

SOME HAEMATOLOGICAL ABNORMALITIES OBSERVED IN HUMAN
IMMUNODEFICIENCY VIRUS (HIV) INFECTION AT KENYATTA NATIONAL
HOSPITAL.

A dissertation presented in part fulfilment for the degree of
Master of Medicine (Pathology) of the University of Nairobi.

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December 1992

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Declaration

I certify that this is my original work and has not been presented for a degree in any other University.

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DEDICATION

To my parents, brothers and wife for their support

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ABBREVIATIONS

AIDS	-	Acquired immune deficiency syndrome
AITP	-	Autoimmune thrombocytopenic purpura
AZT	-	Azidothymidine
BM	-	Bone Marrow
CDC	-	Centre for disease control
DAT	-	Direct antiglobulin test
DNA	-	Deoxyribonucleic acid
EDTA	-	Ethylenediamine tetra acetate
HIV	-	Human immunodeficiency virus
HL	-	Hodgkin's Lymphoma
IgG	-	Immunoglobulin G
IL	-	Interleukin
KNH	-	Kenyatta National Hospital
KS	-	Kaposi's sarcoma
MAI	-	Mycobacterium avium intracellulare
MGG	-	May Grunwald Giemsa
NHL	-	Non Hodgkin's Lymphoma
PCP	-	Pneumocystis carinii pneumonia
PTB	-	Pulmonary tuberculosis
RNA	-	Ribonucleic acid
TB	-	Tuberculosis

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SUMMARY

Peripheral blood and bone marrow changes in 50 HIV positive patients, 16 females and 34 males, were studied.

The study revealed that pancytopenia was present in 6% of cases; 88% of the cases had decreased haemoglobin levels and 30% had abnormalities in the white cells, notably lymphopenia (18%); thrombocytopenia was present in 18% of the cases. 86% of the bone marrows showed abnormality. Prominent was myelodysplasia in all cell lines in 58% of the cases. One case had bone marrow infiltration by Histoplasmosis .

When compared with similar studies done elsewhere there is a close corroboration in the findings with this study. The study therefore suggests that there are consistent changes in peripheral blood and bone marrows of patients infected with the human immunodeficiency virus.

INTRODUCTION AND LITERATURE REVIEW

In 1981 the Centre for Disease Control (CDC), Atlanta, Georgia (U.S.A.) was quick to recognise the onset of a new disease following the initial reports of pneumocystis carinii pneumonia (PCP) and Kaposi's sarcoma (KS) occurring in a group of homosexual males (1). The condition became known as the Acquired Immune Deficiency Syndrome (AIDS) and the etiological agent is the human immunodeficiency virus (HIV) of which, currently, there are two main types HIV 1 and HIV 2 (1,2,3). Whereas HIV 1 has a worldwide distribution, HIV 2 is prevalent in some parts of the world mainly West Africa (2). There is no doubt that for world health, these retroviruses have created a great impact (4).

The HIV is a cytopathic RNA retrovirus and like all retroviruses replicates by forming a DNA provirus using the enzyme, RNA directed DNA polymerase (reverse transcriptase) (2). It is chiefly transmitted sexually and by blood and can also be transmitted transplacentally, from mother to infant and possibly through milk during the neonatal period(2). Several millions of people have been infected with these viruses in the U.S., Europe and Africa resulting in a pandemic with significant impact on health care resources (5).

In 1986 the CDC formulated a clinical classification system for HIV infection. This system includes four groups:-.

- Group I. Acute infection
- Group II. Asymptomatic infection
- Group III. Persistent generalised lymphadenopathy

Group IV. Other diseases i.e. constitutional, neurological, secondary infections, secondary cancers and others (1,3).

The wide ranging clinical and pathological changes in persons infected with the HIV are both fascinating and challenging to physicians and pathologists alike (6). Haematological abnormalities are well recognised in HIV disease and result from diverse influences on the haemopoietic tissues (6,7,8,9,10). Peripheral blood and bone marrow changes are commonly seen and many of these complications have been shown to occur with increasing frequency as the infection progresses (6,7,9,10).

The effect of HIV on haematological practice is very important (7). First the haematologist is on the front line in handling potentially infected blood samples and has to protect not only himself but others; like laboratory personnel and equipment while at the same time providing the haematological services required. The haematologist is also responsible for providing safe blood and blood products for use (1). The haematologist therefore, should be aware of the basic blood, bone marrow and clinical aspects of HIV. Also many of its manifestations are haematological and therapy available to date results in myelotoxicity and significant blood changes (8).

Decreased blood cell counts are common in HIV infection. This may be due to impaired haematopoiesis (dyshaemopoiesis), underproduction or accelerated destruction which may be immune

mediated (9). Ineffective haematopoiesis occurs and may result from direct suppressive effects of HIV, opportunistic infection or tumour infiltration in the marrow or antiretroviral agents used such as azidothymidine (AZT), dideoxycytidine (DDC), phosphonoformate, antimicrobial agents like cotrimoxazole and/or antitumour therapy (9). Discriminating among the multiple possible aetiologies of cytopenia may therefore be difficult. Kaposi's sarcoma, though rarely involves the bone marrow, does result in gastrointestinal and cutaneous lesions which maybe sources of blood loss (8). Further Kaposi's sarcoma causes poor nutrition, frequent other infections and general body apathy resulting in poor physiological response which may affect the haematological system as much.

Peripheral blood changes

Pancytopenia associated with AIDS was first reported in 1983 (7,8,10,11,12). Lymphopenia may be present in over 70% of AIDS patients and CD4 positive, a subset of T lymphocytes are specifically depleted (8,9,10). Thrombocytopenia occurs in 40% of patients and maybe an isolated haematological finding (3,7,8,9,10). Anaemia and granulocytopenia tend to occur simultaneously (3). Anaemia occurs in approximately 70% and the incidence of neutropenia varies between 20-65% in AIDS patients (9,10).

Overall lymphopenia is the predominant haematological finding in HIV infection as the retrovirus is lympholytic and cytopathic for CD4 (T₄) lymphocytes and causes cell lysis during its

intracellular replication (10). A second mechanism for lymphocyte destruction is cell fusion of infected and uninfected CD4 lymphocytes. This syncytial formation produces multinucleated giant cells which die soon after formation. Lymphocytotoxic autoantibodies are present in 95% of cases of AIDS related disease (10). These lyse uninfected CD4 lymphocytes. Also impaired T cell proliferation occurs secondary to interleukin 2 (IL 2) production and lymphokine release by macrophages (10).

Granulocytopenia is a less well recognised feature and a left shift is a common finding (7,8). The incidence of neutropenia has been reported in 20-65% of AIDS patients and may result from concurrent infection, drugs which suppress the marrow or decreased bone marrow production consequent on inhibition of progenitors by a glycoprotein present in the marrow of infected patients (8,10,13). Antineutrophil antibodies have been observed in HIV infection and in some patients with neutropenia, an autoimmune mechanism may be implicated (8,9,10).

Anaemia with nonspecific red cell morphology is present in majority of patients with AIDS and has been attributed to effects of drugs such as cotrimoxazole, pentamidine, azidothymidine, cytotoxic drugs and to underlying chronic infection (8,10,14). Dyserythropoiesis and presence of erythrophagocytic histiocytes in bone marrow also result in anaemia. Also serum erythropoietin levels do not increase appropriately in AIDS patients in response to anaemia similar to anemia of chronic disease(10). Anemia with

haemoglobin levels of less than or equal to 7.5 g/dl occurred in 34% of azidothymidine (AZT) treated patients following 6 weeks of therapy (9,14). In a study by Richman et al. azidothymidine has been associated with myelosuppression and solely pure red cell aplasia in two patients, erythroid maturation arrest and erythroid hypoplasia in three patients have been reported (10,14). Red cell transfusions were required in more than one occasion by 21% of AZT recipients and 4% of placebo recipients in a double blind placebo controlled trial (14). This supports the fact that in some cases severe anaemia ensues.

Richman et al found red cell bound immunoglobulin and/or complement in 18% of anaemic AIDS patients who required transfusion. All of these patients had a positive direct antiglobulin test (DAT) but clinically no haemolysis was observed. They reported that a positive DAT in AIDS patients appears not to be associated with autoimmune haemolytic anemia (15,16).

Thrombocytopenia occurs in 5-20% of asymptomatic HIV 1 infected individuals and increases to 25-50% in AIDS patients (10). The aetiology of thrombocytopenia is complex and includes immune mediated destruction, impaired production, toxic effects from medication and syndromes mimicking the haemolytic uraemic syndrome (9). In majority of the patients with reduced platelet numbers and no splenomegaly, no drug treatment and adequate or increased megakaryocytes in the bone marrow, a presumptive diagnosis of immune thrombocytopenia may be made. This parallels the clinical syndrome of classic autoimmune thrombocytopenic purpura (AITP) (8,9,10). Mechanisms advanced include platelet

autoantibodies, immune complexes, drugs or part of pancytopenia often seen in patients with opportunistic infections (8,9,10,).

Initial reports by Walsh et al, circulating immune complexes capable of binding platelets were found in sera of thrombocytopenic homosexual males (16,17). They suggested that nonspecific deposition of complement and immune complexes rather than specific antiplatelet antibodies account for platelet bound IgG (8,9,10,15,17).

