Simple markers for the detection of severe immunosuppression in children with HIV infection in highly resource-scarce settings: experience from the Democratic Republic of Congo

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Objectives: The decision to initiate the antiretroviral therapy in HIV-infected children living in poor countries is compromised by lack of resources. The objective of this study is to identify simple clinical and biological markers other than CD4+ count and viral load measurement that could help the decision to introduce antiretroviral treatment and to monitor patients.

Methods: A cross sectional study was conducted between January and March 2005 in Kinshasa, Democratic Republic of Congo.

Results: Eighty-four children infected with HIV were recruited. In this cohort, the lymphocytes ($P=0.001$) and CD4 ($P=0.0001$) were significantly lower in children with immunological stage 3 and viral load ($P=0.027$) was significantly higher in children at the same immunological stage. Reticulocytes ($r=+0.440$), white blood cells count ($r=+0.560$), total lymphocytes ($r=+0.675$) and albumin ($r=+0.381$) showed positive significant correlations with CD4. Haemoglobin ($r=-0.372$), Haematocrit ($r=-0.248$), red blood cells ($r=-0.278$) and CD4 ($r=-0.285$) showed negative significant correlations with viral load. Neutropaenia ($P=0.02$), enlarged nodes ($P=0.005$) and oral candidiasis ($P=0.04$) were associated with viral load $>10\,000$ copies/ml. Oral candidiasis ($P=0.02$) was associated with CD4 level $<15\%$.

Conclusion: Oral candidiasis, enlarged nodes, total lymphocytes count, neutropaenia and albumin predict severe immunodepression. These clinical and biological markers may guide the clinician in making the decision to initiate antiretroviral therapy in highly resource-scarce settings.

Keywords: HIV, Children, Simple markers, CD4, Viral load, Antiretroviral therapy, Highly resource-scarce settings, Kinshasa, The Democratic Republic of Congo, Africa

Introduction

Human Immunodeficiency Virus (HIV)/Acquired immune deficiency syndrome (AIDS) is one of the most devastating diseases worldwide. UNAIDS in their 2013 report state that Sub-Saharan Africa contributes significantly to the high global rate of

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morbidity and mortality reported in HIV infection and continues to have the majority of new infections.¹ In 2010, the HIV incidence was estimated to be around 1.9 million (1 700 000–2 100 000) individuals in this part of the world.²

Early detection of the disease, blood screening for HIV and the use of HAART have significantly improved the life expectancy of children infected with HIV.³

In the Democratic Republic of Congo (DRC), the prevalence of HIV is estimated at 1.3%.¹ Despite the high prevalence and incidence, the decision to initiate HAART is compromised by the lack of trained personnel, the inexistence of the appropriate infrastructure and logistics, and the high cost of the anti-retroviral drugs.^{4–7}

Indeed, the CD4+ count and viral load test are possible in only two cities making it difficult to make a decision about the initiation of HAART based on those tests. The decision to initiate HAART is usually related to the presence of an opportunistic infection, which occurs at an advanced stage of the disease.^{8–10}

The lack of the appropriate diagnostic tools remains a challenge for assessing immunosuppression state in resource-limited settings. The World Health Organization (WHO) is promoting the research of simple clinical and biological markers that could be used to start and monitor antiretroviral therapy.¹¹

This paper reports on a cross-sectional study that was conducted in children <3 years-of-age in Kinshasa. The aim of this study was to assess the correlation between immunological parameters (CD4), viral (viral load) and laboratory parameters easy to perform and to identify clinical and laboratory parameters that predict the severe immunosuppression in HIV-infected children.

Patients and Methods

A cross-sectional study was conducted in two hospitals of Kinshasa, DRC, at the University hospital and at the Kalembelembe children's hospital. Eight-four HIV-infected children were recruited.

HIV-infected children aged <72 months-of-age who presented with symptoms of severe HIV infection were recruited and included in the study. HIV infection was confirmed with Determine and Capillus tests where children were aged more than 18 months-of-age and the viral load was used for children <18 months-of-age.

Children were excluded where they had received a blood transfusion in the last 3 months, as well as those receiving HAART and or corticotherapy.

The study received an approval from the Kinshasa School of Public Health IRB. Consent to participate in the study was obtained from parents or legal guardians.

For each participant, their clinical history and clinical records were obtained. Haematology and biochemistry tests, CD4+ count and viral load measurements were performed.

Laboratory collection procedures and blood analysis

Four millilitre of venous blood sample were drawn from each participant into an EDTA tube and were used to determine haematologic parameters at the National Referral Laboratory for AIDS at Kinshasa. Blood count parameters were performed using the BECKMAN Coulter ACT 5 DISS (Miami, FL, USA). Blood samples were collected between 9 a.m. and 12 a.m. Laboratory tests were performed within 2 hours of blood withdrawal in order to minimise the diurnal variations in CD4.¹²

The total proteins were performed by the Biuret Method. Albumin was performed using a spectrophotometer technique with a BIOMERIEUX apparatus (Lyon, France). The kit was donated by Biomérieux (Lyon, France).

