Yield of Bone Marrow Examination in Diagnosis of HIV Infected Patients with Fever of Unknown Origin

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ABSTRACT

Background: HIV-infected patients are commonly found with fever of unknown origin (FUO), leading to high morbidity, prolonged hospitalization, and extensive diagnosis. Other than the noninvasive investigations, such as blood cultures or sputum examination and cultures, bone marrow aspiration or culture and biopsy can be undertaken with specific diagnostic yield for FUO.

Objective: To investigate diagnostic yield of bone marrow examination in HIV infected patients with FUO.

Methods: This study was a retrospective review of bone marrow aspiration, culture and biopsy in 84 HIV infected patients with FUO hospitalized between January 2003 and January 2013 at Bamrasnaradura Infectious Diseases Institute, Nonthaburi, Thailand.

Results: Overall, abnormal findings were found in 46 cases (diagnostic yield, 54%), with specific diagnosis by bone marrow examination in 36 cases (diagnostic yield, 42.8%). Two types of diseases were observed: 1) Infectious diseases in 34 cases [17 with non tuberculous mycobacterial (NTM) infection, 8 with histoplasmosis, 6 with cryptococcosis, and 3 with tuberculosis (TB)], 2) Hematological malignant diseases in 2 cases. The clinical predictor of positive bone marrow examination (BME) was leucopenia ($p< 0.05$).

Conclusion: Bone marrow examination should be recommended as a useful diagnostic tool to yield specific diagnosis for the evaluation of appropriate treatments in HIV-infected patients with FUO.

Keywords: Bone marrow examination, fever, HIV infected patients

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INTRODUCTION

Fever is common in patients infected with HIV and is often accompanied by significant morbidity, prolonged hospitalization, and extensive evaluation.[1] Fever of unknown origin (FUO) in adults is defined as a temperature > 38.3°C, lasting for more than 3 weeks with no obvious source despite appropriate investigation.

There is no consensus definition of FUO in the setting of HIV infection. Many authors use the criteria proposed by Durack and Street[2]: temperature ≥ 38.3°C on multiple occasions; fever of ≥ 3 weeks' duration for outpatients or > 3 days for inpatients, including at least 2 days' incubation of microbiologic cultures; and a diagnosis that remains uncertain after 3 days despite appropriate investigation. Before

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highly active antiretroviral therapy (HAART) was introduced, patients with HIV experienced FUO with relative frequency, most of which was caused by opportunistic infections, such as tuberculosis, or infection with *Mycobacterium avium* complex (MAC). In Thailand, disseminated MAC was the cause of prolonged fever in 25 of 104 patients admitted to a university hospital in Bangkok between March 1997 and June 1998.[3]

In one study at Chiang Mai University Hospital, a tertiary-care hospital in northern Thailand, it was found that disseminated MAC followed by *Penicillium marneffei* was the most and the second common infectious etiology in HIV-infected patients with prolonged fever. Whereas, *Penicillium marneffei* is the third most common opportunistic infection in northern Thailand, it is an uncommon disease in other parts of Thailand.[4] In addition to the recommended noninvasive investigations for diagnosis of FUO such as blood cultures or sputum examination and cultures, bone marrow aspiration or culture and biopsy are frequently undertaken and safe for FUO evaluation. The diagnostic yield of bone marrow aspiration or biopsy reported in many studies ranged from 25% to 34%.[5-8]

The purpose of this study was to estimate the diagnostic yield of bone marrow examination for FUO evaluation in HIV-infected patients at Bamrasnaradura Infectious Diseases Institute (BIDI) during the last decade.

MATERIALS AND METHODS

This study was approved by the Institutional Board. A retrospective review of bone marrow aspiration, culture and biopsy in HIV-infected patients with FUO at BIDI between January 2003 and January 2013 was performed. A total of 132 cases undergoing bone marrow aspiration and biopsy as part of the work up for fever in HIV infected adults at BIDI were enrolled in the study. The inclusion criteria included:[1] 18 years of age or older,[2] known HIV infection,[3] fever ≥ 3 weeks for outpatients or > 3 days for inpatients and a diagnosis that remains uncertain after 3 days despite appropriate investigation (FUO), and[4] only bone marrow examination performed between January 2003 and January 2013.

