

# Symptomatic HIV infection in infancy — clinical and laboratory markers of infection

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*Objective.* To investigate the usefulness of immunological tests in the diagnosis of HIV infection in young symptomatic children (< 15 months of age).

*Design.* Tests were evaluated in HIV-infected (HIV antibody- and PCR-positive) patients and non-infected individuals.

*Setting.* Hospitalised patients in a referral centre (Red Cross War Memorial Children's Hospital, Cape Town).

*Patients.* All admissions under 15 months of age who had HIV antibody requested were eligible, provided there was sufficient serum (150 µl) for further study. Overall, there were 201 symptomatic cases and 49 healthy controls. Twenty of the symptomatic cases were HIV antibody-positive and 19 of these were HIV-infected on the basis of a positive PCR for HIV viral product.

*Results.* Of the tests we evaluated (total IgG, IgM, IgA and rheumatoid factors of the same classes), raised total IgG level (cut-off 18 g/l or above) was the most useful. We used a commercial radial immunodiffusion plate which was found to have excellent reproducibility (inter-assay coefficient of variation 3.2%). The test detected 16 of 19 infected infants (sensitivity 84%, negative predictive value 98%). With the exception of the finding of oral thrush (odds ratio 7;  $P < 0.001$ ), the clinical signs at presentation did not distinguish those who were HIV antibody-positive from those who were negative.

*Conclusions.* In our study of hospital admissions, the finding of elevated IgG and HIV antibody was diagnostic of HIV infection. (The positive predictive value of the combination was 100%.) Likewise, the presence of raised IgG levels and oral candidosis had a high specificity for HIV infection (98%) but the sensitivity was low (37%).

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Measurement of total IgG levels by radial immunodiffusion is simple, relatively inexpensive (< 10% of the cost of PCR), helpful in diagnosing HIV infection in symptomatic infants and able to be performed in areas with minimal laboratory back-up.

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The clinical presentation of HIV infection in early infancy is often associated with non-specific signs and symptoms such as diarrhoea and pneumonia.<sup>1</sup> The presence of maternal antibody may further complicate the diagnosis of HIV infection. In most instances uninfected infants will serorevert by 1 year,<sup>1</sup> although antibody may persist until up to 18 months of age. By combining tests for specific antibody with those that detect viral products (e.g. polymerase chain reaction (PCR), viral culture, p24 antigen) the presence of infection can usually be reliably established by 6 months of age.<sup>2</sup> Such investigations, however, are costly and require a high degree of laboratory sophistication. Delay in diagnosis is likely to occur in more remote areas. A knowledge of the HIV status is useful for counselling and for decision-making with regard to antiviral therapy and the use of antibiotics. Our study was designed to evaluate whether other immunological tests adaptable for use in small laboratories were helpful indicators of HIV infection in symptomatic patients.

## Patients and methods

### Symptomatic infants

These were selected from sequential ward admissions to Red Cross War Memorial Children's Hospital, Cape Town, from June 1993 - June 1994. Study eligibility was restricted to patients < 15 months of age where the admitting doctor requested serological tests for HIV. A minimum of 150 µl of serum remaining after HIV testing was also a requirement. Clinical data were obtained by careful review of the case records. A diagnosis of HIV infection was made if the enzyme-linked immunosorbent assay (ELISA) (Abbott) for HIV antibodies to HIV type 1 or 2 was positive, together with a positive PCR result on 2 occasions. (Primers specific for HIV type 1 and type 2 were used.) Perinatal exposure to HIV infection was present if HIV antibody was positive on ELISA but PCR findings were negative.<sup>2</sup> Individuals whose HIV serology was negative were regarded as uninfected.

### Controls

Serum (generally obtained prior to an elective surgical procedure in otherwise healthy children under 15 months of age) was used to establish reference ranges for the various tests evaluated. The HIV serology in these individuals was negative.

Consent for HIV testing in the two groups of patients described above was obtained by the admitting doctor.

## Serological tests

Apart from total IgG, we developed an ELISA format for the immunological tests. This was because of the small volume of serum required and the lower costs involved.

