

Serum immunoglobulin E levels in human immunodeficiency virus-infected children with pneumonia

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Elevated serum immunoglobulin E (IgE) levels have been reported in association with human immunodeficiency virus (HIV) infection in adults, but there is little information in children. The aim of the present study was to compare serum IgE levels in HIV-positive and -negative children hospitalized with pneumonia in South Africa and to investigate whether IgE may be useful as a marker of specific infections or prognosis in HIV-infected children. History, examination, blood tests, and induced sputum or bronchoalveolar lavage were carried out. Of 122 children [45% female, median age 8 months (3–20 months)], 81 were infected with HIV. A history of allergy or asthma was present in three children (two of whom were HIV positive). Serum IgE was higher in HIV-infected children [83 (33–147) vs. 29 (6–113) IU/l; $p = 0.011$] as was immunoglobulin G (IgG) [49 (37–63) vs. 27.5 (23–34) g/l; $p < 0.001$]. CD4 lymphocytes [600 (330–1210) vs. 1900 (1500–3030) cells/ μ l], percentage CD4 cells [13.6 (9.4–20.3) vs. 40.1 (31.1–44.9)] and CD4:CD8 ratio [0.3 (0.2–0.4) vs. 2 (1.4–2.8)] were lower in HIV-positive children ($p < 0.001$ for all). Bacteremia occurred in 12 (10%) children; other specific pathogens identified included *Mycobacterium tuberculosis* in eight (7%) and *Pneumocystis carinii* in nine (7%). There was no correlation with CD4 count, CD4:CD8 ratio, or the presence of specific pathogens, and IgE level. In-hospital mortality (11%) did not correlate with IgE levels. HIV-infected children with pneumonia have higher serum IgE compared with seronegative patients. In HIV-positive children, IgE levels did not correlate with the degree of immunosuppression or with outcome.

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T-cell dysfunction (manifest as anergy and hypersensitivity) occurs relatively early in the course of human immunodeficiency virus (HIV) infection and precedes T-cell depletion (1,2). HIV-induced immune dysregulation resulting in a shift in cytokine production from a T-helper (Th)1 to a Th2 response has been postulated to account for this (3,4). Increased antibody production, including immunoglobulin E (IgE) is a result of a Th2 dominant response. Elevated IgE has been reported amongst HIV-infected adults and has been associated with more rapid progression of their disease (5,6). Furthermore, elevated IgE has been suggested as a marker of poor prognosis in HIV-infected adults (6,7).

HIV-positive children have also been reported to have higher levels of IgE than seronegative

controls (8–10). The underlying mechanism resulting in IgE production has not been well defined. Elevated IgE production may be part of a polyclonal immunologic dysregulation (11), but may also occur as the result of a specific increase in IgE following HIV infection (12,13). The association of elevated IgE with outcome in children is unclear, with some studies reporting no correlation with allergic disease or immune dysfunction whilst others have found that a shift to a Th2 response correlated with increased IgE production, a decline in CD4 count, and disease progression (8–10). Furthermore, there is little data on the association of specific HIV-associated infections with serum IgE levels.

The aim of this study was to compare serum IgE levels in HIV-positive and -negative children

with pneumonia and to investigate whether IgE may be useful as a marker of specific infections or prognosis in HIV-infected children.

Subjects and methods

Children enrolled were participants in a larger, prospective study to investigate the etiology of acute pneumonia in HIV-infected children hospitalized in four academic hospitals (Red Cross War Memorial Children's Hospital, Somerset Hospital, Conradie Hospital, and Groote Schuur Hospital) in Cape Town, South Africa (14). Children with a primary diagnosis of pneumonia according to World Health Organization criteria (defined as the presence of tachypnoea or lower chest in-drawing), and who were known to be HIV infected, were suspected of having HIV infection based on clinical signs, or had severe respiratory illness necessitating admission to an intensive care unit (ICU), were enrolled during working hours from Monday to Friday. A suspicion of HIV infection was based on the presence of two or more of the following: generalized lymphadenopathy; weight below the third percentile for age; hepatomegaly; splenomegaly; oral candidiasis; enlarged parotid glands; or chronic diarrhoea. As a result of financial constraints, HIV-infected children do not currently receive anti-retroviral treatment. Informed consent for enrollment in the study and for HIV testing was obtained from a parent. The study was approved by the Research and Ethics Committee of the University of Cape Town.

