respond with mucosal IgA RSV-F antibody had high baseline antibody levels and were less than 2 months of age (see table 5). One of the patients was HIV-infected. Only one patient was older than six months of age and he was HIV-infected.

Table 5: Patients who failed to respond with mucosal IgA F antibody following natural RSV-infection.

	Age (Months)	Neutralizing antibody on admission	Neutralizing antibody on day 42
Patient 31	1.7	115.4	75.6
Patient 39	1.26	224	127.1
Patient 51	4.7	10	87.9
Patient 104*	10.6	10	10
Patient 154#	1.1	127.2	95.2

* HIV-infected

HIV exposed but uninfected

Only IgA RSV G-protein antibody responses to subgroup A2 were measured.

This is important when discussing the relatively poor secretory IgA RSV G-protein antibody responses (see table 4) as 69% of children were infected with subgroup B.

Only 22% (6 out of 27) of the patients infected with subgroup B had a response with IgA G-protein antibodies while 100% (all 4) of the patients infected with subgroup A had a response. Twenty-five percent (2 out of 8) of the patients in whom the virus was not subgrouped had an IgA G-protein antibody response (see figure 3).

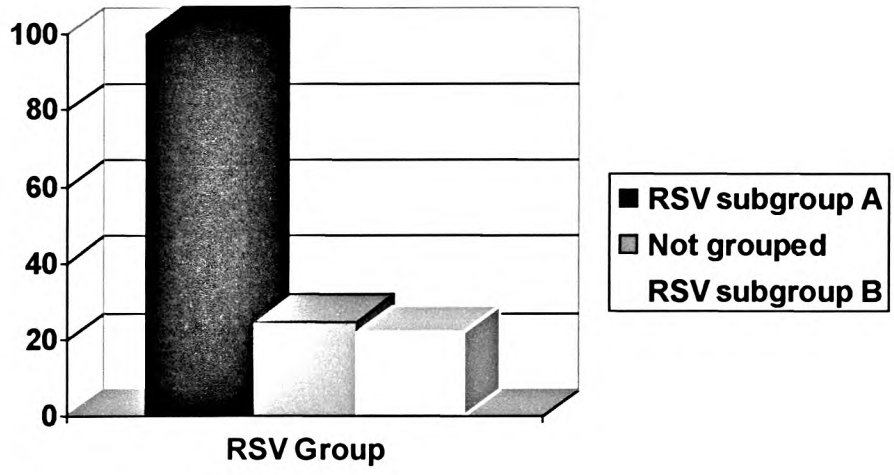

Figure 3: The percentage of children who produced IgA antibody to G-protein following natural RSV infection.

The majority of the children who failed to mount adequate serum neutralizing antibody responses had high baseline antibody levels (13 out of 16 children) and were less than three months of age (also 13 out of the 16 children). See table 6. Two of these 16 children (12,5%) were HIV infected.

The mean age of the HIV infected children who didn't respond was 6.0 months (s.d.= 6.6) and the mean age of HIV-uninfected children who didn't respond was 2.3 months (s.d.= 1.13); P =0.63.

Table 6: Patients who failed to produce a significant increase in serum neutralizing antibody following natural RSV infection

	Age (Months)	Neutr. Ab on admission	Neutr. Ab at day 42
Patient 7	2.26	71.5	81.3
Patient 31	1.7	115.4	75.6
Patient 39	1.26	224	127.1
Patient 42#	2.1	82.8	96.9
Patient 46	1.7	55.9	102.3
Patient 76	2.9	82	97.9
Patient 92	2.1	127.9	183
Patient 94#	3.4	**10**	10
Patient 99	5.3	**10**	10
Patient 102	1.4	158	94.8
Patient 104*	10.6	**10**	10
Patient 121*	1.3	109.5	125.2
Patient 143#	1.1	60.8	54.6
Patient 141	2.7	122.4	139.4
Patient 147	3	56	91
Patient 154#	1.1	127.2	95.2

* HIV infected

HIV exposed but uninfected

The clinical characteristics and antibody responses to natural RSV infection in HIV-infected children are shown in table 7. None of the HIV-infected children were classified by the CDC classification as having clinical stage C disease, although four of them had viral loads > 750 000. Their CD4 counts were not measured. One child failed to produce any response with secretory or serum antibodies and another child failed to produce a response with serum neutralizing antibody, but he had a high antibody level on admission.