### Total IgG

This was measured by radial immunodiffusion (MC plates, Behring, Germany). The inter- and intra-assay coefficients of variation for the assay were 3.2% and 3% respectively.

### Total IgM

Goat antihuman immunoglobulin (anti-GAM) was used to coat microtitre plates. Patient sera (diluted 1:800) were added, after which plates were washed. Plates were then reacted with antihuman IgM, washed and incubated with substrate (2,2' azinobis). The optical density (OD) was read after 1 hour. The ELISA was standardised with a human serum standard. The inter-assay coefficient of variation for the assay was 14% and the intra-assay coefficient of variation was 13%. Spearman's rank correlation coefficient was 0.39 ( $P = 0.03$ ) when ELISA results on 30 samples were compared with those obtained using nephelometry.

### Total IgA

The procedure was essentially the same as that described above. A 1:800 dilution of serum was chosen, as this correlated best with nephelometer readings. Bound IgA was detected with a rabbit antihuman IgA. The inter-assay coefficient of variation was 17% and the intra-assay coefficient of variation 16%. Spearman's rank correlation coefficient was 0.52 ( $P < 0.001$ ) when ELISA results were compared with those of a nephelometer (50 samples).

### Rheumatoid factor ELISAs

IgM rheumatoid factor (RF) was measured by an ELISA as previously described.<sup>3</sup> Human IgG was used to coat ELISA plates. Patient serum was added, the plates washed and reacted with goat F(ab')<sub>2</sub> antihuman IgM, followed by substrate (c.f. IgM ELISA). The ELISA was standardised with an international reference preparation. Low-titre RF-containing sera were used, and the inter- and intra-assay coefficients of variation were 9.9% and 7%, respectively.

For IgG RF, the method was essentially the same except that rabbit IgG was used as capture antigen. Patient sera, diluted 1:40, were pepsin-digested and a goat antihuman IgG conjugate was added. Results were expressed as OD test/OD control x 100. The interassay coefficient of variation for this assay was 15% and the intra-assay coefficient of variation was 12%.

IgA RF was measured using heat-aggregated human IgG to coat the plate. Sera were subjected to pepsin digestion and neutralisation. F(ab')<sub>2</sub> rabbit antihuman IgA was added and the rest of the procedure was the same as for the total IgM ELISA. Results were expressed in the same way as for IgG RF.

## Statistics

Normal ranges for the immunological tests were obtained from the control patients. The 95th centile was defined as the upper cut-off level. This approach was followed because the distribution of antibody concentrations is skewed.<sup>4</sup> Non-parametric statistics were used to compare antibody levels, e.g. Mann-Whitney U test. The relative frequencies of the clinical features in the HIV-infected and non-infected groups were analysed with Epi-Info version 5. Chi-square tests were used, except where the expected cell values were less than 5. (Fisher's exact test was used in these cases.)

## Results

### Symptomatic patients

Of 201 sera screened for HIV antibodies, 181 (90%) were HIV-negative. There were 20 HIV-positive samples; 19 of these were also positive on PCR. One individual was negative on PCR at 4 months and follow-up samples could not be obtained. This patient was regarded as perinatally exposed.

### Controls

Of 49 sera, 16 were from infants less than 6 months of age.

### Serological testing

Reference ranges for the various tests were derived from the control sera. The HIV-positive patients had a lower median age at presentation (3.8 compared with 6 months;  $P = 0.05$ ) and 17 (85%) were < 7 months old. Therefore, we compared results of control infants 6 months of age and younger with those of older controls (7 - 15 months). We did not find statistically significant differences in respect of any of the tests described above ( $P = 0.24$  for IgG, 0.34 for IgM and 0.55 for IgA). Similarly, rank correlations between age and immunoglobulin level of the relevant class demonstrated no significant associations. Therefore, the results of control patients in the different age groups were pooled.