Blood testing, including HIV testing, bacterial culture, a complete blood count, and determination of total IgE and immunoglobulin G (IgG), were carried out. HIV infection was confirmed by two positive enzyme-linked immunosorbent assay (ELISA) tests (Vironostika HIV Uniform II; Organon Teknika, Boxtel, Holland) in children older than 18 months or by a positive ELISA and polymerase chain reaction (PCR; Amplicor HIV-1; Roche Diagnostic Systems, Branchburg, NJ, USA) in those younger. Lymphocyte phenotyping was performed using a two-color monoclonal antibody panel, according to the Center for Disease Control (CDC) guidelines, on a Beckman-Coulter XL flow cytometer (Beckman-Coulter Inc, Palo Alto, CA, USA) (15). When sufficient blood was obtained, an additional specimen for serum IgE was taken. Total IgE was measured by fluorescent immunoassay (UniCAP, total IgE, Laboratory Specialities; Pharmacia, Uppsala, Sweden).

Sputum induction (16) or non-directed bronchoalveolar lavage (BAL) was performed to obtain lower respiratory tract secretions for bacterial, mycobacterial and viral culture, and *Pneumocystis carinii* detection. In non-intubated children, one to three sequential early morning gastric lavages were performed to obtain samples for mycobacterial culture. *Pneumocystis carinii* pneumonia (PCP) was diagnosed when BAL or sputum demonstrated *P. carinii* after staining using a silver methenamine or immunofluorescence (IF) stain (Detect IF *Pneumocystis carinii*; Shield Diagnostics, Dundee, UK).

Results were analyzed using Epi6.04 (CDC, Atlanta, GA, USA). Continuous variables were expressed as median (25th–75th percentile). Comparisons between HIV-infected and -uninfected children were made using the chi-square test for categorical variables and the Kruskal–Wallis test for continuous variables.

Results

Serum IgE levels were analyzed in 122 children (45% female) with a median age of 8 (range: 3–20) months. Eighty-one (66%) children were infected with HIV; 31 (38%) of these children were diagnosed as HIV positive at the time of admission. Seventy-nine (65%) children [48 (61%) of whom were infected with HIV] were under 1 year of age. HIV-positive children were older than HIV-negative patients [10 (range: 4–25) compared to 3 (range: 2–12) months; $p < 0.005$]. Most infants (73%) had been breast-fed; breast-feeding rates did not differ by HIV status (81% and 71% of HIV-positive and -negative infants, respectively; $p = 0.26$). A prior history of asthma was obtained in only three children (two of whom were HIV positive), while a history of a first-degree relative who had been diagnosed as asthmatic was reported in 22 patients [12 (15%) and 10 (24%) of HIV-positive and -negative infants, respectively; $p = 0.33$]. Only one HIV-infected child had a history of allergy, while a positive family history of allergy was obtained in three (4%) and five (12%) HIV-positive and -negative children, respectively ($p = 0.151$).

HIV-positive children had similar white blood cell and eosinophil counts, but a lower number of CD4 cells, percentage CD4 counts and CD4:CD8 ratios compared with seronegative children (Table 1). This pattern of immunosuppression occurred in HIV-positive compared to -negative infants and in older children (Table 1). The majority of HIV-infected infants were severely immunosuppressed, as indicated by a