Table 7: Characteristics and results of HIV-infected children

	Age (months)	CDC classification[12]	PCR viral load	RSV neutralizing antibody		NW RSV-F		NW RSV-G	
				Adm#	42d	Adm	42d	Adm	42d
Patient 78	5.7	B	>750 000	10	1920*	0	22*	0	0
Patient 104	10.6	A	268 000	10	10	0.1	0.1	0	0
Patient 116	2.9	A	>750 000	Not done-child not found at address given					
Patient 121	1.3	A	154 000	110	125	0.1	0.7*	0	0
Patient 124	6.2	B	>750 000	10	360*	0	2.4*	0	0
Patient 137	4.5	N	1510[1]	10	350*	0	0.4*	0	0
Patient 175	2.7	A	>750 000	Not done – patient died during admission					

#Adm. – on admission

* considered to be a significant rise (>= four-fold rise in antibody or any rise from 0)

[1] As this viral load is < 10 000, it should have been repeated to confirm

4.0 DISCUSSION

4.1 Clinical Characteristics

4.1.1 HIV status

The prevalence of HIV infection in this study was 12,8% (five out of the thirty-nine children who completed the study). This is similar to the prevalence of 14,6% found in another study done at this study site.[10]

The community prevalence of HIV infection in children in Soweto is estimated to be about 5%. [10] This indicates that HIV-infected children have a greater than two fold increase in the risk of contracting a severe RSV LRTI when compared to HIV-uninfected children. This is comparable to the previous study done at this site by Madhi et al.[10]

Of the five HIV-infected children who completed the study, two had viral loads that were greater than 750 000 but none were clinical category C by the CDC classification.[15]

Both the children who were HIV-infected but who didn't complete the study had viral loads of greater than 750 000. The one child wasn't found at the address that was given and the other child died during admission.

The fact that none of the children were CDC clinical classification category C could be important in that the children who were in the study were not as severely immunocompromised as they could have been despite the fact that two of the children

had viral loads of greater than 750 000. It would be interesting to look at the CD4 counts to see what immunological category the children in the study would fall under. With time, these infants are more likely to develop severe immune suppression and may not exhibit a significant antibody response at a later stage.

4.1.2 Age

HIV-infected children tended to be older than uninfected children although this did not reach statistical significance. Another study done at this site showed that HIV-infected children were older than HIV-uninfected children.[10]

The small number of HIV-infected children in this study could explain why it didn't reach statistical significance here. HIV-infected children are still prone to developing RSV-associated severe lower respiratory tract infections after the age of six months and would therefore have a higher rate of hospitalisation for RSV infection than HIV-uninfected children of the same age would.

4.1.3 Clinical differences in presentation

There was no difference in presentation between the two groups with respect to gender, cough, rhinitis, tachypnoea, wheezing or oxygen saturation.

HIV-infected children presented significantly more with vomiting when compared to the HIV-uninfected group. They also had a longer duration of hospital stay and a higher mean white cell count. This could be due to that fact that there is more often concurrent bacteraemia in HIV-infected children than in HIV-uninfected children, [10] although none

of the HIV-infected children in this study had a concomitant bacteraemia detected. The longer hospital stay in HIV infected children could also be due to the fact that their cellular immunity is impaired resulting in decreased clearance of the virus.

In this study there was no difference in the presentation with pneumonia or bronchiolitis between the two groups. This is in contrast to the findings in many studies that HIV-infected children are more likely to present with pneumonia rather than with bronchiolitis.[10, 11, 12] The reason for us not finding this now is most likely due to the small number of HIV-infected children included in this study and may also be due to their degree of immunosupppression.

4.2 Nasal wash mucosal IgA RSV antibody to F protein

The response to natural RSV infection with a significant rise (see section 2.3) of mucosal antibody to the F protein in 88% of HIV-uninfected and 80% of HIV-infected infants is consistent with that found in another study done by Yamazaki et al in HIV-uninfected children. [17] In the latter study, in infants less than 6 months of age 10 out of 12 (83%) developed a significant increase in convalescent nasal wash IgA F-protein antibodies. In children 6-16 months of age, 10 out of 10 children developed significant antibody levels.[17]

It has been suggested that very young infants have a suppressed response in the development of IgA F-protein antibody when there are high levels of maternally derived IgG antibody.[17] This maternally derived IgG antibody contains abundant anti-F activity

that suppresses the development of IgA F-protein antibody in the infant during primary RSV infection.

Of the four HIV-uninfected children who failed to develop secretory IgA F-protein antibody, three were less than 2 months old and had high levels of RSV maternally derived serum neutralizing antibodies. (See table 5) The fourth child who didn't develop secretory IgA F antibody was 4.7 months old and had low levels of neutralizing antibody on admission and did develop a significant rise in serum neutralizing antibody.

