THE ROLE OF BONE MARROW ASPIRATE AND TREPHINE SAMPLES IN HAEMATOLOGICAL DIAGNOSES IN PATIENTS REFERRED TO A TEACHING HOSPITAL IN GHANA

G. BEDU-ADDO[1], Y. AMPEM AMOAKO[2] AND I. BATES[3]

[1]School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]Komfo Anokye Teaching Hospital, Kumasi, Ghana, [3]School of Tropical Medicine, Liverpool, UK

Corresponding Author: *Professor G. Bedu-Addo* ***Email:*** *gbeduaddo@gmail.com*

Conflict of interest: *None declared*

SUMMARY

Introduction and methods: A bone marrow examination had been requested by the referring clinician in over half of the 250 patients referred to the haematology clinic at Konfo Anokye Teaching Hospital (KATH) between 1988 and 1998 for investigations for various haematological disorders. Although a full blood count and a peripheral blood film can go a long way to resolve some of these diagnostic challenges faced by doctors in the districts, this information was generally not provided in the referral letter. After careful selection, 80 patients actually underwent a bone marrow examination. The result of the full blood count and peripheral film were available before bone marrow sampling was done.

Results: Lymphoproliferative disorders were the most common diseases that caused infiltration of the bone marrow. 27.5% of lymphomas were diagnosed on morphological examination of the bone marrow as high grade B cell NHL, 13.75% had tropical splenic lymphoma, 10% had chronic lymphocytic leukaemia (CLL) and 5% had disseminated high grade T cell lymphoma and 2.5% had Adult T cell Leukaemia Lymphoma (ATLL). Other disorders diagnosed after bone marrow examination include myelodysplastic syndrome (MDS), aplastic anaemia, megaloblastic anaemia and myelofibrosis. Only 8.75% of these patients had a normal bone marrow.

Conclusions: This study has demonstrated the complexity of using bone marrow examination in clinical diagnosis and emphasizes the need for referring clinicians to consider involving specialist input in difficult haematological cases before requesting bone marrow examination for their patients.

Keywords: Bone marrow aspirate, trephine biopsy, haematological diagnoses, Kumasi

INTRODUCTION

A great variety of cells, haemopoetic and non-haemopoetic are present within normal bone marrow. Bone marrow aspirates and bone marrow biopsy specimens have important and complimentary roles in clinical diagnosis. Bone marrow aspiration provides a small sample, which is diluted with blood, and provides important information about the appearance of cells. Trephine biopsy allows the visualization of the bone marrow structure and the relationship of cells to one another.[1] Marrow aspiration and biopsy have no absolute contraindications but there may be some relative contraindications related to the general conditions of the patient.[2] Complications associated with bone marrow aspiration and trephine biopsy are well recognized but rare.[3] Haemorrhage remains the single most common serious adverse event.

Examination of the bone marrow aspirate or biopsy specimen is a valuable diagnostic procedure in patients with known or suspected haematological disorders and also for diagnosis of metastatic spread of non-haematological malignancies and metabolic disorders. Morphology and immunophenotyping of the cells combined with histological examination of bone marrow trephine biopsy specimen are used for staging lymphomas and for indicating prognosis and monitoring treatment.[4]

It is usual practice in Ghana for patients to be referred to a tertiary centre with a request to perform a bone marrow examination. However there are very few trained physicians available who can perform these procedures to the required standard and there is potential for them to be over-burdened by these requests from District and Regional hospitals. The purpose of this study is to describe referral patterns for patients attending a teaching hospital haematology clinic. The process of bone marrow examination and how this examination affected the final diagnosis is also described in order to demonstrate the need for referring clinicians to consider involving specialist input to difficult haematological cases before requesting bone marrow examination for their patients.

METHODS

The haematology clinic was set up in Komfo Anokye Teaching Hospital (KATH) in 1986 and became a platform for research into patients with splenomegaly. During the period from 1988-1998, 250 Ghanaian patients with suspected haematological problems were referred to the haematology clinic for evaluation and treatment. The reasons for their referral included investigation for anaemia, jaundice and lymphadenopathy. In addition to the Hematologist consultation, more than 50% of the referrals requested for bone marrow biopsy.