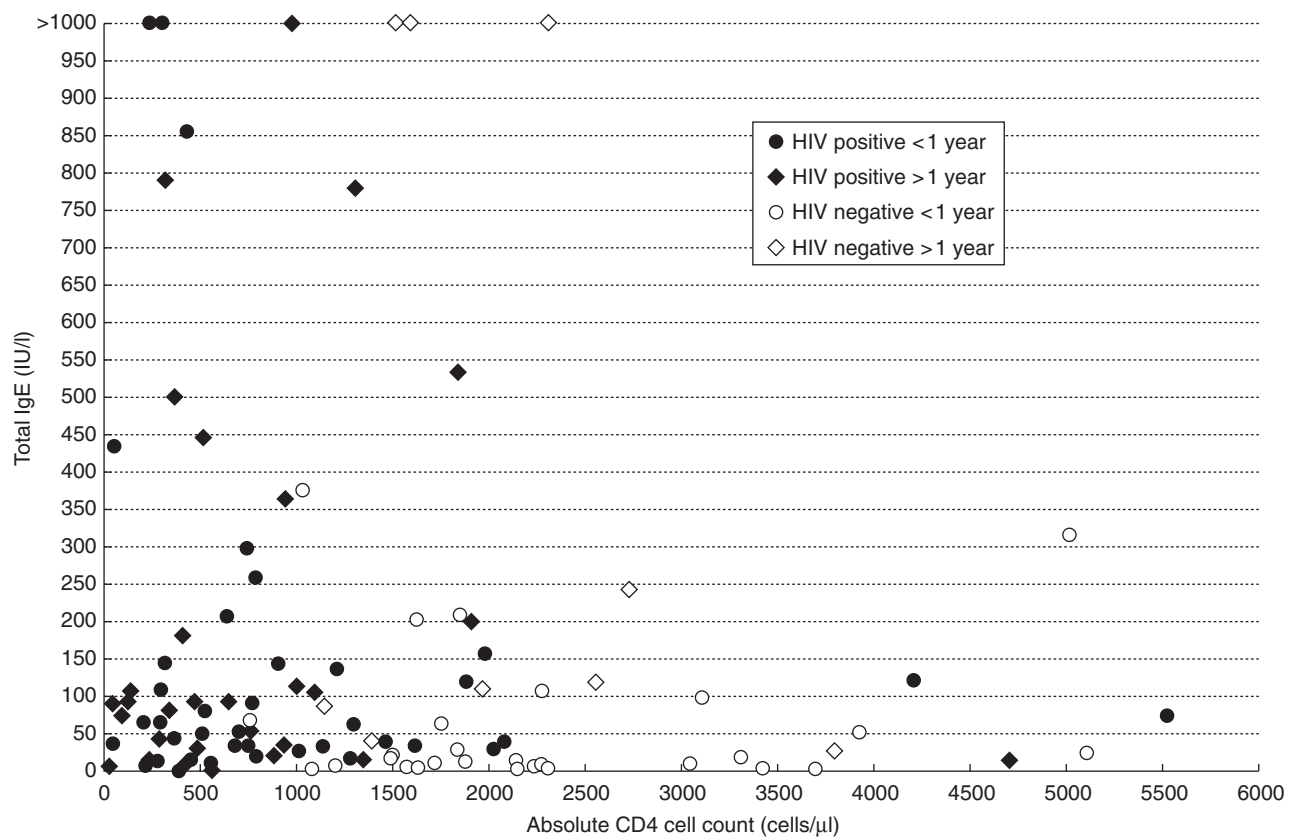


Fig. 1. Total immunoglobulin E versus absolute CD4 cell count in children with pneumonia according to HIV status.