The sample for quantification of viral load has been stored at -70°C . The quantification of viral load was determined using the kits AMPLICOR HIV-1 MONITOR version 1.5 by ROCHE (Johannesburg, South Africa) and was performed by the standard method.

Case Definitions

A diagnosis of anaemia was considered in children aged more than 6 months with a Haemoglobin level below 11 g/dl, while the anaemic threshold was 9 g/dl for children aged <6 months. Those with $<5000/\text{mm}^3$ white blood cells were categorised as having leucopenia and those with more than $15\,000/\text{mm}^3$ were considered as having hyperleukocytosis. Neutropenia was based on a neutrophils count $<3000/\text{mm}^3$ and lymphopenia when the lymphocytes count was $<1500/\text{mm}^3$. Thrombocytopenia was defined as a platelets count $<150\,000/\text{mm}^3$.

Participants were categorised in Clinical (A, B and C) and immunological stages of HIV infection using the CDC clinical classification.¹³

In regard to immunosuppression, patients were regrouped into two categories: (1) the reference category (CD4+ more than 15% and/or viral load $<10\,000$ copies/ml) and (2) Severe immunodepression (CD4+ $<15\%$ and/or viral load more than $10\,000$ copies/ml).

The diagnosis of severe infection was based on the presence of one of the following criteria: (i) pneumonia, (ii) meningitis and (iii) septicaemia.

Data management and analysis

Data entry and analysis were done using the Statistical Package for Social Sciences (SPSS for Windows, version 12.0.1; SPSS Inc, Chicago, IL, USA). Entered data were doubled checked for discrepancies. Continuous variables are described with means and standard

deviation (SD) when the distribution is normal and with median and interquartile range (IQR) when the distribution is skewed.

Frequency of various clinical and laboratory findings are expressed as proportions (%). Student's *t*-test or Mann–Whitney *U* test was used to compare data where appropriate. ANOVA test was used to compare differences among clinical and immunological stages.

The correlation between laboratory findings and CD4/viral load was assessed using Spearman correlation coefficient.

Binary logistic regression was used to predict determinants of severe immunodepression and to measure the strength of association of each determinant (adjusted odds ratio).

Results

Among the 84 HIV-infected children recruited for the study, 48 were boys (57.1%) and 36 girls (42.9%) with a sex ratio M/F of 1.3:1. The median age was 30.7 months (range, 2–72 months). In this cohort, the majority (60.7%) of HIV-infected children were aged <36 months-of-age.

Correlation between clinical stages and values of CD4 and lymphocytes

The relation between clinical stages and the total lymphocytes, CD4+ level and viral load are shown in Table 1. The decrease in the total lymphocytes and CD4+ were significantly associated with the advanced stage of the disease ($P < 0.001$). Viral load increased significantly in the stages B and C ($P = 0.001$).

Correlation between immunological stages and values of CD4+ and lymphocytes

Table 2 shows the evolution of the total lymphocytes, CD4+ and viral load according with the immunological stage. The total lymphocytes and CD4+ T lymphocytes decreased significantly with the evolution of immunological stages, while the viral load increased significantly in stage 3. The difference was

statistically significant between the three stages for lymphocytes ($P = 0.001$), CD4+ ($P = 0.0001$) and viral load ($P = 0.027$).

Correlation of laboratory findings with CD4 and viral load

The correlation of laboratory findings with the CD4+ and viral load is shown in Table 3. The results show that the reticulocytes ($r = +0.440$), WBCs ($r = +0.560$), the total lymphocyte ($r = +0.675$) and albumin ($r = +0.381$) are positively correlated with CD4.

Hb ($r = -0.372$), Ht ($r = -0.248$), RBCs ($r = -0.278$) and the CD4 ($r = -0.285$) are negatively correlated with viral load.

Multivariate analysis

The multivariate regression model shows that oral candidiasis ($P = 0.02$) is associated with CD4 <15% (Table 4). Furthermore, neutropaenia ($P = 0.02$), enlarged nodes ($P = 0.005$) and oral candidiasis ($P = 0.04$) were associated with viral load >10 000 copies/ml (Table 5).

Discussion

In this sample, the majority of HIV-infected patients were children <36 months-of-age. This is consistent with previous studies in Africa.^{14–16} The findings suggest that many children die before their fifth year of life. HIV-infected children <36 months-of-age, particularly the infants <12 months-of-age, are at increased risk of life-threatening severe infections and opportunistic infections because of the immaturity of the immune system during the first year of life.^{17–19} Recent studies show that even in early life, there is sufficient B cell functionality to mount bNAbs against HIV-1.²⁰

A positive correlation was found between the CD4 and the stages of the disease, and between the CD4 and the total lymphocytes in HIV-infected children.^{14,21} In HIV-infected adults, the highly significant correlation between CD4 and the total

Table 1 Evolution of the total lymphocytes, CD4 and viral load according to the clinical stage (A, B, C)

	A	B	C	P
Total lymphocytes (mm ³)				0.09
Median	5 145	4 730	3 730	
P2.5	2 660	2 020	1 650	
P97.5	18 280	13 870	8 770	
CD4 (mm ³)				0.000
Median	1 903	861	526	
P2.5	417	30	125	
P97.5	6 165	2 838	2 002	
Viral load (copies/ml)				0.001
Median	400	55 4970	1 098 328	
P2.5	400	400	400	
P97.5	342 8201	6 530 547	4 366 335	

P: percentile.