The Committee on Human Research, Publications and Ethics of Kwame Nkrumah University of Science and Technology (KNUST) and KATH gave approval for the study of splenic lymphoma with villous lymphocytes (Tropical splenic lymphoma) and consent was obtained before recruitment into the study. Part of the study was based in St Georges Hospital Medical School, London UK.

All patients' had a clinical history and detailed clinical examination documented. At the initial consultation, clinical evidence of the following was specifically looked for: weight loss, fever, tiredness related to anaemia, lymphadenopathy and jaundice. A full blood count (done using a Coulter autoanalyser) and peripheral film were performed. Further tests such as lymph node biopsy liver function test, routine urine analysis, and reticulocyte counts for patients who presented with jaundice were done if clinically indicated.

Bone marrow aspirate and trephine were done using standard procedures[2] on all patients with suspected lymphomas (patients with splenomegaly, lymphadenopathy, a bleeding disorder or nonspecific symptoms such as malaise, weight loss or sweats) or pancytopenia and on any patient whose peripheral blood film did not reveal a diagnosis. The total number of patients in these categories was 80. When appropriate (patients with high WBC count or those with pancytopenia or unknown diagnosis) peripheral blood and bone marrow aspirate and trephine biopsy for patients were sent to St Georges Hospital Medical School, London, UK for immunophenotyping using the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique and immuno-histology.

RESULTS

Eighty patients being investigated for anaemia, jaundice and massive splenomegaly had bone marrow aspirate and trephine biopsy done.

There were 49 females and 31 males. Their ages ranged from 10 – 75 years with a mean age of 32 years. The mean haemoglobin was 7.8g/dl (range 2.4 – 15.6). Anaemia of unknown cause and splenomegaly of unknown cause were the commonest reasons for referral, each accounting for 22.5% of cases. In 14 patients (17.5%), the diagnosis was unknown. Acute leukaemia and pancytopenia of unknown cause accounted for 1.25% and 3.75% of referrals respectively. About one third of referrals were on account of suspicions of Lymphoma and Chronic Lymphocytic leukaemia. Table 1 shows the diagnoses made by the referring doctors.

Table 1 Diagnostic groups based on physicians' referral

Diagnosis	**N (%)**
Anaemia of unknown cause	18 (22.5)
Splenomegaly of unknown cause	18 (22.5)
Possible lymphoma	13 (16.25)
Chronic lymphocytic leukaemia	13 (16.25)
Pancytopenia of unknown cause	3 (3.75)
Acute leukaemia	1 (1.25)
Diagnosis unknown	14 (17.5)
Total	80 (100)

Sixty (60) of the 80 patients (75%) had hepatomegaly and 72 (90%)) had moderate to massive splenomegaly. 18% had conjunctival pallor and 6% were jaundiced. Forty eight (48) patients (60%) had lymphadenopathy, which was mainly limited to the cervical nodes. Twenty-nine patients (36.25%) had leucocyte count greater than 10×10^9/L. Forty percent of patients had absolute lymphocyte count greater than 3.5×10^9/L while 15% had evidence of haemolysis. Laboratory results are presented in Table 2.

Table 2 Laboratory results

Investigation / Result	**N (%)**
Patients with leucocytosis $>10 \times 10^9$/L	29 (36.3)
Patients with absolute lymphocyte count $> 3.5 \times 10^9$/L	32 (40)
Patients with evidence of haemolysis (jaundice, raised reticulocyte count, unconjugated hyperbilirubinaemia, increased urobilinogen in urine)	12 (15)

The diagnoses of the 80 patients who had bone marrow sampling done (Table 3) showed that lymphomas were the most common diagnosis.