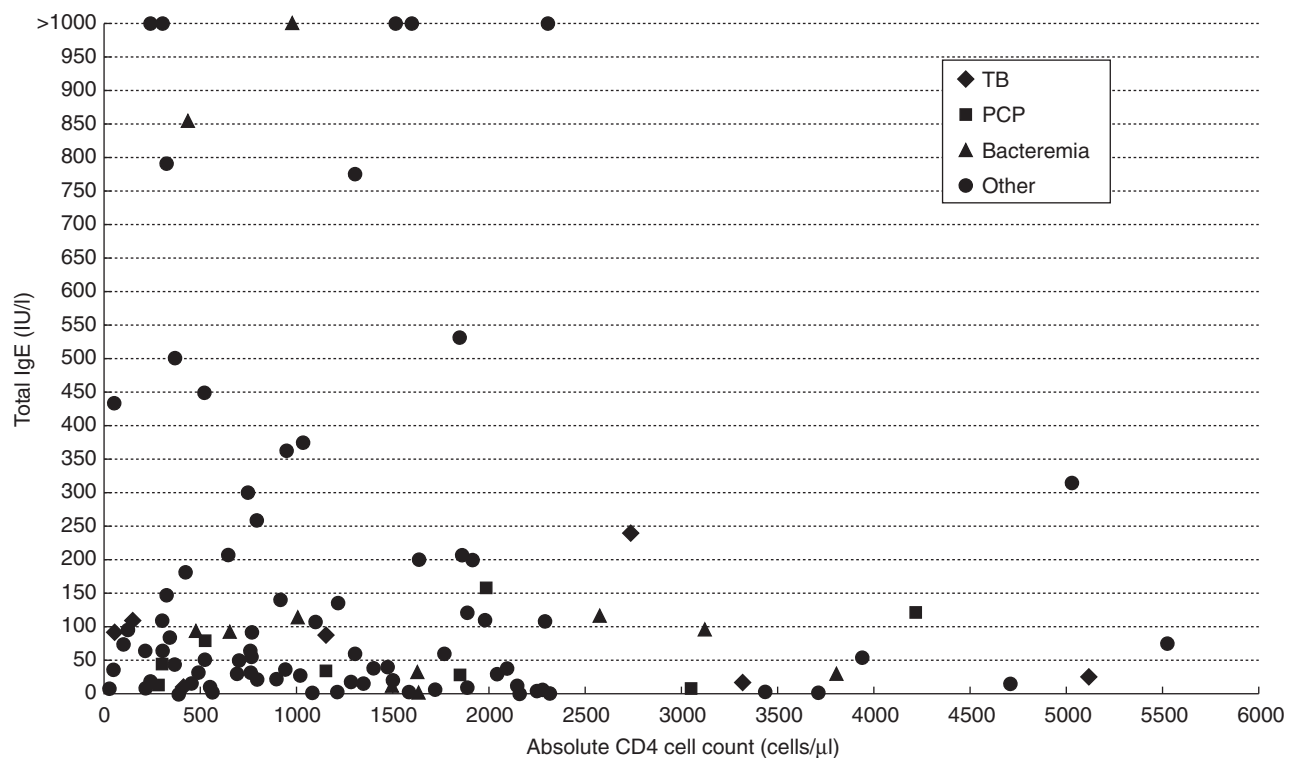


Fig. 2. Total immunoglobulin E versus absolute CD4 cell count by aetiology of pneumonia.

Table 1 Laboratory characteristics of children by human immunodeficiency virus (HIV) status

	HIV positive (n=81)	HIV negative (n=41)	p-value
WCC ($10^9/l$)	12.2 (9.1–17.6)	14 (10.7–22.5)	0.195
Eosinophils (% WCC)	1 (0.5–2)	2 (1–2.5)	0.126
CD4 lymphocytes (cells/ μ l)			
All	600 (330–1210)	1900 (1500–3030)	<0.001
<1 year of age	723 (368–1464)	2010 (1577–3111)	<0.001
>1 year of age	522 (368–1464)	1972 (1523–2563)	<0.001
CD4 (% lymphocytes)			
All	13.6 (9.4–20.3)	40.1 (31.1–44.9)	<0.001
<1 year of age	13.9 (10.6–20.9)	40.9 (31.1–45.2)	<0.001
>1 year of age	12.7 (8.2–19.7)	34.9 (31.6–38.7)	<0.001
CD4:CD8			
All	0.3 (0.2–0.4)	2 (1.4–2.8)	<0.001
<1 year of age	0.3 (0.2–0.5)	2.2 (1.7–3.2)	<0.001
>1 year of age	0.3 (1.1–1.9)	1.3 (1.1–1.9)	<0.001
IgG (g/l)	49 (37–63)	27.5 (23–34)	<0.001
IgE (IU/l)	83 (33–147)	29 (6–113)	0.011

Variables are expressed as median (25th–75th percentile).