The demographic data, age, sex, CD4 level, and radiographic findings were collected from the patients record forms. Bone marrow aspiration and biopsies were performed by puncture at posterior iliac crest with Salah's and Jamshidi bone marrow needle. Bone marrow aspirate was routinely stained with Wright and Ziehl-Neelsen (ZN) stain (acid-fast stain). Bone marrow aspirations were directly inoculated into mycobacterial and fungal culture medium.

Bone marrow examination (BME) was considered positive for infections or malignancy if the aspirated culture grew with significant pathogens, or if there were fungal organisms, acid-fast bacilli (AFB), granuloma formation or evidence of lymphoma or other malignancy on smear or histopathological examination.

Statistical analysis

A logistic regression model was used to determine the relationship, expressed by Odds ratio (OR) and 95% confidence intervals (95% CI). The p-value < 0.05 was considered as statistically significant. Any missing data were not included for calculations.

RESULTS

Among 132 cases who underwent bone marrow aspiration and biopsy as part of the work up for FUO in HIV-infected adults at BIDI between January 2003 and January 2013, the clinical & laboratory records, imaging studies, and pathological reports of only 84 patients were collected due to data missing.

The demographic characteristics of 84 patients have been summarized in Table 1. Of these patients, 82 cases underwent Wright and acid-fast bone marrow smears during the work up of FUO. Meanwhile, bone marrow cultures for fungal and mycobacterial infections were

TABLE 1. Demographic characteristic of 84 HIV-infected cases with fever of unknown origin (FUO).

Characteristic	No (%)
Age, mean ± SD (years)	37.2 ± 8.5
Sex	
Female	32 (38%)
Male	52 (62%)
CD4 (cell/mm^3) median (IQR)**	
< 50	13 (6-30)
≥ 50	69 (82%)
Median duration of fever (weeks)	9 (18%)
(1- 32 weeks)	3
On Antiretroviral therapy (n=84)	
No	48 (57%)
Yes	36 (43%)
< 6 months	30
6-12 months	2
> 12 months	4
Anemia (Hb < 10 g/dl) (n=84)	69 (82%)
Leukopenia (<4,000 /mm^3) (n=84)	70 (83%)
Thrombocytopenia (< 10^5/mm^3) (n = 84)	63 (75%)
Pancytopenia	34 (40%)

*t-test, **CD4 were not done in 6 patients due to patients' death a few days after admission.

performed in 76 patients. Due to missing data, the results of bone marrow biopsy of only 69/84 cases, including 52 males and 32 females (mean age 37.2 years; range 29-46 years), were reviewed. All of the 69 cases had CD4 cell count < 50 cells /mm^3. Final diagnosis of HIV-infected patients presenting with FUO by BME was received in 46 patients (54.8 %). Definite diagnosis confirmed infectious diseases in 36 cases (42.8%), hematologic malignancy in 2 cases (2.4%), granuloma not otherwise specified in 8 cases (9.5%), and undiagnosed in 38 cases (45.2 %). The distribution of infectious causes included 20 cases of mycobacterial infection (17 cases of non tuberculous mycobacterium (NTM) and 3 cases of *Mycobacterium* tuberculosis), 8 cases of histoplasmosis, and 6 cases of cryptococcosis. One case had mycobacterial (positive AFB stain but negative result for culture) and histoplasma coinfection as shown in Table 2.

Bone marrow aspiration

Among the 84 cases with FUO, bone marrow smears were performed in 82 patients (97.6). Of these, 27 patients (diagnostic yield, 33.7%) led to the following diagnosis:19 cases of mycobacterial infection (based on presence of acid fast bacilli : AFB), 5 cases of histoplasmosis, and 3 cases of cryptococcosis. (Fig 1)

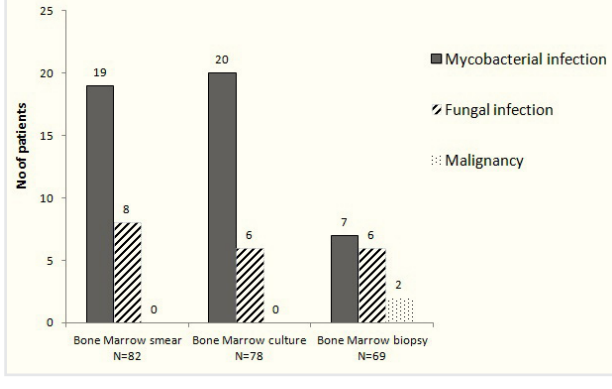

Fig 1. Distribution of etiologies diagnosed by bone marrow aspiration and culture.

