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Comparison of Eosinophil Density in Staging Bone Marrow Biopsies from Malawi and the United States

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To the Editor

Variation in peripheral blood eosinophil count between populations is well documented and generally thought to reflect differences in environmental exposures.^{1,2} However, there is little published literature regarding geographic differences in eosinophil abundance in the bone marrow. Because marrow eosinophilia is associated with a variety of both reactive and pathologic conditions,^{3,4} understanding normal variation between populations is likely important for accurate bone marrow evaluation.

As part of a longstanding collaboration between the University of North Carolina (Chapel Hill, NC, USA) and the Malawi Ministry of Health, we have established a pathology laboratory in Lilongwe, Malawi, the capital city of this southern African nation.^{5,6} A major focus of research and clinical efforts has been the diagnosis and treatment of lymphoproliferative disorders, given the growing burden of HIV-associated lymphoma in sub-Saharan Africa (SSA). As part of this collaboration, unilateral staging bone marrow biopsies are routinely performed. However, due to resource limitations and a primary clinical need to assess the marrow for involvement by lymphoma, manual aspirate differentials are generally not prioritized. Instead, morphologic evaluation and immunohistochemical stains are performed on formalin-fixed, paraffin-embedded tissue sections. As such, we have explored differences in eosinophil abundance on bone marrow core biopsies from patients in Malawi in comparison to similar patients in the United States (US).

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DISCLOSURE STATEMENT

None declared.

We identified 20 negative staging bone marrow biopsies from patients diagnosed with lymphoma in Malawi between June 2013 and May 2014. These cases were then compared to negative staging bone marrow biopsies from 20 patients diagnosed with lymphoma at the University of North Carolina between 2008 and 2014. US cases were limited to the same age range as the Malawi cohort (15 to 78 years) and were matched for HIV status. Otherwise, consecutive cases were selected. From the original 40 cases, specimens were excluded if there were not at least five 40x fields of cellular marrow for evaluation, with a minimum cellularity of 20% in each field. 17 cases from both Malawi and the US met these criteria. Specimens in Malawi were processed similarly to specimens in the United States, with rapid fixation in neutral buffered 10% formalin. In general, the quality of histologic specimens was similar between laboratories (Figure 1A-B).

Clinical, laboratory, and pathologic characteristics of included Malawi and US cases are shown in Table 1. Patients from Malawi had higher absolute lymphocyte counts than their US counterparts (median 2.0 versus 1.0, $p = 0.0008$), but baseline clinical and laboratory parameters, including percentage of patients with non-Hodgkin versus Hodgkin lymphoma, were otherwise similar between the populations. Notably, although increased peripheral blood eosinophil counts have been reported in patients from SSA in prior studies,¹ absolute and relative eosinophil counts in peripheral blood were similar between populations in this study.

Blinded to biopsy origin, two reviewers (NDM and TG) counted the number of eosinophils in the equivalent of five 40x fields from each case. Eosinophil density was then calculated by correcting eosinophil count for marrow cellularity [eosinophils counted / (field area $\times$ percentage marrow cellularity in counted fields)]. Counts between researchers showed moderate agreement (intraclass correlation coefficient = 0.66), with similar Malawi versus US trends (data not shown). Eosinophil density for each case was calculated as the mean of the two independent counts. Using this approach, median eosinophil density among all Malawi cases was 96% higher than US cases (135.4 versus 69.2 eosinophils per mm^2 , $p = 0.0005$) (Figure 1). Similar trends for median eosinophil density were noted when patients with non-Hodgkin lymphoma (Malawi = 140.8, US = 97.2; $p = 0.003$) and Hodgkin lymphoma (Malawi = 104.0, US = 55.0; $p = 0.07$) were considered separately. No correlation was noted between either absolute or relative eosinophil count in peripheral blood and bone marrow eosinophil density (data not shown).

Geographic variation in tissue eosinophil density was previously documented in normal colon biopsies from various regions within the United States.⁷ Surprisingly, however, very little is known about geographic variation in bone marrow eosinophil counts. Our results demonstrate increased abundance of bone marrow eosinophils in negative staging marrows from lymphoma patients in Malawi relative to a similar US population. These findings have important implications for clinicians and pathologists providing care in SSA, particularly for those physicians who also practice in resource-rich nations where bone marrow eosinophils are less abundant.