Table 2 Evolution of the total lymphocytes, CD4 and viral load in relation according to the immunological stage

	1	2	3	P
Total lymphocytes (mm ³)				0.001
Median	5 900	3 870	3 060	
P2.5	2 970	2 540	1 650	
P97.5	18 280	13 870	9 000	
CD4 (mm ³)				0.000
Median	1 939	787	330	
P2.5	1 065	324	30	
P97.5	6 165	1 465	646	
Viral copies (copies/ml)				0.027
Median	11 039	338 462	376 800	
P2.5	400	400	77 143	
P97.5	3 428 201	3 582 120	4 366 335	

P: percentile.

Table 3 Correlation between laboratory findings and CD4 and the viral load

Laboratory parameters	CD4	R
Hb	+0.070	−0.372**
Ht	+0.073	−0.248*
RBCs	+0.066	−0.278*
Reticulocytes	+0.440**	+0.188
WBCs	+0.560**	+0.001
Neutrophils	−0.323**	−0.059
Total lymphocytes	+0.675**	+0.003
Platelets	+0.238*	−0.186
Total proteins	+0.261*	−0.021
Albumin	+0.381**	−0.199
CD4	1	−0.285*

*r: correlation coefficient; *strong correlation; **very strong correlation.

Table 4 Variables selected by logistic regression for the correlation with CD4 < 15%

	B	S.E.	Sig.	Exp (B)	95% Min	CI for Exp (B) Max
Total lymphocytes	0.0003	0.000	0.051	1.000	0.999	1.000
Platelets	0.000006	0.000	0.083	1.000	1.000	1.000
Oral candidosis	1.566	0.680	0.021	4.787	1.262	18.159
Enlarged nodes	1.361	0.726	0.061	3.901	0.940	16.196
Constante	0.425	1.105	0.700	1.530		

B: beta coefficient; S.E.: Standard error; Sig: significance; Exp (B) Antilogarithm in B; C.I.: confidence interval.

Table 5 Variables selected for the prediction of viral load > 10 000 copies/ml

	B	S.E.	Sig.	Exp (B)	95% CI for Min	Exp (B) Max
Neutrophils	0.053	0.023	0.020	1.054	1.008	1.103
Albumin	0.091	0.048	0.058	0.913	0.997	1.203
Oral candidosis	1.364	0.676	0.044	3.912	1.039	14.716
Enlarged nodes	−1.694	0.607	0.005	0.184	0.056	0.639
Constante	2.645	1.918	0.168	14.088		

lymphocytes is a good reflection of CD4 level in case of unavailability of the CD4 count.^{22,23} Furthermore, a positive correlation between leukocytosis and CD4 was also previously reported in Tanzania.²⁴

In HIV-infected women, Feldman *et al.* found a positive correlation between serum albumin and CD4 and between Ht and viral load.²⁵ This situation could be because of the chronic nature of HIV disease leading to an overall malnutrition associated with a decrease in serum albumin in the advanced stages of HIV disease.²⁶ In developing countries,

co-infections and malnutrition may influence the CD4 level or viral load.

CD4 and viral load are the best markers for the initiation of chemoprophylaxis and antiretroviral therapy in HIV-infected children. However, the absence of these two tests led to the establishment of the simple and accessible criteria in low-resource settings.

In this cohort, neutropaenia, enlarged nodes and oral candidiasis were predictors of severe immunosuppression. This is consistent with related studies from other region.^{27,28}

Conclusion

Oral candidiasis, enlarged nodes, total lymphocytes count, neutropaenia and albumin predict severe immunodepression. These clinical and biological markers may guide the clinician in making the decision to initiate antiretroviral therapy in low-resource settings.

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Disclaimer Statements

Contributors

Conceived and designed the experiments: LA, PMT, FK and SE. Performed the experiments: LA, FK and SE. Analysed the data: PKK, LA, MBE, PMT and MNA. Contributed reagents/materials/analysis tools: LA, FK and SE. Wrote the paper: LA, MNA and MBE. PKK revised the paper.

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Conflicts of interest

The authors have declared that no competing interests exist.

Ethics approval

Since all participants were minors, they provided assent and their legal guardians provided consent for study participation. This consent procedure was reviewed and approved by the National Ethical Committee of the Public Health School of the University of Kinshasa, Kinshasa, DRC.

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