Elevated levels of platelet bound immunoglobulins have been detected in HIV infected patients. Van Der Lelie et al eluted the immunoglobulins with ether and these were shown to bind to normal platelets but not to type I Glanzmann's disease platelets, indicating the presence of specific platelet autoantibodies (8,9,10,15). Primary sequestration in the spleen also occurs (10). The specific mechanism of platelet autoimmunity remain controversial (18). Van Der Lelie et al suggest that the mechanism is similar to that in classic AITP but Walsh et al suggest that it is different from that in AITP (8,9,10,15,17).

Statistically significant increases in the platelet counts have been observed in AIDS patients on treatment with AZT (14). This suggests that the virus participates in thrombocytopenia. In a study by Walsh et al, sixteen out of seventeen patients with thrombocytopenia had a moderate to excellent response while on corticosteroid treatment and ten out of ten patients had an excellent response to splenectomy which has persisted (19).

Bone marrow findings.

Several studies have addressed bone marrow (BM) morphology in patients with AIDS and findings of interest which may be typical, if not specific have been reported (3,4). BM aspiration and biopsy range from decreased, normal to increased cellularity (3,8,10,11). Myeloid: Erythroid ratios are usually normal but may be increased or decreased. BM aspiration may be difficult and may not be adequate for assessing cellularity as reticulin fibrosis is frequent and has been reported in 21-90% of patients (8,10,11,20).

Myeloid, erythroid and megakaryocytic dysplasia occurs in 30-50% of AIDS/ARC patients (10). Myeloid dysplasia often presents as a marked left shift in maturation with large myelocytes, metamyelocytes and juvenile band forms which are consistent with lack of synchrony between nuclear and cytoplasmic maturation. Dysgranulopoiesis affects eosinophils as well and marrow eosinophilia has been observed in 9-45% of cases (10). Dyserythropoiesis presents mainly as megaloblastic features, not due to folate or B12 deficiency, with binucleated erythrocytes, fragmented nuclei and ringed sideroblasts (8,10,11). Megakaryocytic dysplasia is exhibited by dwarfism, nuclear hyposegmentation and nuclear fragmentation (multiple discrete nuclei) (11,21).

Other marrow changes include lymphoid aggregates and plasmacytosis (3,8,11,20,21). Pseudogaucher cells in BM of patients with co-existent mycobacterium avium intracellulare

(MAI) infection have been described (3,20). Plasmacytosis occurs in majority of AIDS patients (10). The presence of lymphoid aggregates does not presage the development of but may mimic a malignant lymphoma (10,11,20).

MAI may be cultured from bone marrow aspirates in disseminated infection and is associated with anemia and red cell hypoplasia. Cryptococci and histoplasma have also been shown in marrow aspirates (8,10,11,20). One case of pneumocystis carinii involvement of the marrow has been reported (8,20,22). Kaposi's sarcoma rarely involves the BM and only a few cases have been reported (3,10,11,20). Reticuloendothelial iron blockade a characteristic of anemia associated with chronic infections, inflammatory and neoplastic disease, with increased bone marrow iron stores have been found in 14-55% of patients infected with HIV (8,11). Malignancies like non-Hodgkins and Hodgkins lymphoma and acute leukemia have also been documented (8,10).

Patients with both inherited and acquired abnormalities of cellular immunity have a higher incidence of Non-Hodgkin's lymphoma than the general population (23). Infection with HIV is associated with the development of B cell high grade lymphomas (8,11,20,23). Biggar et al, demonstrated a significant rise in the incidence of Non-Hodgkin's lymphoma (NHL) among unmarried single males between the ages of 20 and 49 in Sanfrancisco (23). The aetiology of NHL in the setting of HIV infection is uncertain and future studies are required to identify the precise mechanisms (23).

The morphological appearances of NHL in HIV in majority of cases are classified as diffuse large cell tumours of either the intermediate grade or high grade immunoblastic type (11,23). About one third of patients present with tumours of the high grade, small non-cleaved cell variety (11,23). The commonest clinical feature of NHL with HIV infection is extranodal disease (20,23). In a recent study of 89 patients from New York University, 87% had extranodal disease at presentation and the most common sites included the gastrointestinal tract, central nervous system, bone marrow and liver (23). Rectal lymphomas presenting as perirectal abscesses have been reported. Four cases of NHL involving the heart have been described. Lymphoma confined to the CNS is frequently observed in HIV infected patients and the clinical and radiological findings in these patients are often indistinguishable from those of cerebral toxoplasmosis (8,11,23).

The therapeutic outcome and survival of these patients has been disappointing. Complete response is achieved less frequently, relapse rates are higher and survival generally shorter than those in non HIV infected patients with NHL (23).

Hodgkin's disease while not causally linked to the presence of immunodeficiency appears to have a more aggressive clinical form which often presents with advanced stage and marrow involvement, in patients with HIV infection (11,20,23). Majority of patients presented with extranodal disease in a New York University study (23). The most common histological subtype was mixed

cellularity, followed by nodular sclerosis and lymphocyte depleted respectively (23). The response to therapy has been poor with associated short survivals. Poor BM reserve and the occurrence of intercurrent opportunistic infections has made it difficult to administer many of the standard chemotherapeutic regimes now used for the treatment of Hodgkin's disease (23).

The high frequency of pneumocystis carinii pneumonia argues strongly for antibiotic prophylaxis against this common opportunistic pathogen (20,23).

There are in the world today three basic epidemiological patterns of AIDS. In the first pattern, seen in North America and Europe, transmission is predominantly through homosexual contact although heterosexual transmission is increasing and through sharing of unsterilised needles and syringes by intravenous drug abusers (24). In the second pattern seen in tropical Africa and the Carribean, transmission is mainly heterosexual, vertically from mothers to infants and through blood transfusions, with some transmission from injections, possibly scarifications and other transfer of blood by unsterilised instruments. In the third pattern, seen in Asia and North Africa, infections have been identified in only a few, most of whom have had contact with other parts of the world and show high risk behaviour such as male homosexuality, female prostitution or intravenous drug abuse (24).

All three patterns are observed on the African continent and the Central part of Africa, including Kenya, is in the midst of an epidemic of AIDS. As many as 5-10% of young adults in some urban areas may be having antibodies against HIV (24,25).

The epidemiology, the major risk groups and the clinical presentation of HIV infected populations are very different in Western Europe/United States and in regions of Central Africa and Haiti. There is no reason why the same should not apply to haematological findings. It is the aim of this study to determine this aspect.

AIMS AND OBJECTIVES.

1. To define the prevalence of blood cell and bone marrow abnormalities in HIV infected patients as seen at the Kenyatta National Hospital
2. To compare the haematological abnormalities in Western population as derived from literature/information with those found in HIV infected patients at Kenyatta National Hospital.

ETHICAL CONSIDERATIONS

- o This study did not raise any ethical issues as the indications for the haematological work up are no different from those in patients with other diseases.

- o Secondly it was performed on patients whose HIV status had been confirmed previously.

- o The identity of the patients was also not revealed at any stage of the study.

- o However informed consent was obtained from all patients and only those willing to participate were included in the study. Those unwilling to participate were accorded adequate medical attention.

- o The ethical committee had gone through the protocol and found it ethically viable and gave its consent for the project to go ahead.

JUSTIFICATION

- o The central part of Africa including Kenya is probably in the midst of an epidemic of AIDS. The number of people infected by the HIV is quite high and is increasing. AIDS affects individuals who are in their most productive years (20 - 40) of their lives. Blood changes are noted frequently so that a study to put these changes in figures are necessary.

- o Several studies have been carried out to characterise the blood and bone marrow changes in HIV related disease in Western countries. This study was carried out to study the blood and bone marrow changes in HIV related disease in our environment.

- o The epidemiology, the major risk group and the clinical presentations of HIV infected populations are very different in Western Europe/United States and in regions of Central Africa and Haiti. There is no reason why the same should not apply to haematological findings. It is the aim of this study to determine the aspect.

MATERIALS AND METHODS

This was a prospective study carried out between May 1991 and March 1992 at the Kenyatta National Hospital. The study was approved by the Ethical review committee of Kenyatta National Hospital and the University of Nairobi.

The patients were informed of the purpose of the study and verbal consent was obtained. Clinical history and physical examination was performed on the patients who fulfilled the inclusion criteria.

Two millilitres of venous blood was collected into an EDTA bottle from the cubital vein. Full blood counts were done using the Coulter counter (Model S IV Plus) and a thin blood film was made and stained with the May Grunwald Giemsa stain. (Appendix IV)

A sternal crest marrow aspiration was performed in the orthodox way, (Appendix III) on all patients. The marrow smears were stained with the MGG and Perls Prussian blue stain for iron (Appendix V).

Morphological evaluation of the peripheral blood film and marrow smears was carried out. The slides were examined by the investigator first and then an experienced haematologist. The red cells were examined for rouleaux formation, anisopoikilocytosis, anisochromia or any inclusions or parasites. A differential count of the white cells was obtained by counting 100 white cells and the neutrophils were examined for any

cytoplasmic vacuolations, toxic granulations or for any dysplastic changes like hypogranularity or rounding off of the nuclear lobes.

The bone marrow smears were assessed for cellularity of the particles and the myeloid: erythroid ratio was obtained by counting 500 nucleated cells. Erythropoiesis was quantified as hypoplastic, normal or hyperplastic and its quality of maturation was assessed to see if it was normoblastic, megaloblastic, dysplastic or micronormoblastic.

