Original Article

Histomorphology of Bone marrow from Adult Pancytopenic Patients at the University Teaching Hospital in Lusaka, Zambia

Francis Musonda,[1] Clemence Marimo,[2] Sumbukeni Kowa[3]

[1]University Teaching Hospital, Department of Pathology and Microbiology, PO Box RW1X, Lusaka Zambia: email franciskaoma@gmail.com
[2]Cavendish University, School of Medicine.
[3]University of Zambia, School of Medicine

ABSTRACT

Background: Pancytopenia is a haematologic condition characterised by leukopenia, anaemia and thrombocytopenia. Pancytopenia is not a diagnosis and has to be qualified by determination of its cause. The aetiologies of pancytopenia are diverse, and study of bone marrow histomorphology via cytology and histology are key components that assist in the determination of the underlying cause. Pancytopenia is encountered regularly in medical practice in Zambia, however, no studies have been conducted on pancytopenia to date. This was a descriptive cross-sectional study done on adult pancytopenic patients admitted to the medical wards of the University Teaching Hospital (UTH) over an eight-month period. The aim of this study was to determine the histomorphology of the bone marrows of adult pancytopenic patients admitted to the UTH.

Methods: A total of 45 bone marrow biopsies were collected over the study period. In all cases the indication was pancytopenia that had been confirmed by a full blood count done at the UTH and the biopsy site was either the anterior superior iliac spine or the posterior superior iliac spine. Demographic and clinical details were obtained using data collection sheets and from review of patient records. The collected data was analysed using the Statistical Package for the Social Sciences (SPSS) version 21. A Chi square test was used to measure association between categorical variables. A p value of < 0.05 at 95% confidence interval was considered statistically significant.

Results: There were 32 females (71%) and 13 males (29%), and the age ranged from 15 to 72 years with an average age of 35 years. Forty percent (n=18) of the study participants had human immunodeficiency virus (HIV) and all of these all were on highly active antiretroviral therapy (HAART). There were 6 histologic patterns found the commonest being megaloblastosis seen in 38% of the patients, followed by malignancy and myelodysplasia both at 17.0%. Bone marrow aplasia accounted for 13.0%, non-megaloblastic erythroid hyperplasia accounted for nine percent and myelofibrosis for four percent. A chi square test was used to determine if there was association between each histomorphology and HIV status, the only significant result was obtained from the Chi test applied to HIV status and myelodysplasia which gave a p value of 0.026. The Chi square test involving the other histomorphologies all yielded a p value greater than 0.05.

Conclusion: The bone marrow biopsies of the study population showed six histomorphologic pictures which in order of frequency were megaloblastosis, malignancy and myelodysplasia, bone marrow hypoplasia, non-megaloblastic erythroid

Keywords: *Pancytopenia, Bone marrow, Trephine and Histomorphology*

hyperplasia and myelofibrosis. Association was found between HIV status and myelodysplasia via the Chi square test.

INTRODUCTION

Pancytopenia is defined as a deficiency of all three cellular components of the peripheral blood, namely erythrocytes, leukocytes and platelets. Except for rare instances, pancytopenia in adults is caused by acquired disorders. Variation in aetiology of pancytopenia and therefore on the bone marrow histomorphology is influenced by geography and differences may be appreciated not only in different countries but also in different regions of a single country. Review of literature consistently states the commonest causes of pancytopenia stretching from Asia, through the Middle East and into Northern, Eastern and Southern Africa as megaloblastic anaemia, malignancy and aplastic anaemia. Causes quoted with less consistency or stated as being rare were myelodysplastic syndrome, infections (e.g. tuberculosis, malaria, HIV leishmaniasis, parvovirus B19), Waldenstrom's macroglobinaemia and hemophagocytic syndrome. Whatever the cause of the pancytopenia, examination of bone marrow morphology is frequently required to determine the cause.