WCC, white cell count.

median CD4 count of 723 cells/ μ l and percentage CD4 count of 13.9%. Similarly, the majority of HIV-infected children older than 1 year of age were severely immunosuppressed, as reflected by a CD4 cell count and percentage CD4 count of 522 cells/ μ l and 12.7%, respectively. Serum IgE and IgG concentrations were significantly higher in HIV-positive children. No correlation was found between age and IgE levels ($r=0.09$). For all patients, there was no correlation between serum IgE and the degree of immunosuppression, as measured by CD4 count ($r=-0.07$, Fig. 1), percentage CD4 cells ($r=-0.03$) or CD4:CD8 ratio ($r=-0.06$). Amongst the group of HIV-infected children, no correlation with IgE and CD4 count ($r=-0.1$, Fig. 1), percentage CD4 cells ($r=0.01$), or CD4:CD8 ratio ($r=0.03$) was found.

Bacteremia, present in 12 children (10%), occurred in eight (10%) HIV-positive and four (10%) HIV-negative patients. IgE levels did not differ by the presence of bacteremia (96 IU/l in those with bacteremia compared to 61 IU/l in those with no bacteremia; $p=0.3$, Fig. 2). Amongst the subgroup of HIV-positive children, there was a trend towards higher IgE levels in those with bacteremia compared to those without [114 (95–200) vs. 75 (31.5–145) IU/l, $p=0.08$]. A culture-confirmed diagnosis of pulmonary tuberculosis (TB) was made in eight (7%) children, four of whom were infected with HIV. IgE levels were similar in those with and without pulmonary TB [89.5 (20.5–101) compared to 63 (19–147) IU/l, respectively; $p=0.881$] (Fig. 2). Amongst HIV-infected children, IgE levels did not differ accord-

ing to the presence of TB [92 (52–101) compared with 81 (33–158) IU/l in those with and without TB, respectively; $p=0.7$].

PCP occurred in nine children (7%), seven of whom were HIV infected. Two seronegative patients with malnutrition also had PCP. Serum IgE was similar in children with and without PCP [46 (29–121) compared to 66 (19–147) IU/l; $p=0.68$] (Fig. 2). HIV-positive children with PCP had levels of IgE similar to those of seropositive patients without PCP [81 (34–143) compared to 83.5 (33–182) IU/l; $p=0.89$]. Cytomegalovirus (CMV) was cultured from the lower respiratory tract secretions of 18 children (15%), with a similar rate in HIV-positive (16%) and -negative patients (12%). There was no difference in IgE levels in children with CMV (median 112, 33–364 IU/l) compared to those without (median 56, 19–143 IU/l) ($p=0.1$), nor was there a difference in IgE amongst the subgroup of HIV-positive children with CMV compared to those without [114 (33–531) vs. 77 (33–143) IU/l; $p=0.23$]. Two children (one of whom was HIV positive) was culture positive for herpes simplex virus (HSV), while parainfluenza virus was cultured from a sputum specimen obtained from an HIV-negative child.

Eleven HIV-positive children (13.6%) died in hospital compared to two (4.9%) seronegative patients [relative risk (RR)=2.33 (95% CI: 0.63–8.53); $p=0.143$]. The serum IgE level in those who died was similar to that in survivors [49.5 (19–97) compared to 66 (88–147) IU/l, respectively; $p=0.57$]. Amongst HIV-positive children, IgE

levels did not differ by outcome in those who died (median 61, 33–97 IU/l) compared to survivors (median 66, 18–147 IU/l) ($p=0.89$).

Discussion

In this study of children with community-acquired pneumonia, HIV-infected patients were found to have higher serum IgE levels than seronegative patients. This finding is consistent with published pediatric data (8,9,12). In addition, the present study has shown that IgE levels are elevated in very young HIV-infected infants compared to seronegative controls. No correlation was found between the degree of immune dysfunction or presence of specific pathogens, and the IgE level. Pathogens identified as causative of pneumonia, including *P. carinii*, *Mycobacterium tuberculosis* and bacteria, have been consistently reported as causing community-acquired pneumonia in HIV-infected children in sub-Saharan Africa. IgE levels did not correlate with outcome, as measured by the in-hospital mortality.