Granulopoiesis was assessed for its quantity and for maturation of the series by estimating the various proportions of myeloblasts, promyelocytes, myelocytes, metamyelocytes and segmented forms. The population sizes of eosinophils, lymphoid cells and plasma cells were obtained by differential counts of the nucleated cells. Foreign cells such as carcinoma cells were also searched for.

The number and activity of megakaryocytes was assessed. Dysplastic changes like dwarfism, nuclear hyposegmentation and nuclear fragmentation (multiple discrete nuclei) were sought.

The smears were also checked for parasites, pigment or fungal elements and the iron status was assessed as reduced, normal or increased in the Perls Prussian blue stain. The iron granules were checked to see if they were in normoblasts or reticulum cells. The presence of ringed sideroblasts was noted.

Inpatients were investigated and managed in their respective wards in collaboration with other ward staff. Other investigations included chest Xrays, liver function tests, Urea and Electrolytes, Alkaline Phosphatase and urinalysis. Histological specimen of a lymph node or a skin were obtained for a diagnosis where necessary.

The relevant information about the blood and bone marrow characteristics was extracted onto a proforma (Appendix I). The frequency of the characteristics was obtained and compared with similar characteristics derived from western literature.

INCLUSION CRITERIA

- i. Only patients above the age of 14 years with a positive Elisa for HIV were included
- ii. Patients who had been on any kind of anti-HIV medication were included.

EXCLUSION CRITERIA

- i. Patients who were very sick requiring intensive care were excluded
- ii. Patients who had very fulminant secondary infections like miliary tuberculosis were excluded from the study.

RESULTS

A total of 57 patients were initially interviewed and enrolled for the study. Of these 50 patients fulfilled the inclusion criteria as 7 patients refused to consent. One patient had a failed marrow tapping on two occasions and refused consent for a trephine biopsy. Hence only 49 patients had complete data. Also there were some patients on whom certain parameters could not be obtained due to technical reasons for example only 30 specimens were available for stainable iron by Perl's stain. This has been adjusted for in the study.

The study composed of 34 males and 16 females (M:F ratio of 2.1:1). The age range was 19 years to 52 years. Median age was 30 years and mean age was 31.7 years (Fig 1)

The reasons for hospital admission of 50 patients were variable as shown in Table 1. 22 patients (44%) had chest symptoms. Of these 9 patients (18%) were suspected to have tuberculosis clinically and were empirically on anti-TB treatment. 3 patients (6%) had proven TB with definite bacteriological evidence of mycobacteria. 10 patients (20%) had non-tuberculous pneumonias. 10 patients (20%) had diarrhoeal diseases characterised by loose watery stools of more than two weeks duration. (Table 1)

9 Patients (18%) had haematological conditions. Of these 7 patients (14%) had anaemia and 2 (4%) had lymphoproliferative disorders. (Table 2)

Of the remaining 12 patients, (6%) had Kaposi's sarcoma, (6%) Central nervous system involvement, (4%) extensive and infected scabies and (2%) cardiovascular disease.(Table 1)

Further analysis revealed that 44 patients had haematological problems. Pancytopenia was present in 3 patients (6%), anaemia in 38 patients (88%), leucopenia in 10 patients (21.5%) and lymphopenia in 9 patients (19.5%).(Table 2)

Thrombocytopenia was present in 8 patients (18%).(Table 2). The reference range of the blood cell counts used at Kenyatta National Hospital is given in Appendix II.

The mean corpuscular volume ranged between 86fl and 102 fl. The peripheral blood film of 37 patients (74%) had moderate to marked rouleaux and anisopoikilocytosis (P1). 33 patients (66%) had toxic changes in the granulocytes characterised by cytoplasmic vacuolations and toxic granulations (P2). 9 patients (18%) had dysplastic changes in the granulocytes such as nuclear abnormalities, Pseudopelger forms and cytoplasmic changes like hypogranularity (P3).

42 bone marrows (85%) showed abnormality. Of these, 10 (24%) were hypercellular and 32 (76%) were normocellular. Cellularity was not easily definable in 7 (14%) as there were few particles in the prepared marrow smears. The myeloid: erythroid ratio was increased in 10 cases (24%), reversed in 2 cases (5%) and normal in 30 cases (71%). (Table 3).

Erythropoiesis was dysplastic in 10 cases (20%), micronormoblastic in 3 cases (6%) and normoblastic in 34 cases (70%). Granulopoiesis was dysplastic in 1 case (2%), left shifted without excess of blasts in 14 cases (28.6%) and orderly in 25 cases (51%). Plasma cells ranging between 10% to 20% (Normal - upto 10%) were present in 22 cases (45%) and eosinophilia (Normal upto 3%) in 9 cases (18%).(P4,P5,P6).

Megakaryocytes were dysplastic in 23 cases (47%) and increased in 7 cases (14%).(P7,P8). Stainable, iron was increased and mainly in the reticuloendothelial system (P9) in 16 cases (53%), normal in 11 cases (37%) and reduced in 3 cases (10%). Ringed sideroblasts were present in 3 cases (10%) who were all on anti-TB therapy.(Table 3).

Other abnormalities observed included:-

- o Brown pigment (malarial or schistosomal) in 2 cases (4%).
- o Infiltration by histoplasmosis (fungal infection) in 1 case (2%).
- o Sea blue histiocytes and lymphocytosis in 1 case (2%) each.

Karcher and Frost (1990) studied the bone marrows of 178 patients infected with HIV and reported the incidence of hypercellularity in 53%, hypocellularity in 13%, dysplasia in 69%, plasmacytosis in 25%, eosinophilic in 6% and dysplastic megakaryocytes in 31%.(Table 3).

Table 3 shows the results of this study on bone marrow

characteristics of patients infected with the HIV compared with those from Western derived literature.

DISCUSSION

The haematological features particularly the bone marrow morphological changes associated with HIV related disease have been well characterised in some previous studies. However the incidences of these abnormalities have varied widely in these studies. (Table 3)

These variations could be the result of the relatively small number of patients in many of these studies, the lack of uniform criteria among the different authors, the stage of the disease when samples assessed, the different chemotherapeutic agents administered to the patients and their effects on the bone marrow or the different second pathologies that occur in different parts of the world eg. Tuberculosis, Pneumocystis carinii pneumonia etc.

By applying uniform and widely accepted criteria to a large group of patients, a better comparison of the characteristics may be made.

Majority of patients with AIDS manifest with anaemia, granulocytopenia and about one third have thrombocytopenia. Our findings confirm these observations. (Table 2).

Anaemia was present in 38 cases (88%) in this study. 2 cases were receiving cytotoxic drugs, 1 case was on AZT and 11 cases were on anti-TB treatment with isoniazid, thiacetazone and

streptomycin. Many of these drugs are myelotoxic. 3 patients had Kaposi's sarcoma which may lead to blood loss especially in the gastrointestinal tract. 1 patient was diagnosed to have refractory anaemia with excess blasts. (RAEB). In 20 cases (46%) no secondary cause of anaemia was apparent. It has been suggested that erythropoiesis could be suppressed by the action of a retrovirus on progenitor cell maturation.

Anisocytosis and poikilocytosis (often marked) are commonly seen with moderate to marked rouleaux on the peripheral blood film. (P1).

Leucopenia occurred in 21.5% and lymphopenia in 19.5% of the cases. The lymphopenia is mainly due to the cytopathic and lympholytic effects for the CD(T_H) lymphocytes of the retrovirus. Also impaired T cell proliferation occurs secondary to interleukin 2(IL 2) production and lymphokine release by macrophages.

Dysplastic granulocytes present in 9 cases (18%) could be due to myelotoxic agents, secondary infections or myelodysplasia. (P3). Neutropenia in HIV infected patients maybe due to inhibition of granulocyte precursors by a glycoprotein present in the marrow of these patients.

All thrombocytopenic patients had adequate or increased megakaryocytes in the marrow. The megakaryocytes showed dysplastic changes but platelet budding was normal. Thus an

immune mediated peripheral destruction of platelets maybe the cause of thrombocytopenia.

The common bone marrow characteristics in our study included plasmacytosis (45%), myelodysplasia (59%), increased reticuloendothelial iron blockade (53%) and eosinophilia (18%). (Table 3).

Zon et al (1987) and Karcher et al (1990) reported an incidence of myelodysplasia in 50% and 69% respectively of their cases. These are close to our incidence 59%. Karcher et al did not find any correlation between myelodysplasia and drug toxicity. Thus myelodysplasia associated with HIV infection is probably a direct result of the disease. Indeed myelodysplasia may play a significant role in the causation of anaemia and leucopenia.

Plasmacytosis and lymphoid aggregates have been reported to occur in a large proportion of HIV infected patients. These may represent a physiological response to an unusual antigenic stimulation or dysregulated B cell proliferation due to HIV. We could not document the frequency of lymphoid aggregates as they are better seen in biopsy rather than aspirate specimen. These aggregates are widely accepted as benign.

Marrow hypercellularity has been encountered in patients infected with HIV with a reported frequency of 15-59% (Table 3). We found BM hypercellularity in 24% of our cases. Zon and colleagues showed a greater incidence of hypercellularity in HIV infected people with active opportunistic infection or ongoing drug

therapy compared to those with neither of these clinical features. Hypercellularity in this environment may be due secondary infections like tuberculosis or malaria.