### Total IgG

In the controls, the median level was 4 g/l (range 2 - 24 g/l). Sixteen of the 19 patients with HIV infection had IgG levels equal to or above 18 g/l (the 95th percentile). Three PCR-positive individuals had levels within the normal range, as did the PCR-negative patient. When the latter case of indeterminate HIV status was excluded, the sensitivity was 84% (Table I). The median IgG concentration was 24 g/l (range 5.2 - 48 g/l). As shown in Fig. 1, the IgG levels in the symptomatic seronegative group were significantly lower than in the HIV-infected patients (median 13.5; range 0 - 35 g/l;  $P < 0.001$ ). Of 46 seronegative cases with raised IgG levels, the majority (34) had diarrhoea, lower respiratory tract infection or a combination of the two. IgG concentrations were elevated in 6 of the 12 patients with tuberculosis, i.e. including pulmonary and extrathoracic disease.

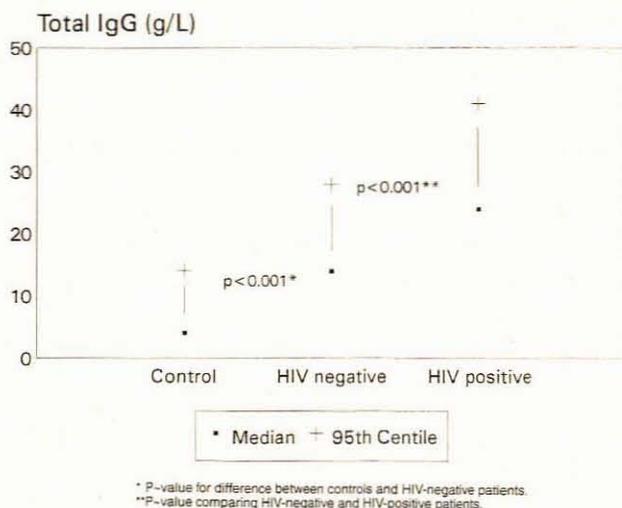


Fig. 1. Total IgG levels (median and 95th centile) in controls, HIV seronegative patients and HIV-infected children under 15 months of age.

### Total IgM and IgA

Overall, the total IgM level was higher in the HIV-infected patients (median 1.29 g/l, range 0.8 - 4.7 g/l) than in seronegative cases (median 0.96 g/l, range 0.2 - 4.1 g/l;  $P = 0.005$ ). However, raised IgM levels (above the 95th percentile) were found in only 3 of 19 HIV-infected patients (sensitivity 16%, specificity 97%). Total IgA concentrations were elevated in the HIV-positive group compared with controls (median 0.56 g/l, range 0.1 - 2.6 g/l in the HIV-positive group compared with 0.15 g/l, range 0 - 1.6 g/l in the controls;  $P < 0.001$ ). However, when IgA levels in the HIV-positive group were related to those in the symptomatic HIV-negative patients (median 0.38 g/l, range 0.05 - 5.6 g/l) the levels were not significantly increased ( $P = 0.27$ ). Raised IgA levels (above the 95th percentile) were present in 8 of the HIV-infected infants (sensitivity 42%, specificity 73%).

### Rheumatoid factor

The sensitivities of these tests in the diagnosis of HIV infection were lower than those for measurement of total IgG (Table I). However, the specificities for IgM and IgG RF were relatively high (84% and 99% respectively). Only 2 of 181 symptomatic HIV-negative patients had raised IgG RF levels. One had cytomegalovirus infection and the other pulmonary tuberculosis. Raised IgM RF was present in diverse conditions such as diarrhoea, pneumonia, tuberculosis and congenital syphilis. Owing to technical difficulties (high background values), the IgA RF ELISA was not performed in the HIV-negative symptomatic patients. Detectable IgA RF was noted in 21% of the HIV-infected children.

