Haematologic features of the Human Immunodeficiency Virus (HIV) infection in adult Zimbabweans

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Objective: To describe the haematologic features of the HIV infection in adult Zimbabweans and compare the features in the different clinical stages of the disease.

Design: Descriptive cross sectional study.

Setting: Parirenyatwa Hospital, a tertiary and referral medical centre in Harare, and the blood donor clinics of the Blood Transfusion Service in Harare.

Subjects: Patients attending HIV outpatients clinics or receiving inpatient care at Parirenyatwa Hospital and asymptomatic persons donating blood at the BTS Harare.

Main Outcome Measures: Full blood counts and bone marrow cell counts and morphology.

Results: Blood cytopenia was found in 47.5% of adults with HIV infection. The most frequent abnormalities were lymphopenia (31.5%); anaemia (30.8%); neutropenia (29.6%); thrombocytopenia (24.7%); eosinophilia (23.5%) and leucopenia (11.7%). Frequency of anaemia in the AIDS and symptomatic groups (43.4% and 24.5% respectively) was greater than in the carriers (6.7%), while the frequency of other cytopenias and of eosinophilia was about the same in all groups. There was also a general lack of association between the severity of haematologic abnormalities and the clinical stage of the disease.

Conclusion: Severe haematologic changes occur frequently in HIV infection and AIDS but routine full blood count may not be helpful in the monitoring of the disease or the prediction of onset of AIDS.

Introduction

Haematologic abnormalities associated with the human immunodeficiency virus, (HIV) infection and the acquired immunodeficiency syndrome (AIDS) have been described in various studies.[1,2] Immune thrombocytopenia has been shown to often predate clinical manifestations of the infection, while frequency of other cytopenias and absolute CD4 positive lymphocyte counts are known to correlate with clinical stages of the disease.[3-5] The present study was undertaken to describe the haematologic features of the HIV infection in adult Zimbabweans and compare the features in the different clinical stages of the disease.

Materials and Methods

The study was carried out over a 12 month period, with informed consent on consecutive patients attending the HIV clinic at the Parirenyatwa Hospital in Harare. The HIV positive status of subjects was established by ELISA and confirmatory Western blot testing for HIV antibodies. The clinic patients included a few asymptomatic cases but more were included in the study from among apparently healthy blood donors at the National Blood Transfusion Service (NBTS) in Harare. The informed consent of donors was obtained at the time of donation for their haematologic profiles to be included in the study without prejudice to the

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anonymity and confidentiality of their HIV test results which were routinely determined for all donors by the BTS. Subjects were classified into three categories namely AIDS, symptomatic non-AIDS cases (which were formerly included in the AIDS-related complex group) and asymptomatic HIV positive subjects also called "carriers".

AIDS was diagnosed according to the Centers for Disease Control (CDC) criteria which were in use at the time of the study.[6] Our AIDS cases had the following case-defining diseases, Kaposi sarcoma 30%; wasting syndrome 20%; extrapulmonary and atypical tuberculosis 20%; oesophageal and bronchial candidiasis 14%; cryptococcal meningitis 8%; persistent *Herpes* ulcers, atypical salmonellosis; high-grade non-Hodgkin's lymphoma (NHL) and systemic fungal infections 8%. Symptomatic patients who did not meet the CDC criteria for AIDS were categorised as symptomatic non-AIDS. These included cases of persistent generalised lymphadenopathy (PGL), pulmonary tuberculosis and other non-AIDS-defining conditions.

Tests carried out on all subjects included nine-parameter automated blood counts (coulter-JS) and blood film examination. Bone marrow aspiration was done in some of the cases presenting with cytopenia or with atypical lymphoid cells in the blood film. Myeloid-Erythroid (M-E) ratios, BM lymphocytes, eosinophils and plasma cells were enumerated by differential counting of at least 500 marrow cells. Erythroid dysplasia was semi-quantified by counting the number of dysplastic cells in 200 erythroid precursors. Serum immunoglobulins were determined by rate nephelometry (Beckman) on a quarter of the subjects randomly selected from each group.

Study subjects were not on antiviral agents and patients on cytotoxic therapy for Kaposi's sarcoma and NHL were excluded. Analysis of the results of the three clinical groups was done by Students' *t*-test and the Kruskal-Wallis one way analysis of variance for non-normally-distributed parameters. However, cases in some of the clinical groups were too few for valid statistical comparison.

Results

One hundred and sixty two HIV antibody positive subjects were studied, of whom 83 had AIDS, 49 were symptomatic non-AIDS and 30 were asymptomatic carriers. The subjects were aged 17 to 58 years with a mean age of 32.2 years (SD: 8.4) and comprised 103 males (63.6%) and 59 females. The proportions of subjects showing different abnormalities of blood counts are shown in Table I.