There are a number of mechanisms that may have resulted in increased IgE production. Infection with viruses such as respiratory syncytial virus (RSV) or Epstein–Barr virus (EBV) may stimulate IgE production (17,18). However, these viruses were not cultured from any child in our study. Although CMV may be associated with increased production of IgE (8), no relationship between CMV infection and IgE production was found in our study population. Parasitic infections, especially gastrointestinal worm infestation, may also result in elevated IgE levels. Although we did not perform stool studies for these pathogens, the young age of children in our study (median age 10 months) makes infection with these unlikely. Elevated IgE levels may be associated with atopy. However, an atopic history was obtained in only a minority of children. Furthermore, published studies of HIV-infected children have not demonstrated an association between elevated IgE levels and atopic disease (9,12,19). Moreover, consistent with the results of other studies, eosinophilia, another marker of atopy, was not found to be a feature of HIV infection, even in the presence of raised IgE (19,20). Current evidence suggests that elevation of IgE in HIV-infected children is not a result of specific IgE antibodies against common aeroallergens but rather the result of polyclonal activation (10,11). In support of this, children in our study were also found to have elevated serum IgG levels. However, distinct mechanisms may

underlie production of different immunoglobulin classes (21).

Immune dysregulation resulting from infection with HIV may cause a shift to a Th2 pattern of cytokine production response (3,22). The production of a Th2 profile of cytokines, including interleukin (IL)-4 and IL-6, increases synthesis of IgE. Evidence for this is provided by *in vitro* data in which HIV-1 envelope glycoproteins gp120 and gp160 have been shown to promote IL-4-induced IgE synthesis (23). Moreover, HIV may induce synthesis of IL-6, resulting in polyclonal IgE production; increased production of IL-6 has been suggested as a distinct mechanism affecting production of IgE but not the other immunoglobulin subclasses (12). Elevation of IgE may also be caused by production of antibody specific to the virus itself (13,19).

IgE elevation was not associated with the degree of immunodeficiency, as manifest by CD4 lymphocyte numbers. This contrasts with data from HIV-infected adults in whom increased IgE levels have been reported to correlate closely with declining CD4 cell counts (6). However, consistent with our results, prior studies of HIV-infected children have also failed to demonstrate an association between IgE level and the degree of immunosuppression (8–10,19). One explanation for this may be that HIV infection produces changes in immune function which precede the decline in CD4 lymphocytes. Qualitative defects in T-helper cells may occur even when the numbers of CD4 cells are normal (3); such defects may explain the absence of an association between cell count and serum IgE level. This is further supported by reports of opportunistic infections, including PCP in HIV-infected children with relatively well maintained CD4 cell counts (24). In addition, acute infection may have a substantial, but variable, impact on CD4 counts because of migration into tissues and changes in lymphocyte trafficking. Thus, CD4 counts may have been lowered transiently, secondary to acute pneumonia, which may explain the lack of correlation between IgE and CD4 counts. Further study of changes in CD4 counts over time and in the absence of acute infection are warranted. Another possibility is that elevated IgE levels may be correlated with the decline in CD4 count over time (9); a limitation of our study was the inability to perform longitudinal follow-up of children to investigate long-term changes in IgE as the study was a cross-sectional design.

No correlation was found between the IgE level and specific pathogens or with outcome, as measured by in-hospital mortality. However, subsets of children with specific pathogens and

the number of patients dying was relatively small and thus it is possible that a type II error occurred. Moreover, a lack of association between outcome and IgE level may be a result of the enrollment criteria, which selected for HIV-infected patients with severe disease. Additional, larger studies are needed to investigate the relationship between specific infections, IgE and outcome in HIV-infected children. The current data do not, however, support the use of IgE as a marker of specific infections or prognosis in HIV-infected children.

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