Bone marrow eosinophils may be increased in a variety of reactive and pathologic conditions. While drug effects and other allergies are the most common cause of bone

marrow eosinophilia in resource-rich settings,⁸ in patients from resource-limited countries, infectious causes are frequently implicated.⁹ All patients in this study were new lymphoma patients initiating treatment and were comprehensively evaluated using medical record review, history, physical exam, and appropriate diagnostic testing available in each setting. No patients had evidence of active allergic or parasitic disease at the time of bone marrow biopsy, although we acknowledge important local limitations in laboratory capacity in Malawi to diagnose clinically occult allergic or parasitic disease. Hematopoietic neoplasms of both myeloid and lymphoid lineage may also increase marrow eosinophils.^{3,4} Among lymphoid neoplasms, classical Hodgkin lymphoma and T-lymphoblastic leukemia/lymphoma are frequently implicated. Such associations are relevant in the context of staging bone marrow biopsies, because eosinophilia may raise concern for involvement by lymphoma.

In our cohort, no correlation was noted between peripheral eosinophil count and bone marrow eosinophil density. These results are consistent with previous studies demonstrating that peripheral blood and bone marrow eosinophil counts are not invariably related.¹⁰ Such discordance could reflect differences in the tendency of marrow eosinophils to either enter or remain in the circulation. For instance, increased marrow eosinophils may not lead to increased absolute eosinophil counts in the peripheral blood if circulating eosinophils rapidly redistribute to peripheral tissues. These possibilities are not easily distinguished with available data.

To our knowledge, the current study represents the first published comparison of bone marrow eosinophil density between patients from SSA and a developed nation. Conclusions are limited by a relatively small sample size and evaluation of patients with a disease (lymphoma) known to cause bone marrow eosinophilia. We have attempted to overcome these limitations by selection of a well-matched comparison population from the United States. Given the paucity of published literature on this topic, these data represent an important first step in characterizing geographic variation in bone marrow eosinophil density.

Our collaborative pathology experience in Malawi and the US suggests that familiarity with regional variation in eosinophil abundance is important in the evaluation of bone marrow biopsies. Eosinophil density that would be considered atypical in one setting may be within the expected normal range in another. While a mature literature exists regarding regional variation in peripheral blood parameters, little published data addresses variation between populations in cellular constituents of the bone marrow. Our work emphasizes the need for such studies and the importance of establishing local reference ranges/morphologic expectations in clinical and pathology laboratory testing.

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FIGURE 1. Increased eosinophil density in bone marrow biopsies from Malawi relative to the United States

(A and B) Representative hematoxylin and eosin stained slides show increased eosinophils in a bone marrow biopsy from Malawi (A) relative to US counterparts (B). Original objective magnification 60x. (C) Scatter plot of eosinophil density from negative staging marrows performed on patients in Malawi (n=17) and the United States (n=17) with a confirmed tissue diagnosis of lymphoma. Median values are indicated by a horizontal line (Malawi = 135.4, United States = 69.2; $p = 0.0005$).

TABLE 1

Comparison of baseline clinical, laboratory, and pathology characteristics of patients from Malawi and the United States

	Malawi (n=17)	United States (n=17)	P value
Age (years), median (range)	47.0 (15.1-77.4)	45.1 (19.1-74.7)	0.89
Male, n (%)	14 (82.4)	11 (64.7)	0.24
HIV positive, n (%)	7 (41.2)	8 (47.1)	0.73
Hodgkin lymphoma	3 (17.6)	4 (23.5)	0.67
Non-Hodgkin lymphoma	14 (82.4)	13 (76.5)	0.67
<i>High grade B-cell lymphoma</i>	<i>12</i>	<i>11</i>	
<i>Low grade B-lineage lymphoma</i>	<i>1</i>	<i>2</i>	
<i>Extranodal NK/T-cell lymphoma</i>	<i>1</i>	<i>0</i>	
Hemoglobin (g/dL), median (range)	11.0 (4.3-11.3)	12.5 (8.4-15.7)	0.14
Platelets (10 ³ /μL), median (range)	320 (155-764)	260 (118-597)	0.17
White blood cells (10 ⁹ /L), median (range)	5.7 (2.6-36.8)	6.5 (3.1-16.2)	0.62
Absolute neutrophil count (10 ⁹ /L), median (range)	2.5 (0.5-29.9)	4.7 (1.0-13.5)	0.97
Absolute lymphocyte count (10 ⁹ /L), median (range)	2.0 (0.8-4.0)	1.0 (0.3-1.7)	0.0008*
Absolute monocyte count (10 ⁹ /L), median (range)	0.4 (0.1-0.5)	0.4 (0.1-1.0)	0.32
Absolute eosinophil count (10 ⁹ /L), median (range)	0.2 (0.0-1.9)	0.2 (0.0-0.8)	0.62
Percent eosinophils, median (range)	3.1 (1.0 – 30.6)	3.7 (0.0-20.5)	0.62