Karcher et al (1990) reported an incidence of dysplastic megakaryocytes in 31% of their cases. Using similar morphological characteristics, we found dysplastic megakaryocytes in 47% of our cases.(P7,P8). They may represent myelodysplasia associated with HIV related disease.

A variety of opportunistic microorganisms including atypical mycobacteria, cryptococcus, histoplasma and pneumocystis spp. have been identified by morphological examination of bone marrow specimens. We were able to identify histoplasma organisms within the cytoplasm of numerous histiocytes in one patient (2%). Karcher et al (1990) reported BM infiltration by histoplasma in 2% and cryptococcus in 2% of their specimens. We did not do the special stains necessary for demonstration of cryptococci. Both our and their study did not find any case of Pneumocystis carinii involvement of the BM.

Infection with HIV is associated with the development of a number of neoplasms including certain intermediate grade to high grade B cell lymphoma and Kaposi's sarcoma. In our study one patient had BM involvement by Non Hodgkin's lymphoma but none of the patients had Kaposi's sarcoma in the BM. Probably a more sensitive method like trephine biopsy may have detected KS in the BM. Consideration should be given to treatment of these

malignancies with reduced doses of cytotoxic agents.

Although there are many drugs to which patients infected with HIV are exposed to such as Zidovudine (AZT) etc which certainly play some role in the production of haematological abnormalities, some of the haematological defects are directly related to the HIV infection and its complication. This is supported by the fact that very few patients at KNH are put on AZT as it is very expensive. Only one patients in this study was on AZT.

Improved understanding of the pathogenesis of the haematologic abnormalities could yield insights into assisting therapy of AIDS patients and to lead a comfortable life. This is because haematologic pathology may be playing a significant role in the morbidity and mortality of some of these HIV infected individuals.

CONCLUSIONS

Peripheral blood and bone marrow changes are common in HIV infected patients at KNH.

The common abnormalities in blood include pancytopenia, anaemia, leucopenia and thrombocytopenia. The cause of the cytopenia is multifactorial.

Among the common bone marrow findings in HIV related diseases are myelodysplasia, plasmacytosis and reticuloendothelial iron blockade.

Discriminating among the multiple possible aetiology of cytopenia is very difficult.

RECOMMENDATIONS

HIV infection should be considered in the differential diagnosis of any patient being evaluated for mono, bi or pancytopenia.

This may cut the expensive and costly evaluations for unexplained leucopenia, anaemia and thrombocytopenia that some patients may undergo.

Evaluation of a patient with known HIV infection should involve blood counts and bone marrow evaluation when necessary. Also particular attention should be paid to haematological changes of intercurrent therapy. It will be prudent to ensure that infections, neoplastic and/or drug treatment causes of these haematological abnormalities are ruled out.

Table I: Conditions for which the patients were admitted to KNH

CONDITION	No. of Patients	Percentage(%)
1. Chest ailment		
i) Suspected TB	9	18
ii) Proven TB	3	6
iii) Non-Tuberculous	10	20
2. Diarrhoeal disease	10	20
3. Haematological		
i) Anaemia	7	14
ii) Lymphoproliferative disorder	2	4
4. Kaposi's Sarcoma	3	6
5. CNS Disease	3	6
6. Generalised Scabies	2	4
7. Cardiovascular disease	1	2
TOTAL	50	100

Table 2: Peripheral blood abnormalities found in HIV infected cases

Abnormality	This study 1992	Karcher et al 1990	Zon et al 1988
Pancytopenia	6	-	-
Anaemia	88	89	71
Leucopenia	21.5	58	65
Thrombocytopenia	18	45	43

Key:

All figures are in percentage

Table 3: Results of studies on Bone Marrow characteristic of HIV infected patients

AUTHOR	THIS STUDY	KARCHER	ZON	CASTELLA
YEAR	1992	1990	1987	1985
No. of Patients	50	178	34	55
Hypercellular	24	53	59	15
Normocellular	76	34	35	67
Hypocellular	0	13	6	17
Dysplastic	59	69	6	-
Erythroid dysplasia	20	56	47	-
Myeloid "	2	18	29	-
Plasmacytosis	45*	25	53	83
Increased megakaryocytes	14	64	50	37
Dysplastic megakaryocytes	47	31	-	-
Increased iron	53	65	53	44
Adequate iron	37	-	47	31
Lymphoid aggregates	-	35	21	10
Eosinophilia	18	6	9	-
Histoplasma	2	2	-	-

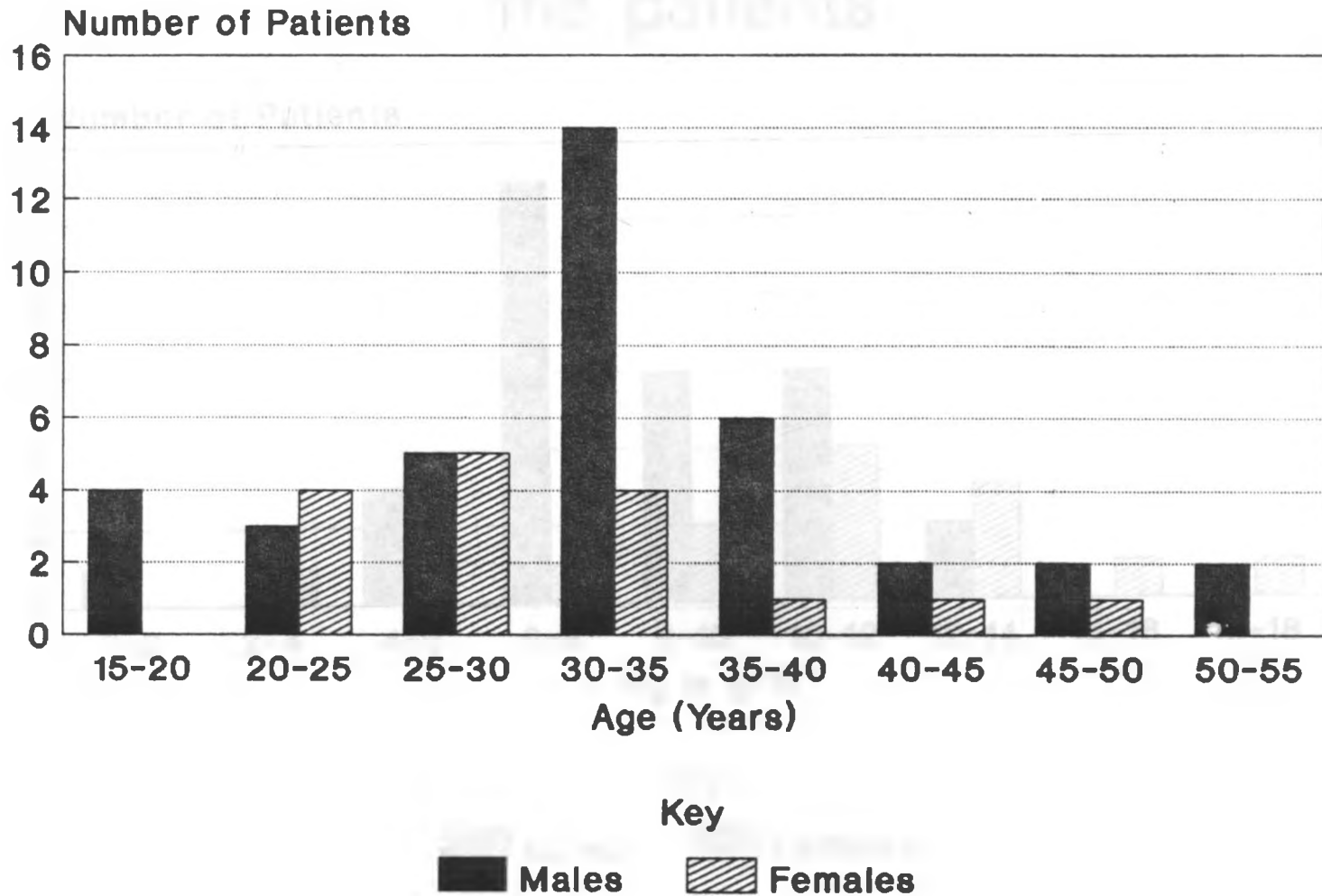
Key:

All figures are in percentages

- not evaluated in the study

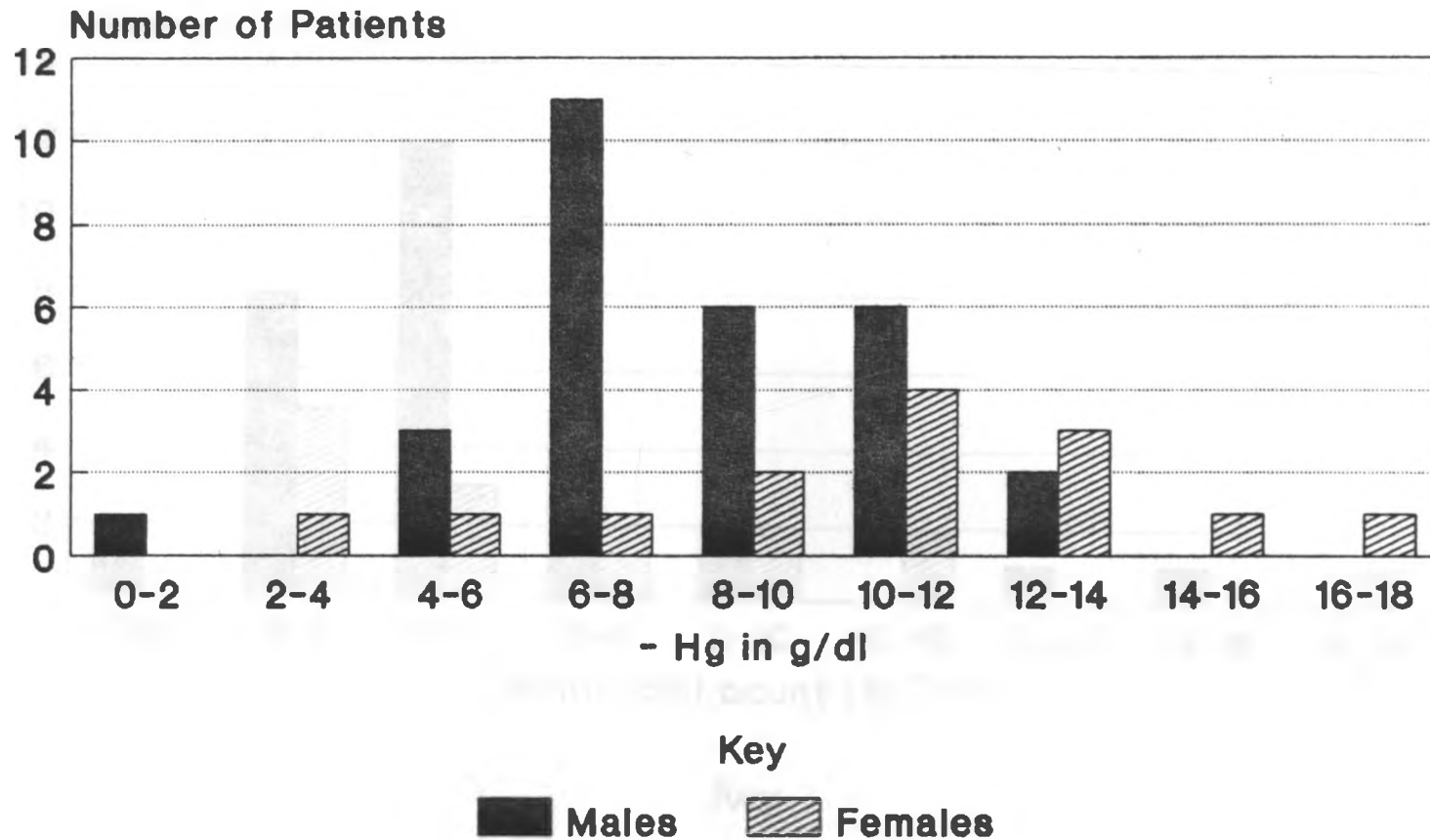
* Plasmacytosis = >10% plasma cells in BM

Fig 1 Age and Sex Distribution of Patients



Median Age 30 years Mean Age 31.7 years

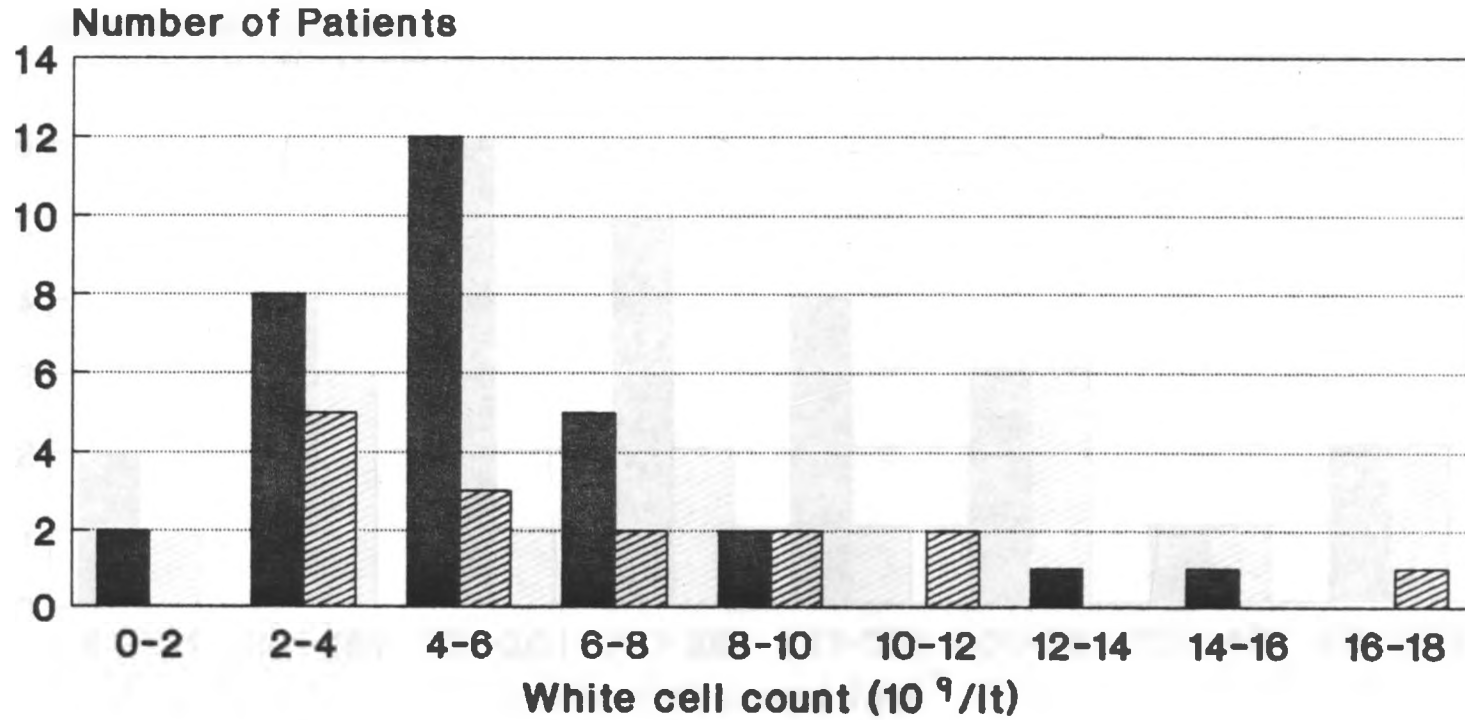
Fig 2 Haemoglobin (g/dl) distribution among the patients