Table 3: Final diagnosis after bone marrow sampling

Diagnosis	**N**	**%**
High grade B cell NHL	22	27.50
Tropical splenic lymphoma	11	13.75
Chronic lymphocytic leukaemia	8	10.00
Disseminated high grade T cell lymphoma	4	5.00
Multiple myeloma	3	3.75
Adult T cell leukaemia/lymphoma	2	2.50
Hodgkin lymphoma	1	1.25
Acute leukaemia	2	2.50
Metastatic prostate carcinoma	1	1.25
Myelodysplastic syndrome	1	1.25
Aplastic anaemia	5	6.25
Megaloblastic anaemia	2	2.50
Myelofibrosis	3	3.75
Erythroid hyperplasia	8	10.00
Normal marrow	7	8.75
Total	**80**	**100**

Table 4 Comparison of "referral diagnosis" and "final diagnosis" after bone marrow sampling

Referral diagnosis	Number of patients	Final diagnosis	Number of patients
Anaemia of unknown cause	18	AA MDS Metastatic prostate carcinoma Megaloblastic anaemia Acute leukaemia High grade B cell NHL Normal marrow	4 1 1 2 1 3 6
Splenomegaly	18	TSL High grade B cell NHL MF	11 4 3
Possible Lymphoma	13	High grade T cell lymphoma High grade B cell NHL Normal marrow	4 8 1
CLL	13	CLL HD High grade B cell NHL	8 1 4
Pancytopenia of unknown cause	3	AA ATLL High grade B cell NHL	1 1 1
Acute leukaemia	1	Acute leukaemia	1
Diagnosis unknown	14	MM ATLL High grade B cell NHL Erythroid hyperplasia	3 1 2 8
Total	**80**		**80**

Legend

AA- aplastic anaemia, MDS-Myelodysplastic syndrome, MF-Myelofibrosis, TSL-tropical splenic lymphoma, CLL-chronic lymphocytic leukaemia, HD-Hodgkin's Lymphoma, NHL-non Hodgkin's Lymphoma, MM-multiple myeloma, ATLL-adult T cell leukaemia lymphoma

Table 4 represents a comparison between the referring Physicians diagnosis and that made after examination of the bone marrow. Of the 18 patients referred as 'anaemia of unknown cause', the exact causes of the anaemia were determined to be aplastic anaemia (4), myelodysplastic syndrome (1) and metastatic prostate carcinoma (1). Others in this category had megaloblastic anaemia (2), acute leukaemia (1) and high grade B cell NHL (3). Six of the patients referred under this category had a normal bone marrow examination. Eleven of the 18 patients referred with a diagnosis of 'splenomegaly' were found to have tropical splenic lymphoma while there were 4 and 3 patients with high grade B cell NHL and myelofibrosis respectively. Fourteen patients referred with unknown diagnosis were determined to have erythroid hyperplasia (8), multiple myeloma (3), high grade B cell lymphoma (2) and ATLL (1).

In all, twenty-two patients (27.5%) had grade B cell lymphoma, 13.75% has Tropical splenic lymphoma whilst 5% had disseminated high-grade T cell lymphoma and 2.5% had Adult T cell leukaemia/lymphoma (ATLL). One patient had nodular sclerosing-lymphocyte depleted Hodgkin's disease. Chronic lymphocytic leukaemia (CLL) accounted for 10% of the lymphoid malignancies and 1 patient suffered from Myelodysplastic syndrome. Two patients (2.5%) had acute leukaemia, one was acute myelomonocytic leukaemia and the other was acute myeloid leukaemia. Uncommon diagnoses on bone marrow examination included metastatic prostate carcinoma, aplastic anaemia, megaloblastic anaemia and myelofibrosis. Eight patients (10%) had erythroid hyperplasia while 7 (8.75%) had normal bone marrow.

DISCUSSION

This study demonstrated that in this group of haematology clinic patients selected for bone marrow examination using specific criteria, bone marrow examination was very helpful in determining the diagnosis. The bone marrow was normal in 8.75% of these carefully selected patients. Bone marrow examination was able to elucidate the underlying cause of several syndromes that are common reasons for referral to haematologists in Africa such as anaemia, pancytopenia and splenomegaly. Lymphoproliferative disorders were the most common diagnoses and a detailed description of a selection of these patients has been published previously.[6] Important but unsuspected diagnoses, such as myelodysplastic syndrome, aplastic anaemia, megaloblastic anaemia, and myelofibrosis would have been missed without a bone marrow examination.