METHODS

A cross sectional study was conducted at the University Teaching Hospital (UTH) in the Department of Pathology and Microbiology from July 2016 to March 2017. Adult male and female patients (15 years and above) admitted to the UTH within the study period with a finding of pancytopenia on full blood count were enrolled into the study. Pancytopenia was determined as per UTH protocol as a white cell count less than 4.0 $x10^9$/L, haemoglobin less than 10.0 g/dL and platelet count less than 150 x 10^9/L. For the patients recruited, at least one bone marrow biopsy was collected and processed to produce haematoxylin and eosin stained slides following UTH histopathology laboratory protocols, were indicated Ziehl Neelsen and Gomori methenamine silver stains were also used. Data collected was analysed using SPSS version 21. Descriptive statistics were used to define the study population and as continuous variables. Frequency listings were used for qualitative variables and a Chi square test was used to determine associations between variables in the data. A p-value less than 0.05 at 95% confidence interval was considered as statistically significant.

RESULTS

A total of forty-five patients were enrolled in this study. The age range was 15 to 72 years with a mean and median of 35 and 32 years respectively. Forty percent (n=18) of the study participants had human immunodeficiency virus (HIV) and all of them were on highly antiretroviral therapy (HAART), only 34.0% of the CD4 counts were available and thus no statistically valid conclusions could be drawn regarding the CD4 counts in our study population. Review of medical charts showed that none of the enrolled patients presented with fever and 2 (4.44%) had splenomegaly. There were six histologic pictures found in this study, these were;

1. Megaloblastosis (38.0%)
2. Malignancy (18.0%)
3. Myelodysplasia (18.0%)
4. Bone marrow aplasia (13.0%)
5. Non-megaloblastic erythroid hyperplasia(8.0%) and
6. Bone marrowfibrosis (4.0%).

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A chi square test was used to determine if there was association between each histomorphology and HIV status, the only significant result was obtained from the Chi test applied to HIV status and myelodysplasia which gave a p value of 0.026. The Chi square test involving the other histomorphologies all yielded a p value greater than 0.05.

DISCUSSION

Bone marrow examination combined with a detailed clinical history, is often required for an accurate

diagnosis in determining the underlying cause of pancytopenia. The potential clinical significance of each histomorphology is summarised in table 1.

Table 1: Histologic findings and their potential clinical significance

Bone Marrow histomorphology (n,%)	Clinical significance in appropriate Clinical context
Megaloblastosis (17, 38.0%)	Investigate the patient for megaloblastic anaemia. Investigate the patient for Myelodysplastic syndrome
Bone marrow space infiltration (8, 18.0%)	Investigate the patient for malignancy, is it primary or metastatic. Endeavour to fully characterise the malignancy.
Myelodysplasia (8, 18.0%)	Investigate the patient for myelodysplastic syndrome, nutritional deficiencies and AML
Bone marrow aplasia (6, 13.0%)	Investigate the patient for aplastic anaemia, hypocellular AML and hypocellular MDS
Non-megaloblastic erythroid hyperplasia (4, 9.0%)	Investigate the patient for causes of peripheral blood destruction/ sequestration (e.g. splenomegaly, autoimmune disease, portal hypertension)
Myelofibrosis (2, 4.0%)	Primary or secondary myelofibrosis