Normal range Males 12.5 - 18 g/dl
 Females 11.5 - 16.5 g/dl

Fig 3

Total white cell count distribution among the patients



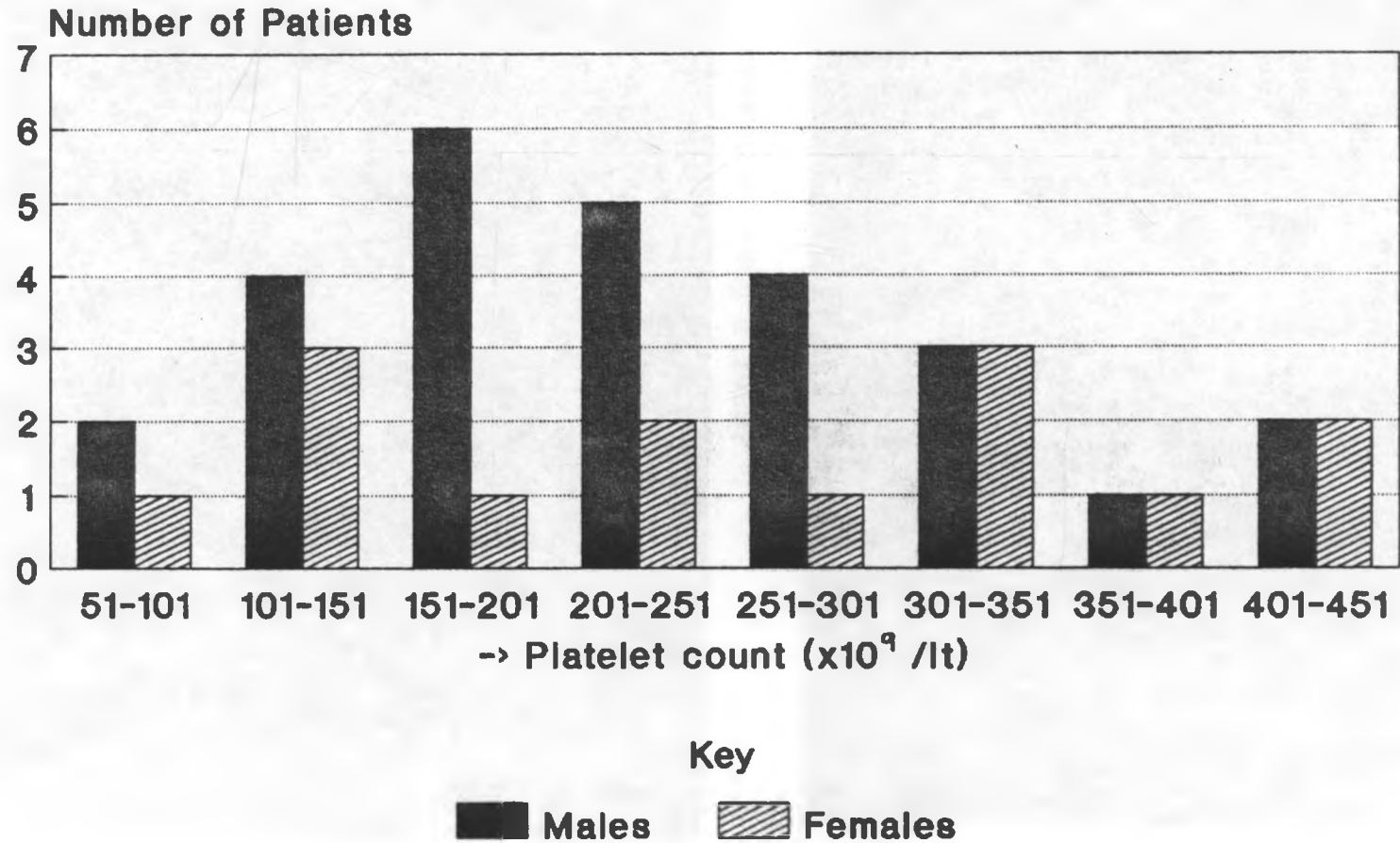
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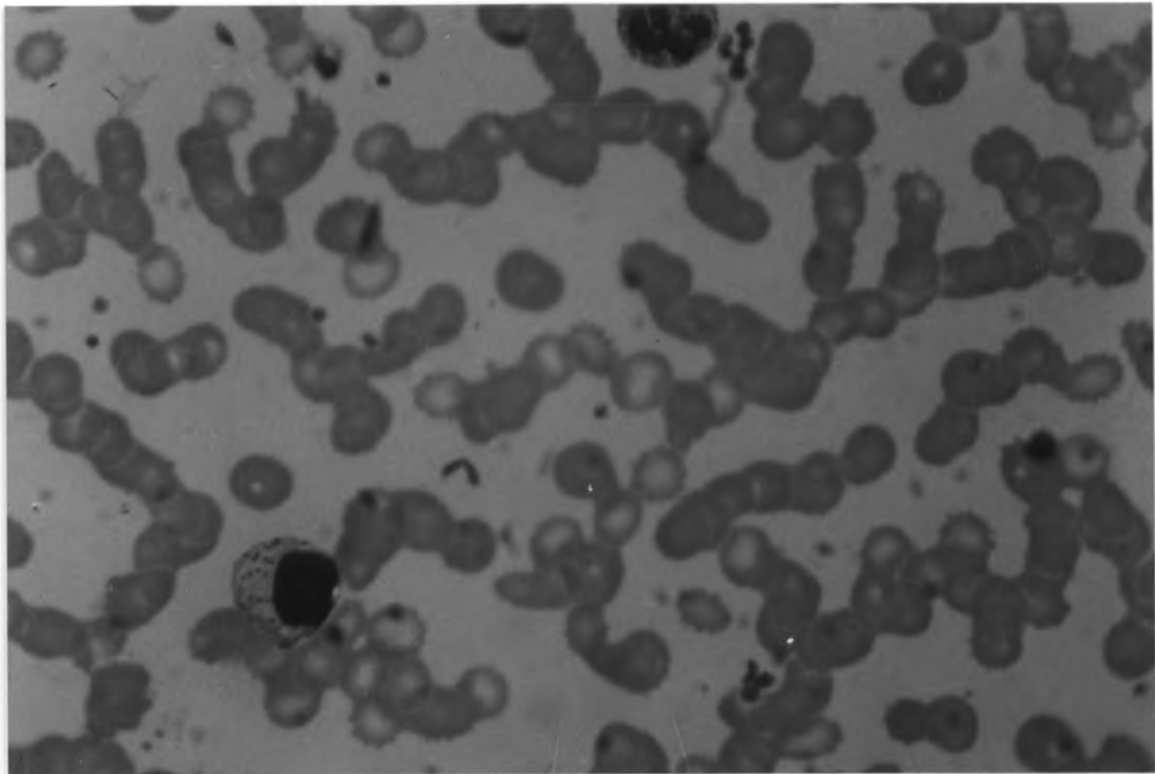
■ Males ▨ Females