A bone marrow biopsy specimen may be diagnostic in patients without lymphadenopathy or when there is no conclusive histological evidence from other sites. Bone marrow involvement is a frequent finding in lymphoproliferative disease and can be detected by a variety of techniques involving morphologic examination of bone marrow biopsies and aspirate smears, flow cytometric analysis of aspirate samples and immunochemistry of tissue samples (e.g. lymph nodes, bone marrow) for B and T cell markers and molecular genetic analysis using polymerase chain reaction (PCR).[7] Discordance between the type of lymphoma found on lymph node biopsy and that found in the marrow has been reported.[8] Some of these investigations are not available in Ghana but through a partnership with an external laboratory it is possible to receive results on samples from patients in Kumasi sent to an international centre of excellence within a couple of weeks. This is an adequate time frame to influence the clinical care of the patients.[9]

Bone marrow and trephine biopsy should only be carried out when clinically indicated.[1] The results of the full blood count and blood film should be available before a bone marrow examination is undertaken since these more simple tests may be sufficient to provide a diagnosis. An abnormal blood count or film does not necessarily indicate a primary haematological disorder because it may reflect a non-haematological condition. A primary blood disease should be considered when a patient has splenomegaly, lymphadenopathy a bleeding disorder or nonspecific symptoms such as malaise, weight loss or sweats, which are symptoms characteristic of lymphomas or leukaemias. An accurate diagnosis of the sub-type of lymphoma is critical since it guides the treatment and prognosis.

The key lessons from this study are that bone marrow aspirate and trephine are not a routine procedure and should only be performed when indicated. Patients referred to a specialist should have recent full blood count and film comments available. Interpretation of bone marrow findings is complicated and needs to take into account the patient's clinical history, examination and laboratory results. A high quality bone marrow sample is needed and this needs to be processed by a laboratory experienced in dealing with bone marrow samples. For all these reasons the decision to recommend bone marrow sampling should be taken by a physician with haematology expertise and bone marrow processing should be undertaken in a specialist haematology unit. An onward referral system needs to be in place for complicated cases, which cannot be resolved locally, and this may include facilities for sending samples overseas.

REFERENCES

1 Bain BJ. Bone marrow trephine biopsy. *J Clin Pathol* 2001; 54: 737-42.

2 Malempi S, Joshi S, Lai S, Braner DAV, Tegtmeyer K. Videos in clinical medicine: Bone marrow aspiration and biopsy. *N Engl J Med*, Oct 8 2009; 361 (15) e 28

3 Bain BJ. Short report. Bone marrow biopsy morbidity and mortality. *Br J Haematol* 2003; 121(6): 949-951

4 Bain BJ. Bone marrow biopsy morbidity: review of 2003. *J Clin Pathol* 2005; 58 (4): 406- 408

5 Trisch B, Barti R. Histological classification of chronic lymphocytic leukaemia. A retrospective and prospective study of 530 cases. *Acta Haematology* 1988; 79:140-52

6 Bedu-Addo G, Bates I. Causes of massive tropical splenomegaly in Ghana. *Lancet* 2002; 360: 449-454

7 Bain BJ, Clark DM, Lampert IA. Bone marrow pathology. 2nd edition 1996 Blackwell Science.

8 Kluin PM, van Krieken JH, Kleiverda K, Kluin-Nelemans HC. Discordant morphological characteristics of B cell lymphomas in bone marrow and lymph node biopsy. *Am J Clin Pathol* 1990; 94(1): 59-66

9 Stevens E, Owen RG, Bedu-Addo G, Opare-Sem O, Ekem I, Adomakoh Y, Bates I. UK based real time LPD diagnostic service to improve the management of patients in Ghana. *J Haemopathol* 2009 Jul; 2 (30): 143-9 ✪