TABLE 2. Results of bone marrow examination in all 84 cases.

BM findings	No. of cases
Bone marrow smear (N=80)	80/84 (95%)
Positive findings	27 (33.7%)
AFB	19/80 (23.7%)
Histoplasma spp.	5/80 (6.3%)
Cryptococcus spp.	3/80 (3.7%)
Bone marrow culture: (N=78)	78/84 (92.8%)
Positive findings	26/78 (33.3%)
Non tuberculous mycobacterium (NTM)	17 (21.8%)
Mycobacterium tuberculosis	3 (3.8%)
Histoplasma spp.	2 (2.5%)
Cryptococcus spp.	4 (5.1 %)
Bone marrow biopsy: (N= 69)	69/84 (82%)
Missing data	15/84 (18%)
Cellularity	14 (20.3%)
Hypocellular	10 (14.5%)
Hypercellular	39 (56.5%)
Normocellular	6 (8.69%)
Supoptimal specimen	24/69 (34.8%)
Positive findings from bone marrow biopsy	
Granuloma	15/69 (21.7%)
Organisms found from bone marrow biopsy	
Positive AFB (Acid fast bacilli) with granuloma	3/69 (4.3%)
Histoplasma spp.	5/69 (7.2%)
Cryptococcus spp.	2/69 (2.9%)
Suspected Lymphoma	2/69 (2.9%)

Bone marrow culture

Bone marrow culture was performed for detecting mycobacterial and fungal infections in 79 from 84 cases (92.3%). The final diagnosis was done by bone marrow culture in 26 cases (diagnostic yield, 21.8%). Twenty (25.6%) microorganisms were isolated from the mycobacterial cultures, with diagnosis of 8 non tuberculous mycobacteria (final identification of the species were not done), and 3 *Mycobacterium tuberculosis* complex. Fungal microorganisms were identified in 6 cases (7.7%).

Histoplasma capsulatum and *Cryptococcus neoformans* were isolated in 2 and 4 cases (2.5% and 5%), respectively.

In Fig 1, there were 4 cases of NTM, 1 case of *M.tuberculosis* and histoplasmosis found by culture despite negative bone marrow smear and biopsy.

Bone marrow biopsy

Among 84 HIV-infected patients with FUO, bone marrow biopsy was done in only 69 cases (82%). Of these, 24 (diagnostic yield, 34.8%) were diagnosed with histoplasmosis (5 cases), cryptococcosis (2 cases), granuloma (15 cases with 3 positive AFB stain and 1 cryptococcosis), and lymphoma (2 cases, but without performing immunohistochemical reactions due to patients' death before confirmation). As shown in Table 2 and Fig 1, there were 2 cases of histoplasmosis and 1 cryptococcosis diagnosed by bone marrow biopsy despite negative results by other methods.

In 69 HIV-infected patients whose bone

marrow biopsy could be collected, granuloma was noted in 15 cases (21.7%), with 7 (46%) positive AFB, 1 (8%) cryptococcosis, and 7 (46%) no specific causes identified. Bone marrow culture was positive in 6 (40%) of 15 cases. All of these patients with positive blood culture had NTM infection and CD4 < 50 (mean = 12) cell/mm^3.

Predictive factors of positive bone marrow examination (BME) contributing to diagnosis of causes of FUO

The clinical laboratory predictors have been shown in Table 3 for the yield of BME in the work up of FUO. It was found that the groups with and without BME contribution did not differ significantly in age, sex, hepatosplenomegaly or baseline hemoglobin and platelet counts. However, leukocyte levels were lower in the group with positive BME results. ($p<0.05$)

DISCUSSION

FUO still remains a critical problem in HIV-infected patients. Our retrospective study of 84 HIV-infected patients with FUO and or cytopenias who underwent bone marrow examination [bone marrow aspiration (N = 80), culture (N = 79), and biopsy (N = 69)] during 10 years led to the diagnosis of opportunistic infection and hematologic malignancies in 46

TABLE 3. Clinical and laboratory predictors of positive bone marrow examination.