Normal range $3-10 \times 10^9$ /lit

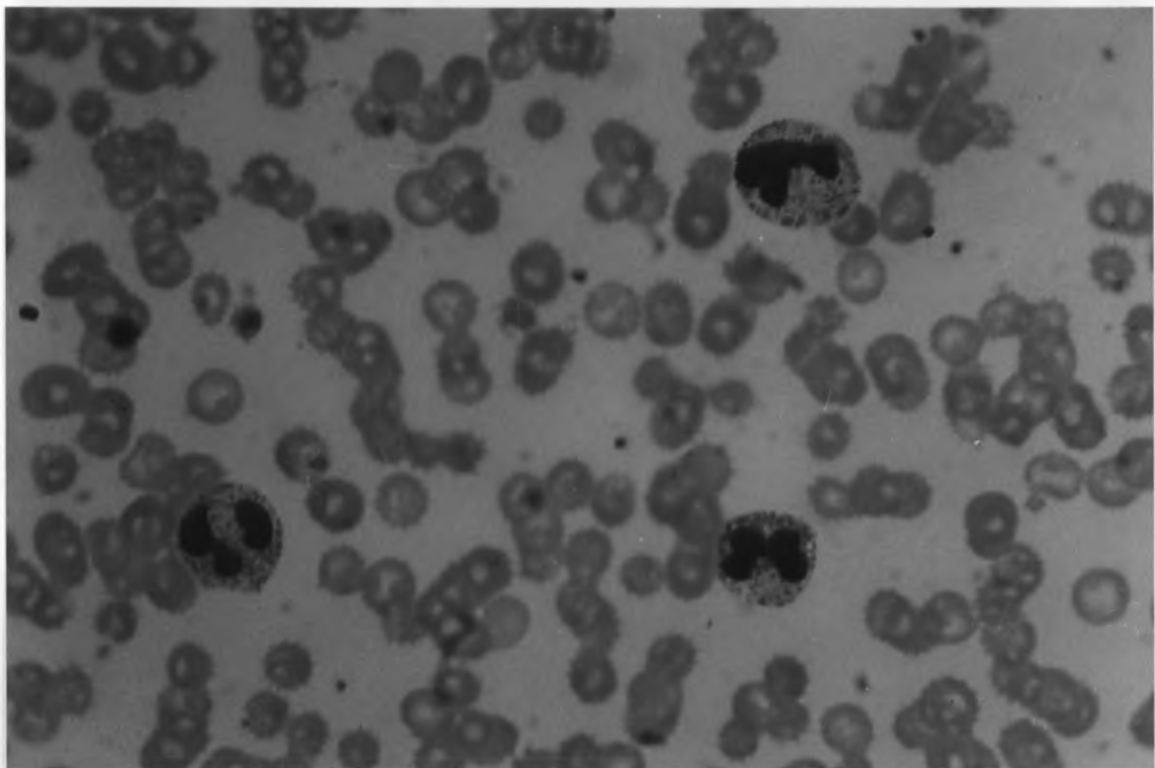
Fig 4

Platelet Count distribution among patients

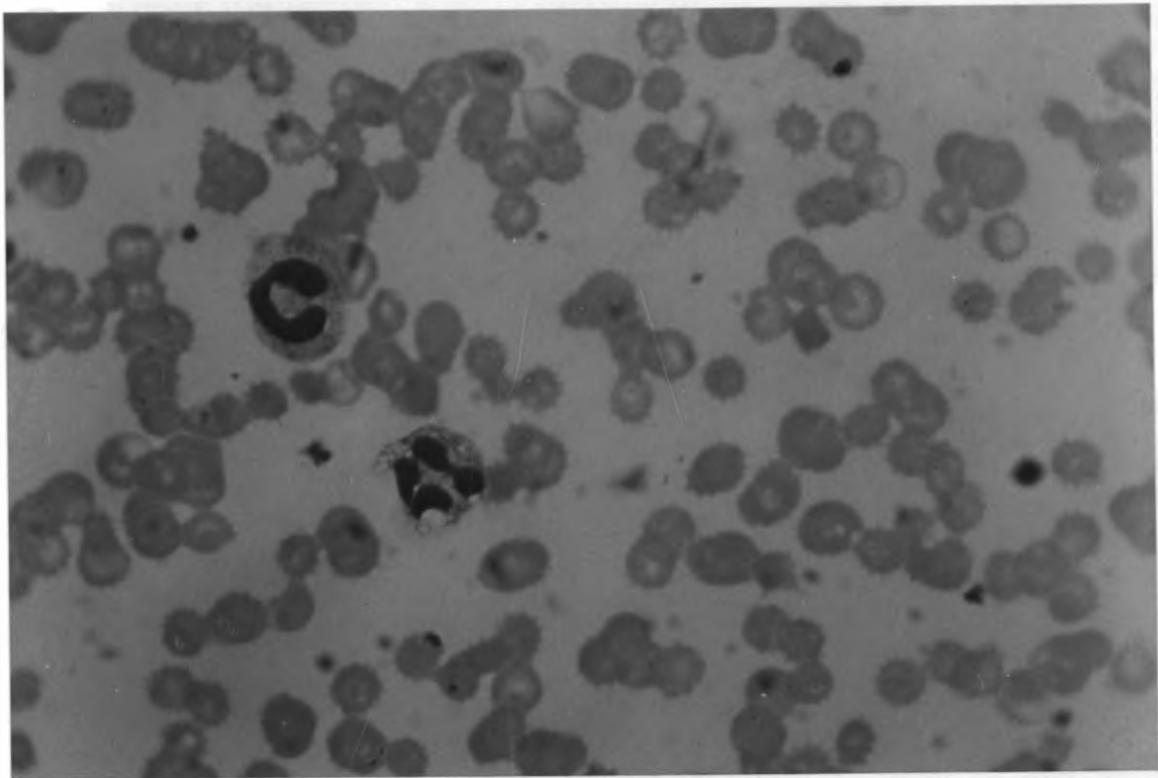




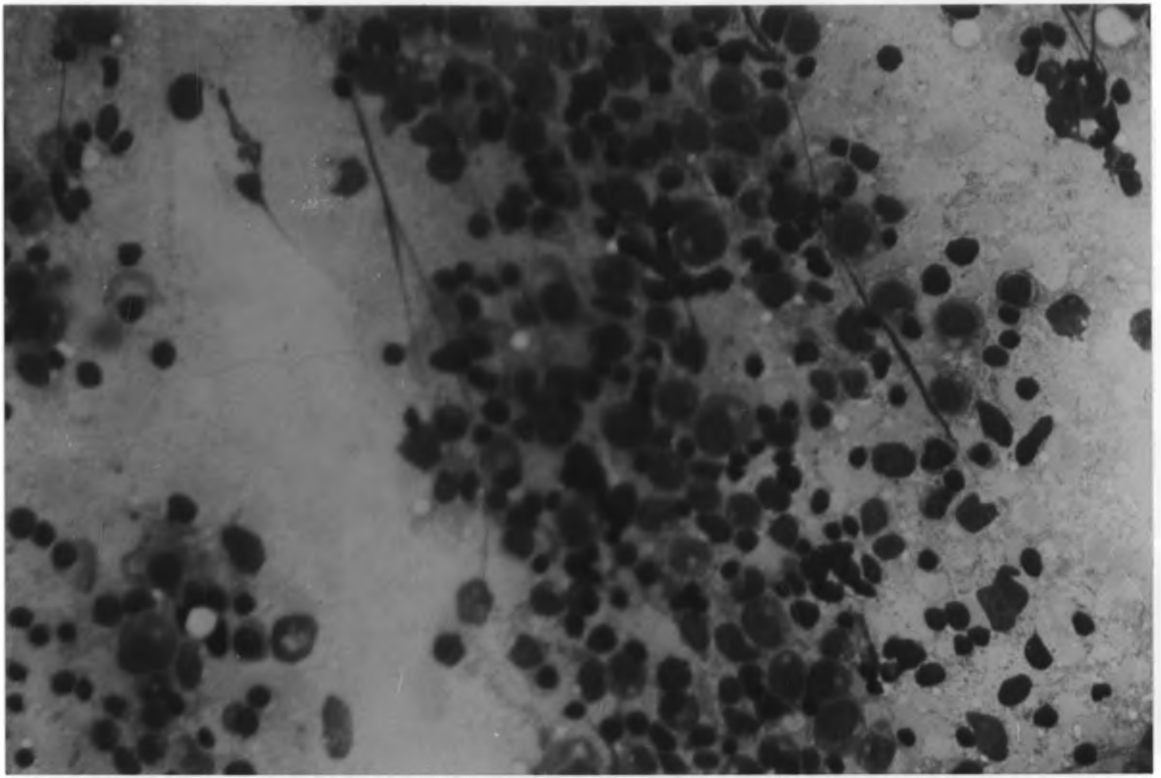
(P1) ROULEAUX FORMATION (MGG x 1000)



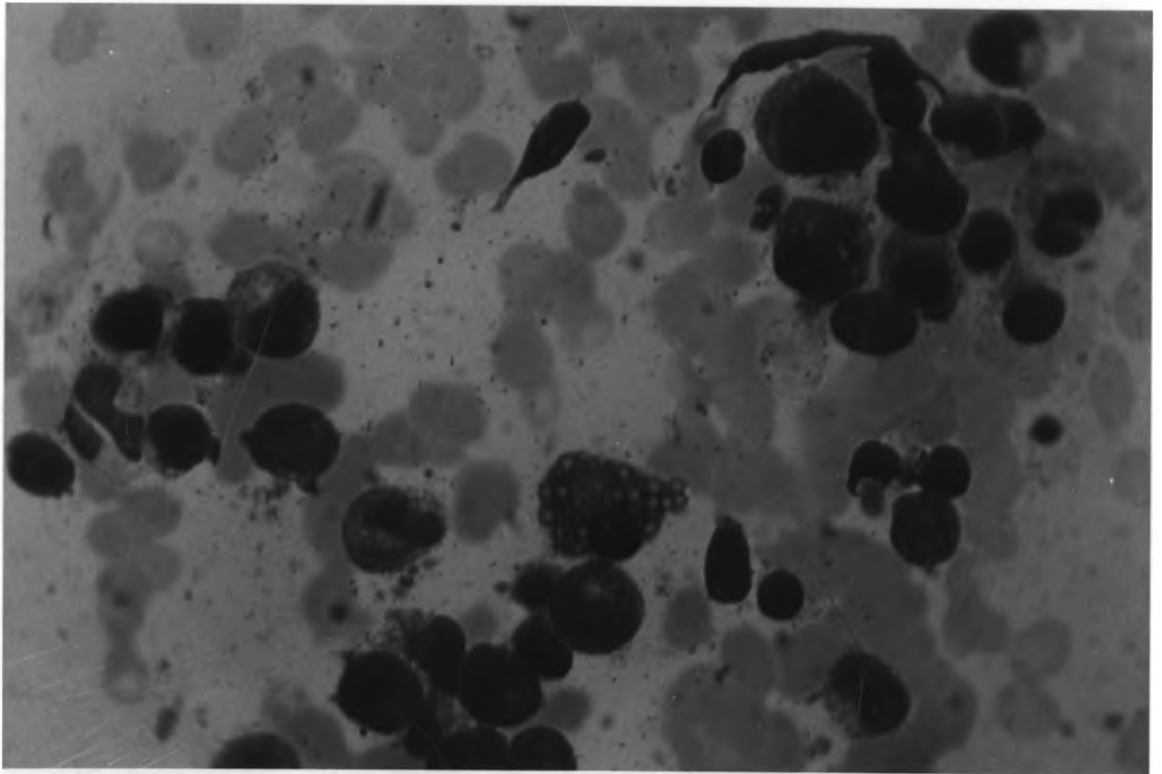
(P2) NEUTROPHILS WITH TOXIC GRANULATIONS (MGG x 1000)



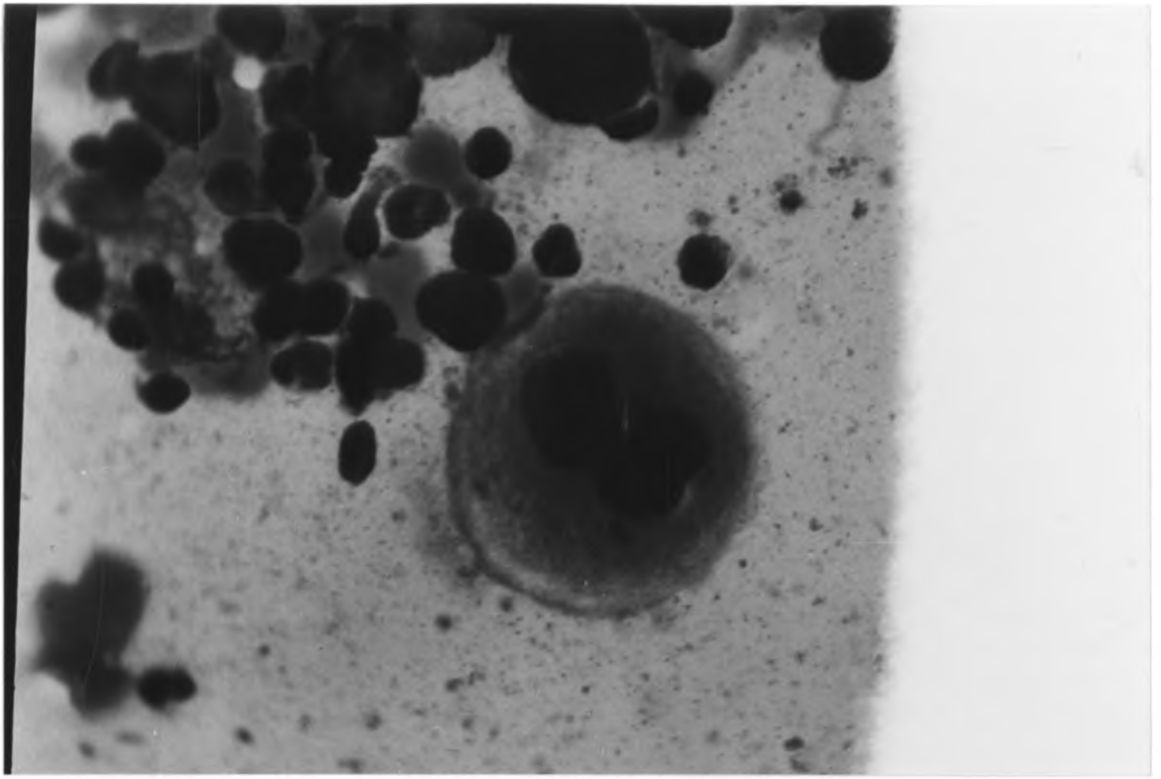
(P3) Features of dysgranulopoiesis. Pseudopelger forms, non-specific nuclear abnormality and cytoplasmic vacuolation (MGG x 100).