Table I. Performance of immunological tests in the diagnosis of HIV infection in children under 15 months of age

	Sensitivity	Specificity
Total IgG (g/l)	84%	75%
Total IgM (g/l)	16%	97%
Total IgA (g/l)	42%	73%
IgG RF	37%	99%
IgM RF (IU/ml)	16%	84%

## Clinical findings

Overall, the clinical presentations of the patients who were HIV seropositive was similar to those who were HIV seronegative (Table II). There was a statistically significant difference, however, in respect of the presence of oral thrush, which was commoner in those who were HIV-positive ( $P < 0.001$ ). Nevertheless, the sensitivity of this physical finding was only 45% (specificity 90%). Dehydrating diarrhoea and lower respiratory tract infection were the commonest clinical conditions that resulted in serological screening for HIV. Indeed these are among the commonest causes of presentation to the hospital (R Els — personal communication).

Table II. Clinical features at presentation in patients under 15 months of age

	HIV +ve (N = 20)	HIV -ve (N = 181)	Odds ratio	P-value
Age (mths) (median)	3.8	6	—	0.05
Gastro-enteritis	14	116	1.3	0.78
Oral thrush	9	19	7.0	<0.001
Pneumonia	9	70	1.3	0.76
Failure to thrive	6	41	1.4	0.65
Hepatosplenomegaly	1	1	9.5	0.19
Lymphadenopathy	0	2	0	1.0
Other (including tuberculosis)	2	21	0.8	0.87
Previous admission	4	24	1.6	0.5
Duration of illness (days) (median)	3.0	2.0		0.47

Because of the relatively younger age of the HIV-infected patients (median 3.8 months), the clinical findings in infants < 7 months were analysed separately. The presence of thrush remained the only statistically significant finding in the HIV-seropositive group (odds ratio 4.7;  $P = 0.001$ ).

The combination of elevated IgG and oral candidiasis was commoner in the HIV-infected group (7/19 v. 3/181; odds ratio 34.6;  $P < 0.001$ ). The specificity of the combination for the diagnosis of HIV infection was 98% and the negative predictive value 94%.

Given that an infant has thrush at presentation, how useful is elevated IgG in the diagnosis of HIV infection? Oral candidiasis was noted in 9 HIV-infected patients, 7 of whom had elevated IgG compared with 17 with thrush and 3 with raised IgG in the non-infected group (positive predictive value 70%, negative predictive value 88%).

## Discussion

Standard diagnostic tests for HIV in infancy include the detection of HIV (IgG) antibody by ELISA or Western blot and demonstration of viral products (by PCR, culture or p24 antigen assay). The presence of HIV antibody does not distinguish between active infection and passive transfer of maternal antibody. In contrast, PCR and viral culture and p24 antigenaemia have high specificity and sensitivities of between 88% and nearly 100% for the diagnosis of HIV infection by 6 months of age.<sup>2,5,6</sup>

Of the tests we evaluated, measurements of the total IgG had the highest sensitivity (84%) for the detection of symptomatic HIV infection. The specificity was, however, lower (75%). These findings are in keeping with a number of reports which indicate that elevated IgG is a frequent finding in symptomatic HIV-infected infants.<sup>1,4</sup> The presence of raised immunoglobulin levels may precede other evidence of infection in antibody-positive infants and may be of prognostic significance.<sup>1,7</sup>

Are these findings useful clinically? Elevated IgG and HIV antibody in a symptomatic patient virtually establish the diagnosis of HIV infection, rendering further testing unnecessary. Tests for viral antigen are costly (e.g. PCR costs R270), not readily available in outlying areas and results may be subject to delay. Radial immunodiffusion for IgG is a simple method with excellent reproducibility; it could easily be performed in small laboratories at modest cost. (Reagent price is R13 per test.)

Indeed, knowledge of the total IgG on its own without the HIV antibody result may be of some value, at least while awaiting the latter. An IgG level within normal limits in a symptomatic infant has a high negative predictive value (98%) and virtually excludes the diagnosis of HIV infection. Conversely, raised IgG levels will detect most HIV-infected infants. Although measuring IgG may have some usefulness as a screening test, the positive predictive value is low (26%) so that the diagnosis of HIV infection must be confirmed. Furthermore, not all cases would be detected. For these reasons, as well as cost, measurement of HIV antibody (e.g. by ELISA) remains the screening method of choice. (The ELISA costs approximately R18 per test.)