Anaemia.

Moderate to severe anaemia with haemoglobin (Hb) concentration below 11g/dl (range 2.2 to 10.9g/dl) was seen in 30.8 % of all HIV positive subjects. AIDS cases had the highest frequency of anaemia at 43.4%, far above the 24.5% for symptomatic non-AIDS and 6.7% for asymptomatic subjects, ($x2 = 15.25$; $df = 2$; $p<0.001$). It should, however, be noted that the frequency of anaemia in our asymptomatic subjects may be falsely low since they were mostly blood donors for whom anaemia was one of the exclusion criteria. The mean Hb concentrations of the AIDS, non-AIDS, and asymptomatic groups were 8.8 (SD: 1.8), 8.1 (SD: 2.0) and 9.5 (SD: 1.2) g/dl respectively. The differences between theAIDS and non-AIDS groups were not statistically significant ($x2= 1.42$; $df =1$; $p= 0.233$). Anaemia was normocytic in two thirds of cases (42/50) and macrocytic (MCV>100 fl) in the remaining third (18/50), of whom 14/18 had AIDS.

Thrombocytopenia.

Platelet counts of less than 150 x 10^9/L (range 11-149 x 10^9/L) were recorded in 24.7% of HIV positive subjects.

The proportion of thrombocytopenic cases in asymptomatic subjects (20%) was about the same as in symptomatic non-AIDS subjects (18.3%). The AIDS group had a somewhat

Table I: Frequency and severity of haematologic abnormalities in HIV subjects.

Abnormal Parameter	AIDS (Total=83) n	% of Group	Mean	SD	Symptomatics (Total=49) n	% of Group	Mean	SD	Carrier (Total=30) n	% of Group	Mean	SD	All HIV pos. (Total=162) n	% of Group	Mean	SD
Anaemia																
HB<11G/dl	36	43.4	8.8	1.77	12	24.5	8.1	2.0	2	6.7	9.5	1.2	50	30.8	8.6	1.86
Macrocytosis																
MCV>100fl	14	16.9	104.4	3.67	2	4.1	109.8	9.45	2	6.7	102.6	0.8	18	11.1	104.8	5.04
Leucopenia																
WBC<2.9x10⁹/L	13	15.7	2.04	0.70	3	6.1	2.45	0.37	3	10.0	2.46	0.09	19	11.7	2.17	0.64
Neutropenia																
Neut<1.8x10⁹/L	25	30.1	1.15	0.43	14	28.6	1.10	0.45	9	30.0	1.30	0.23	48	29.6	1.17	0.42
Lymphopenia																
ym<1.5x10⁹/L	29	34.9	1.05	0.30	13	26.5	1.20	0.15	9	30.0	1.23	0.20	51	31.5	1.12	0.27
Eosinophilia																
Eos>0.45x10⁹/L	24	28.9	0.81	0.39	9	18.4	0.80	0.33	5	16.7	0.55	0.10	38	23.5	0.77	0.37
Thrombopenia			Median	Q_1/Q_3			Median	Q_1/Q_3			Median	Q_1/Q_3			Median	Q_1/Q_3
Plt<150x10⁹/L	25	30.1	115	60/136	9	18.4	92	45/133	6	20.0	119	95/134	40	24.7	109	60/15

higher proportion of thrombocytopenic cases (30.1%) but the difference fell short of statistical significance (x2 = 2.72; df =2; p = 0.256). Thrombocytopenia was mild to moderate only, in the three groups, the median (Q_1,Q_3) platelet counts being 115 (60,136), 92 (45,133), and 119 (95,134) x 10^9/L for the AIDS, non-AIDS and carrier groups respectively.

Leucopenia.

Leucopenia (WBC < 2.8 x 10^9 /L) was observed in only 11.7% of HIV positive subjects. The AIDS group had a slightly higher prevalence of leucopenia (15.7%) than symptomatic non-AIDS (6.1%) and asymptomatic subjects (10.0%). The differences in frequency were not significant (x2= 2.81; df =2l; p= 0.245), and the non-AIDS cases were too few for comparison of mean white cell counts (Table I).

Table II: Patterns of cytopenias in HIV infection.