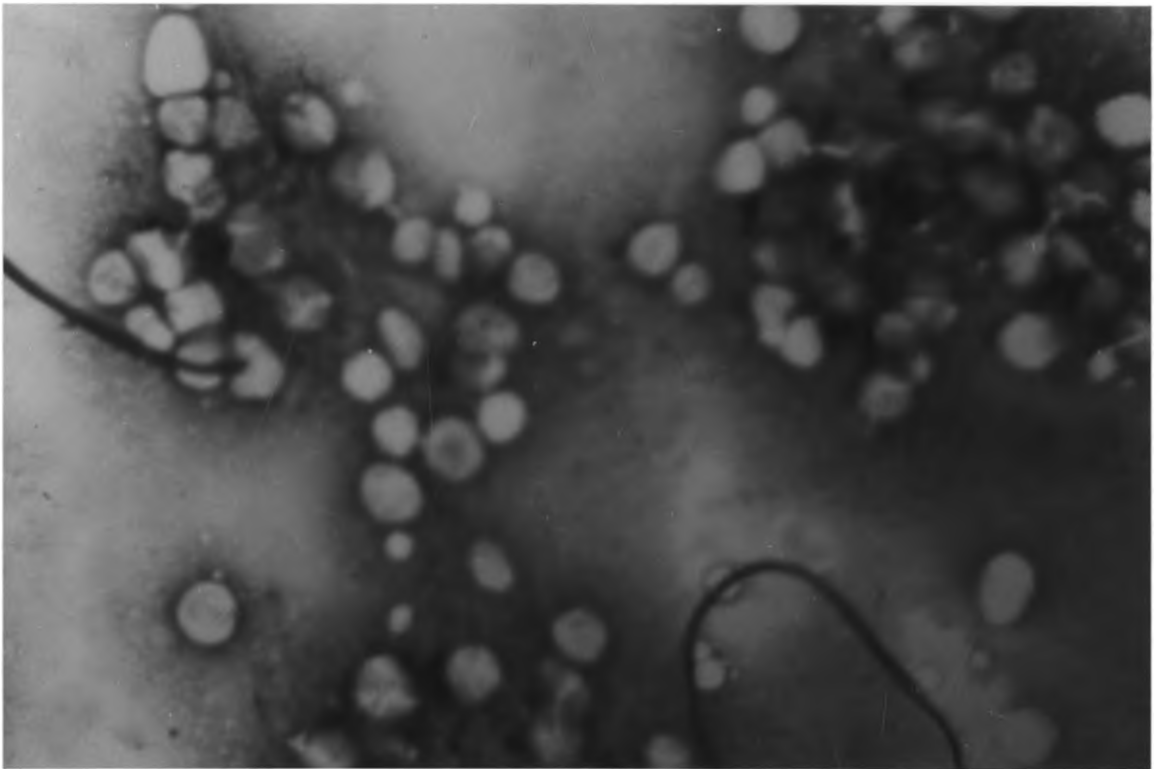
(P4) Plasmacytosis (MGG x 400)



(P5) Conspicuous plasma cells and foam cell. Some features of Dysplasia (MGG x 1000)



(P8) Megakaryocyte showing poor budding with complete walling off (MGG x 1000)



(P9) Increased stainable iron in the reticuloendothelial system.
(Perls prussian blue X 400)

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APPENDIX I

STUDY PROFORMA SHEET

NAME:

AGE:

SEX:

IP NO:

WARD:

CLINICAL HISTORY

History of:

- i) Previous transfusion Y/N
- ii) Diarrhoea Y/N
- iii) Weight loss Y/N
- iv) Cough Y/N
- v) Skin rash Y/N
- vi) Fever Y/N

Social history: occupation Smoking Y/N Drinking Y/N

Physical examination:

Pallor Y/N Fever Y/N Wasting Y/N

Oral thrush Y/N Lymphadenopathy Y/N

Skin Lesions Y/N (if yes describe)

Short clinical examination:

Provisional clinical diagnosis:.....

Treatment (if any)

Laboratory :-

- (i) HIV serology Positive Negative
(ii) CxR.
(iii) Urine c/s

Haemogram

(a) Hb _____ g/dl MCV _____ MCH _____ MCHC _____

(b) WBC _____ x10⁹/lt

Differential N ___% L ___% M ___% E ___%

Others (specify)

(c) Platelet count _____ x10⁹/lt

Film

Bone marrow aspirate

Particles: Present/Absent

Cellularity:

Myeloid/Erythroid ratio:

Erythropoiesis:

Granulopoiesis:

Plasma cells ___% Eosinophil ___%

Megakaryocytes:

Iron:

Abnormal cells:

Parasites / Fungi:

Comment

APPENDIX II

Reference range used at KNH

Haemoglobin Males 12.5 - 18 g/dl

Females 11.5 - 16.5 g/dl

Total Leucocyte count 3 - 10 x 10⁹/lt

Platelets 100 - 400 x 10⁹/lt

Lymphocytes 1500 - 3500 x 10⁹/lt

Mean corpuscular volume 76 - 96fl

Mean corpuscular haemoglobin 29.0 μ g 3.0 picograms

Mean corpuscular haemoglobin concentration 34.0 μ g 2.0 g/dl

APPENDIX III

Marrow aspirate technique:

Bone marrow aspirates were obtained aseptically from the sternal crest using the Klima needle and the following technique:-

1. After scrubbing, two pairs of sterile gloves were worn.
2. With the patient lying supine the sternal crest was cleaned with spirit and draped with a sterile towel with a hole
3. Using a 21 Gauge needle, the skin, subcutaneous tissues and periosteum were infiltrated with a local anaesthetic (5% Procaine hydrochloride)
4. The Klima needle was then pushed through the cortex into the marrow cavity by smooth clockwise and anticlockwise motion
5. Using a 10ml or 20ml syringe, about 0.5 - 1 ml of bone marrow was aspirated and smears were made by expelling particles from the syringe onto clean labelled microscope slides. The excess blood was removed using a suction bulb or 5 ml syringe. The pale, white marrow particles were left behind and a second slide was then placed on top of them and a smear made by pulling slides apart in a sliding motion.

6. The Klima needle was then removed and the puncture wound dressed with pressure dressing.
7. Analgesics were rarely required following aspiration
8. The smears were stained with the May Grunwald Giemsa and Perls Prussian blue stains
9. The stained preparations were then examined together with an experienced cytomorphologist.

(Adapted from Practical Haematology by Dacie and Lewis)

APPENDIX IV

May Grunwald Giemsa Stain

1. Prepare blood films and bone marrow smears and squashes on clean, dust and grease free slides
2. Air dry the films and squashes
3. Fix the specimen by immersing in a jar of methanol for 5 - 10 minutes
4. Put the slide into a jar containing May-Grunwald's stain freshly diluted with an equal volume of buffered water for 10 minutes.
5. Transfer without washing into jar containing Giemsa's stain freshly diluted with 9 volumes of buffered water for 5 - 10 minutes
6. Wash rapidly in 3 or 4 changes of water and allow to stand in water for differentiation to occur (2 - 5 minutes)
7. Stand slide upright to dry
8. When thoroughly dry, mount slides with cover glass and mountant (DPX). (Dibutylphthalate xylene)

(Adapted from Practical Haematology by Dacie and Lewis).

APPENDIX V

Pearls Prussian Blue reaction (For iron)

Solutions:

- 1) 4% aqueous potassium ferrocyanide (solution I)
- 2) 4% aqueous hydrochloric acid (solution II)
- 3) 0.5% neutral red

Technique

- 1) Rinse smears with distilled water
- 2) Flood the slide with a freshly prepared mixture of equal parts of solutions I and II for 15 minutes
- 3) Rinse in distilled water
- 4) Counterstain with 0.5% neutral red for 30 seconds
- 5) Air dry and mount in dibutylphthalate xylene (DPX)
(Adapted from Practical Haematology by Dacie and Lewis).



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Dr. Amit Goyal,
Dept. of Pathology,
C.H.S.

22nd April, 1992.

Re: Research Proposal entitled "Haematological Abnormalities in Human Immunodeficiency Virus (HIV) Infections at Kenyatta National Hospital" (P197/9/91)

This is to inform you that the Kenyatta National Hospital Ethical and Research Committee (KNH-ERC) has reviewed your above cited research proposal and made the following suggestions:

1. Compare the haematological parameters of HIV patients with other non-HIV patients without haematological abnormalities.
2. Separate the inclusion and exclusion criteria
3. Indicate the number of cases to be studied
4. Indicate whether the budget of Kshs.21,000 is based on sample size or not.

Recommendation

The Committee recommended that your study be APPROVED. However, you are requested to furnish the office of the undersigned with evidence that you have considered the above suggestions.

Dr. Anastasia N. Guantai
SECRETARY - KNH-ERC

cc. Prof. F.E. Onyango - Dept. of Paediatrics
Dr. M.S. Riyat - Dept. of Haematology and B.T.
Director - KNH.