Predictor	Positive Bone marrow (N=46)	Negative results (N=38)	OR (95% CI)	p-value*
Age, mean years (±SD)**	36 (± 6.938)	38.5(± 9.848)		
Group I	10	14	1	
Group II	9	10	0.7 (0.22 – 2.36)	0.581
Group III	26	14	0.4 (0.14 – 1.09)	0.072
Sex				
Female	19	13	1	
Male	27	25	1.6 (0.65 – 3.84)	0.315
Hepatomegaly				
No	28	25	1	
Yes	18	13	0.8 (0.33 – 1.98)	0.642
Splenomegaly				
No	32	29	1	
Yes	14	9	0.7 (0.27 – 1.89)	0.491
Anemia (Hb < 10g/dl)				
No	8	7	1	
Yes	38	31	0.9 (0.30 – 2.86)	0.902
Leukopenia (<4,000 /mm^3)				
No	8	16	1	
Yes	38	22	0.3 (1.11 – 0.79)	0.015
Thrombocytopenia (< 10^5/mm^3)				
No	27	23	1	
Yes	19	15	0.9 (0.39 – 2.23)	

**Chi-square test*

***Group I refers to the age of 36-43 years, Group II refers to the age of ≥ 44 years, and Group III refers to the age of 18-35 years.

cases (diagnostic yield = 54%). Since the majority of the patients in our study had advanced HIV infection with low CD4 count, the yield of BME was higher, comparable to other studies of 25-42%.[5,9-14]

The comparative results of this study revealed the usefulness of BME in diagnosis of FUO in HIV-infected patients, in contrast to one study that reported the lack of utility of this procedure.[16] Bone marrow smear stained with acid-fast bacilli and Wright stain allowed diagnoses in 23.7% and 10%, similar to one study in Brazil.[19]

Mycobacterial and fungal cultures were diagnosed in 33% of 79 patients, higher than 20% in a study by Nichols et al.[11] The differences may result from several factors like the higher prevalence of some opportunistic infections such as MAC and tuberculosis in Thailand.[17] The most common cause of FUO in the patients of our study was similar to previous studies in Thailand,[3,4] NTM (final identification of the species were not done due to limitation of culture method), assuming that MAC is the most common infectious etiology, followed by histoplasmosis, cryptococcosis, and tuberculosis. The results in our study were different from other studies in Europe and South Africa where tuberculosis was more common than MAC.[17,18] MAC is the most common cause of FUO in the United States (31%) followed by tuberculosis (6.5%).[19] The etiology of prolonged fever in HIV-infected patients may vary depending on the prevalence of opportunistic infections in that geographic area.

In 69 patients with bone marrow biopsy, lymphoma was observed in 2 cases (8%), similar to 6% in one study in London.[12] Since this study was a retrospective review and some previous investigation results, the immunohistochemistry confirmation of bone marrow biopsy was not requested due to the death of some patients. Therefore, only 2 patients could be classified as suspected lymphoma.

The primary objective of diagnostic evaluation for opportunistic organisms in HIV-infected patients is to timely identify the pathogens so that appropriate treatment can be instituted. BME has the advantage of specific diagnosis leading to the differentiation between infectious and non-infectious diseases, especially mycobacterial and fungal infections that are commonly found in HIV-infected patients.

The highest yield of bone marrow examination in other studies has been found in patients with fever, extremely advanced and cytopenia[13,15] which is consistent with the findings in this study. However, the author could not explain why only leukopenia was the predictive factor of positive BME whereas anemia and thrombocytopenia were not significant predictive factors.

In advanced HIV-infected patients with FUO and/or peripheral cytopenias, BME and culture provided diagnostic information were available in half of the patients studied.

The limitation of this study was the low number of cases studied due to the missing of BME results in 48 patients (36%), which may affect to the precision of the results in this study. With more cases, we would be able to better investigate clinical predictors relating to positive BME. Hence, more data collection of BME in HIV-infected cases with FUO is recommended for future investigation.

CONCLUSION

Our study revealed the usefulness of bone marrow examination for diagnostic yield in HIV-infected patients with FUO, which should be considered as a helpful tool in the evaluation of appropriate treatments.

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