The fact that these children had high levels of maternally derived serum neutralizing antibodies on admission but still developed RSV illness severe enough to require hospitalisation is most likely due to the fact that there are two subgroups and a number of strains within each of the subgroups which are heterogenous in the nucleotide sequence of the G protein. The degree of antigenic relatedness between F proteins of groups A and B has been estimated to be 50% and between G proteins of groups A and B, only 5%. [17] Immunity to one subgroup of RSV does therefore not necessarily protect from infection with another subgroup, or even within a subgroup, as immunity to strains within each of the subgroups may vary.

There was no statistical difference in response between HIV-infected and HIV-uninfected children in this study. These results however have their limitations as the number of HIV-infected children was too small to comment on this fact, and a future study with larger numbers needs to be done.

Children infected with HIV are known to have deficiencies in humoral immunity[9] and also to have lower concentrations of mucosal IgA.[18] Despite the frequent presence of increased serum immunoglobulin, the ability to produce specific serum antibody to antigens is impaired. The severities of these deficiencies are related to the degree of immunodeficiency.

The one HIV-infected child who didn't have a significant increase in mucosal IgA F-protein antibody had category A disease by the CDC classification[15] and had a viral PCR load of 268 000 (see table 7). He was 10 months old. In children older than 6 months of age in a study done by Yamazaki et al, 100% (10 out of 10) of HIV-uninfected children had a significant rise in IgA F-protein antibody.[17]

4.3 Nasal wash mucosal IgA RSV antibody to G protein

Only 35% of the HIV-uninfected group and none of the HIV-infected group had a significant rise in secretory RSV IgA G-protein antibody. This is far lower than that seen in other studies.[8, 17] In a study done by Yamazaki et.al, 11 out of 12 infants (91%) less than 6 months of age and 7 out of 10 children (70%) aged 6-16 months of age developed secretory IgA-G protein antibodies.[17] As the degree of antigenic relatedness between G proteins of group A and B is only about 5%, it is necessary to distinguish between the two groups when analysing antibody activity.

Secretory IgA against G protein from RSV group A and not group B was measured. However, in this study RSV group B was responsible for the majority of infections (69%). All four of the patients who were infected with RSV subgroup A had a

significant development of IgA G-protein antibodies as measured whereas only 22% of the patients (6 out of 27) infected with group B had a response. Twenty-five percent (2 out of 8) of the patients who were ungrouped had a response (See figure3). It is therefore highly likely that we are underestimating the true response rates compared to if we had evaluated for IgA responses to subgroup B as well.

4.4 Serum neutralizing antibody

There was no difference in the proportion of HIV-uninfected (59%) and HIV- infected children (60%) who had a significant increase in neutralizing antibody. (See table 4). Serum neutralizing antibody is important in the prevention of the development of lower respiratory tract infection in children infected with RSV. In infants less than 6 months of age, the antibody titers that develop are usually low. This blunted response may be secondary to the relative immunological immaturity of the infants and/or to a suppressive effect of maternally transmitted transplacental antibodies.[7]

Of the 16 patients in total who failed to produce a significant increase in neutralizing antibody, only three (19%) had insignificant levels of maternally derived neutralizing antibody. One of these three was HIV-infected. (See table 6).
Only one child out of the group who didn't produce a significant increase in neutralizing antibody was more than six months of age, and that child was HIV-infected. He also failed to develop a significant secretory IgA F-protein antibody response.

Although the numbers are small, there appears to be no difference in the humoral immune response to natural RSV infection between HIV-infected and HIV-uninfected children. This could be because these children had not had their humoral immunity affected by HIV infection as yet.

5.0 STUDY LIMITATIONS

The major limitation of this study was the small sample size of HIV-infected children. A study with larger numbers would be needed to evaluate as to whether there is statistical differences in the immune responses between HIV-infected and -uninfected children.

As we only included children who were sick enough to be admitted into hospital into our sample, we may have been biasing the results as children who are sick enough to be admitted might develop different antibody responses to children who are able to cope with their RSV infection in the community.

The fact that we were only testing for RSV subgroup A means that we are unable to interpret the mucosal IgA response to G protein with any accuracy. It would be important in any future studies to test for both subgroup A and subgroup B.

6.0 CONCLUSIONS

In this study, it appears as if HIV infected infants do not have an impaired humoral (both secretory and serum antibody) immune response to natural RSV infection when compared to HIV-uninfected children of a similar age.

HIV-infected children are at a greater risk than HIV-uninfected children from developing a severe LRTI following RSV infection as evidenced by the greater than two times prevalence of HIV infection in this study when compared to the HIV prevalence in the community.

The small study numbers however make it difficult to reach any conclusion and our findings needs to be corroborated in a larger study.

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