	AIDS Total=83		Symptomatic Non-AIDS Total=49		Asymptomatic Carriers Total=30		ALL HIV-pos. Total=162	
	n	%	n	%	n	%	n	%
Single line cytopenia								
Anae only	16	19.3	9	18.4	1	3.3	26	16.0
T-penia only	9	10.8	4	8.2	5	16.7	18	11.1
L-penia only	2	2.4	2	4.1	2	6.7	6	3.7
Sub-total	**27**	**32.5**	**15**	**30.6**	**8**	**26.7**	**50**	**30.9**
Bi-cytopenia								
Anae/ T-penia	10	12.0	3	6.1	1	3.3	14	8.6
Anae/ Lpenia	4	4.8	0	0.0	0	0.0	4	2.5
L-penia/ T-penia	1	1.2	0	0.0	2	6.7	3	1.9
Sub-total	**15**	**18.1**	**3**	**6.1**	**3**	**10.0**	**21**	**13.0**
Pancyto-penia	6	7.2	0.0	0.0	0.0	0.0	6	3.7
Grand total	**48**	**57.8**	**18**	**36.7**	**11**	**36.7**	**77**	**47.5**

Anae=anaemia; T-penia=thrombocytopenia; L-penia=leucopenia.

Neutropenia.

Forty eight HIV positive subjects (29.6%) had absolute neutrophil counts equal to or less than 1.8 x 10^9/L, the proportions of neutropenic subjects being similar in the three groups at 30.1 %, 28.6% and 30.0 % in AIDS, symptomatic non-AIDS, and asymptomatic categories respectively. Neutropenia was almost equally severe for neutropenic subjects in the three groups, the mean counts being 1.15 ± 0.44x 10^9/L in the AIDS cases, 1.10 ± 0.47 x 10^9/ L in the symptomatic -non-AIDS and 1.33 ± 0.24 x 10^9/L in the carriers.

Lymphopenia.

Lymphopenia (lymph < 1.5 x 10^9/L) was a very common haematologic abnormality observed in our subjects, occuring in 31.5 % of HIV positive subjects. The frequency of lymphopenia varied only slightly from 30.0% in asymptomatic HIV subjects to 34.9% in AIDS cases. Among the lymphopenic subjects, the mean lymphocyte count in the AIDS group was 1.05x 109 /L (SD: 0.30). This was slightly lower than for the non-AIDS (1.20 x 10^9/L; SD: 0.16), and the asymptomatic cases (1.22 x 10^9/L; SD: 0.21). The difference fell just short of statistical significance (x2= 3.65; df= 2, p= 0.163).

Multilineage Cytopenias.

As shown in Table II, 30.9% of HIV antibody positive subjects had single cell-line cytopenia with anaemia only leading the group (16.0%), followed by thrombocytopenia-only, (11.1%) and leucopenia only, trailing at 3.7%. However, thrombocytopenia alone was by far the commonest finding among the asymptomatic carriers (16.7 %). Thirteen percent of subjects had bilineage cytopenia. Those with anaemia and thrombocytopenia formed the bulk of the group (8.6 %), while those with anemia and leucopenia constituted 2.5%. Leucopenia with thrombocytopenia but no anaemia occurred rarely (1.9%). Only 3.7% of HIV positive subjects were found to have pancytopenia and significantly all of them were in the AIDS group.

Eosinophilia.

Eosinophil counts greater than 0.45 x 10^9/L were found in nearly a quarter (23.5%) of HIV positive subjects. The frequency was slightly higher in the AIDS cases (28.9%) than in the non-AIDS (18.4%) and the asymptomatic groups (16.7%), although these proportions were not statistically significantly different (x2=2.85; df=2; p=0.240). Eosinophilia was moderate with a mean count of 0.55 ± 0.10 x 10^9 /L in asymptomatics but slightly greater in the other groups with mean counts of 0.80 ± 0.33 and 0.81 ± 0.39 x 10^9/L in the symptomatic non-AIDS and AIDS cases respectively. However, these differences in mean eosinophil counts were not statistically significant.

Peripheral Blood Film.

Blood smears of HIV positive subjects showed some characteristic features. There was increased rouleaux formation in most cases. The red cells were normocytic in the asymptomatic and non-AIDS subjects, but in AIDS cases there was often macrocytosis and occasional dysplastic features such as basophilic stippling.

White cells showed cytoplasmic vacuolations in monocytes and occasional atypical lymphocyte particularly in AIDS, in which eosinophils were also often increased.

Bone Marrow Findings.