If the combination of elevated IgG and HIV antibody is used for the diagnosis of HIV infection in symptomatic infants, what is the incidence of false positives? Unfortunately, our data do not allow accurate determination of the false-positive rate as we only had 1 antibody-positive, PCR-negative patient. In this regard, the findings of the European Collaborative Study<sup>1</sup> are helpful. The false-positive rate in uninfected infants (< 6 months) was only 3% (although the findings were not limited to symptomatic patients). In contrast, the study by Kline *et al.* indicated that approximately half of the HIV-uninfected infants had raised immunoglobulin levels (class unspecified) in the first 6 months of life.<sup>8</sup> It was not stated whether these infants were otherwise healthy. The low specificity for hyperimmunoglobulinaemia described by these authors may reflect the use of lower cut-off values than ours. (The control population is not stated.) It may be important to use a higher cut-off level, particularly when evaluating symptomatic patients where, as we note above, the IgG level is raised but usually not to the very high concentrations seen in HIV infection. We did not find a significant correlation between age and immunoglobulin level among the controls; therefore we did not use age-dependent cut-off levels in the first year of life. Most of the reported variation in IgG, IgA and IgM levels occurs within the first 4 - 6 weeks of life; the IgG concentration is higher at this time, the IgM and IgA concentrations lower.<sup>9</sup> We had relatively few controls in this age group so that lower cut-off points for IgM and IgA may be appropriate before 6 weeks.<sup>10</sup>

Consistent with other reports,<sup>4,8,11</sup> we found the IgM and IgA concentrations to be significantly higher in the HIV-

infected group. The sensitivities were low, however, as were the positive predictive values.

Rheumatoid factors have been noted in HIV patients but the finding has not been reported in detail.<sup>12</sup> It is perhaps surprising that IgM RF was not detected more frequently. Congenital infection with syphilis, cytomegalovirus and rubella all result in its production,<sup>13</sup> possibly as a consequence of stimulation of CD5 B cells by immune complexes. The majority of RF was of IgG class, which suggests that class switching occurred in RF-producing B cells. This is likely to be due to the specific cytokine profile of the infants and is an area for further study.

Apart from the immunological tests described above we also analysed the usefulness of the clinical signs (at the time of presentation to hospital) in diagnosing HIV infection. As not all children under 15 months of age were screened for HIV antibody there may have been selection bias in choosing which patients to test. However, the clinical presentation of the infants with HIV infection was similar to those without the infection, suggesting that this was not the case. The limitations of chart review to determine clinical features should also be considered.

In general, the physical findings did not appear to be very helpful in distinguishing patients with HIV infection from those without. Oral candidosis was the only clinical feature in the present study which occurred significantly more often in the HIV-infected group. In longitudinal studies, other workers have noted that oral lesions (especially candidiasis) are found in up to 72% of HIV-infected children and that the prognosis is worse when candida occurs.<sup>14</sup> Our data indicate that oral candidosis associated with elevated IgG is highly specific for HIV infection (specificity 98%), but has a low sensitivity (37%).

Follow-up studies have also indicated that HIV-infected infants are more prone to develop acute diarrhoea, recurrent diarrhoea (2 or more episodes) and persistent diarrhoea (14 days or longer). In addition, an earlier onset (< 6 months) has been noted in HIV-infected infants.<sup>15</sup> In the present study of symptomatic hospital admissions, however, we did not find these features to be useful discriminators. The overall number of patients with recurrent diarrhoea in our study was 15 (4 HIV-positive) while 19 had persistent diarrhoea (1 HIV-positive).

In the present study, 19 out of 20 symptomatic antibody-positive hospital admissions had HIV infection (diagnosed by PCR). This finding, which is somewhat surprising, should be tested in a larger unselected sample of all hospital admissions < 15 months of age. If substantiated, it indicates the heavy load placed on hospital facilities by young HIV-infected infants.

In conclusion, measurement of total IgG by radial immunodiffusion is useful in establishing HIV infection in symptomatic infants. A level below 18 g/l virtually excludes infection. Conversely, concentrations of 18 g/l and above in the presence of HIV antibody were diagnostic of HIV infection.

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