The finding in this study of the top two bone marrow histomorphologiesbeing consistent with megaloblastosis (38.0%) and malignancy (18.0%)is not surprising given that these are the morphologies expected to be seen in megaloblastic anaemia and malignancy respectively which as stated earlier are in the top three causes of pancytopenia across a wide geographic distribution stretching from Asia, the middle east, northern, eastern and southern Africa. Considering that these pathologies have an epidemiologic aspect associated with the prevailing environment, this result is expected, as several countries in these geographic locations share within them areas with a socio-economic environment similar to that found in Zambia. The histomorphology of myelodysplasia was demonstrated in 8(18.0%) of the study population,6 of these (13.0%) were HIV positive and below the age of 50, the remaining 2 were HIV negative and aged 54 and 62 years. The patients with myelodysplasia who were HIV negative and above 50 years were candidates for ancillary testing for myelodysplastic syndrome which is a disease of the elderly, and very rare before the age of 50 years. Pancytopenia is said to occur as a rule in advanced HIV infection via direct suppression of bone marrow stem cells by infected T-cells and disruption of cytokine homeostasis through the destruction and dysregulation of T-cells, B-lymphocytes and monocytes and indeed it has been shown that myelodysplasia may be the first manifestation of HIV disease it is therefore possible that given the ages of the HIV positive patients with myelodysplasia (less than 50 years) that some or indeed all of them had HIV induced myelodysplasia. This would be in tandem with the findings of a study on pancytopenia done in Zimbabwe which listed HIV as the third most common cause of pancytopenia. A search for other published studies that were similar to this one and done in Africa revealed that these were few and did not list HIV as a cause. A possible explanation for this may be the low incidences of HIV documented in the countries in which those studies were conducted (Egypt and Morocco).In this study non megaloblastic erythroid hyperplasia was found in 4 patients (9.0%), this low frequency is similar to that quoted in similar studies done in India, other studies however did quote this morphologic finding in the top 3 bone marrow morphologies in pancytopenic patientsin their studies. These two opposing sets of results can be explained by the fact that non megaloblastic erythroid hyperplasia is a bone marrow histomorphology associated with peripheral destruction of the cellular components of blood and not a diagnosis, therefore, any pathology causing the peripheral destruction or sequestration of the cellular components of blood can result in the histologic picture of erythroid hyperplasia, these are varied and

include any pathology causing haemolytic anaemia, hypoxia and renal disease and also include hereditary diseases like hemoglobinopathies and familial polycythaemia. The epidemiology of non-megaloblastic erythroid hyperplasia is therefore directed by the epidemiology of the said pathologies. The patients with a bone marrow histomorphology of myelofibrosis had both suffered from chronic illness associated with persistent cytopenias with eventual development of pancytopenia, we were however unable to determine the primary pathology in both cases. There was no histologic evidence of infiltration of the bone marrow by opportunistic infections (OIs) found in this study, this is not surprising given that the incidence of OIs has greatly reduced since the advent of HAART and all patients with HIV in this study were on HAART, furthermore, literature states that the chances of positive histomorphologic evidence of OIs (i.e. granulomata, lympho-histiocytic infiltrates) being found in the bone marrow are increased if a patient presents with fever and none of our cohort presented with fever.

CONCLUSION

We conclude that the histomorphology of the bone marrows from pancytopenic adults at the University Teaching Hospital in Lusaka in order of frequency is megaloblastosis, malignancy, myelodysplasia, bone marrow aplasia, non-megaloblastic erythroid hyperplasia and myelofibrosis. The finding of myelodysplasia was associated with HIV status by Chi square test, this finding along with data from literature supports working up patients with myelodysplasia for HIV. There was no bone marrow infiltration by opportunistic infections identified in the study population.

LIMITATIONS

Our inability to collect all CD4 counts and viral loads from the HIV positive population in our cohort left us unable to corelate CD4/viral load to bone marrow histomorphology. Another limitation was that due to our inability to perform ancillary studies (such as serum folate and vitamin $B_{12,}$ flow cytometry, immunohistochemistry and cytogenetic investigations) on all the patients, characterisation of diagnoses past histomorphologic appearance was not possible in the entire patient population. It is however hopeful that future follow up studies will address this shortcoming.

RECOMMENDATIONS

Clearly massive training of haematologists, haemato-oncologists and pathologists is required to assist with early diagnosis of pancytopenic patients. There is also an urgent need to invest in reliably available ancillary studies, that is serum folic acid, serum vitamin B_{12}, flow cytometry, immunohistochemistry and cytogenetic investigations in order to rapidly and completely diagnose these patients.

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