Bone marrow (BM) aspirates and trephine biopsies were obtained in 28 cases, 18 in the symptomatic non-AIDS and 10 in AIDS. Erythrodysplastic features seen in BM smears included nuclear irregularity; lobulation or multinuclearity; gross cell membrane irregularity; cytoplasmic inclusions; basophilic stippling and ring sideroblasts. Myeloid, including megakaryocyte, dysplasia was observed as cell and nuclear membrane irregularity, nuclear immaturity or multinuclearity; cytoplasmic hypogranularity and vacuolation and haemophagocytosis. As shown in Table III, 80% of bone marrows examined in both non-AIDS and AIDS were normocellular or hypercellular. Sixty five percent of cases had normal ME ratio, suggesting proportional erythroid and myeloid hyperplasia. Erythrodysplasia was, however, very severe in the AIDS cases reaching up to one dysplastic red cell precursor in four, compared to one in 12 in non-AIDS cases.

Other salient findings in the bone marrow are shown in Table III.

Table III: Bone marrow findings in HIV subjects.

BM feature	AIDS (Total=10) Number affected	Average number score	Symptomatics (Total=18) Average affected	score
Normo/Hypercellular	8	2+	15	2+
Hypocellular	2	1+	3	1+
ME ratio raised	3	1+	4	1+
ME ratio normal	7	N	11	N
Erythro-dysplasia	7	1 in 4	10	1 in 12
Myelodysplasia	6	3+	10	2+
Megaloblastic change	4	2+	4	1+
Plasmacytosis	3	8%	4	5%
Lymphocytosis	2	22%	6	35%
Lymph aggregates	2	1+	3	1+
Lymphoma	1	HG*	0	—
Fibrosis	2	2+	2	1+
Granuloma	3	1+	2	1+
Eosinophilia	2	8%	3	6

** HG = High grade.*

Serum Immunoglobulins.

Serum IgG was above the historical reference range in all the 38 HIV positive subjects examined. Mean serum IgG was 3 487.57 ± 960.39 mg/dl (range 1 710 to 5 510 mg/dl) in HIV subjects compared to the upper limit of normal of 1 680 mg/dl in our laboratory. Though limited numbers of subjects were examined, Serum IgG levels appeared highest in the asymptomatic subjects and lowest in the AIDS group, but the difference was not statistically significant (p= 0.245).

Discussion

This study has confirmed the occurrence of blood and bone marrow changes in Zimbabwean subjects with HIV infection similar to those reported in other groups.[1,2] Peripheral cytopenia and BM morphologic abnormalities were the prominent changes. The most frequent abnormalities in our HIV subjects were Lymphopenia (31.9%), anaemia (30.8%), neutropenia (26.9%); thrombocytopenia (24.7%); eosinophilia (23.5%) and leucopenia (11.7%). These figures are lower than reported in some other studies.[7] It was, however, not always clear in those studies, how much of the reported abnormalities were attributable to antiviral therapies which were not being received by our subjects.

In our study, we found that the frequency of anaemia varied significantly with the clinical stage of HIV infection, anaemia being more common the more advanced the disease. Thrombocytopenia, neutropenia and lymphopenia on the other hand were almost equally common in all clinical stages. This would suggest that HIV infection has a full impact on white cell and platelet development, and/or survival, very early in the course of the disease. Pancytopenia was distinctly associated with AIDS and not HIV infection in general. We found eosinophilia to be nearly as common in our HIV subjects as thrombocytopenia. This abnormality has not been as commonly reported as others and its relationship to HIV infection is not entirely clear. On the whole, there was a lack of correlation between severity of cytopenias, as judged by cell counts, and clinical stage of the disease. This makes routine blood counts, except perhaps in the presence of pancytopenia, unhelpful in monitoring HIV infection or predicting onset of AIDS. Special blood counts such as the CD4 and CD8 - positive subsets of lymphocytes have however been accepted and used for staging HIV infection and predicting AIDS.[8,9]

It may be the progressive depletion of CD4 positive lymphoctyes that was reflected as lymphopenia in our HIV/AIDS subjects. Marrow findings in our subjects were similar to reports in other studies.[10] Uniform finding of BM normo- or hypercellularity in the presence of blood cytopenias would support the belief that peripheral destruction of cells, as has been clearly demonstrated in the case of platelets, and intramedullary ineffective haemopoiesis, are the probable mechanisms for the abnormalities.[3] Bone marrow changes were nearly as well established in symptomatic non-AIDS as in AIDS, confirming early involvement of the haemopoietic system in HIV infection. Infectivity of myeloid cell lines *in vitro* by HIV has been demonstrated,[11] and more recently it has been shown that megakaryocytes express CD4 molecules on their surface membrane[12] and may be infected by HIV in seropositive patients with immune thrombocytopenic purpura.[13] Our results would appear to lend further support to the growing evidence that ineffective haemopoiesis in HIV infection may be due, at least in part, to direct viral infection of myeloid progenitor cells.

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