Journal of Bone Marrow Research

Kumar Nath, et al., J Bone Marrow Res 2014, 2:3
DOI: 10.4172/2329-8820.1000153

Research Article Open Access

Hemophagocytic Syndrome in HIV-Infected Patients: A Prospective Clinicopathological Tertiary Care Centre Study from India

Uttam Kumar Nath*, Dipanjan Haldar and Bhattacharyya M

Institute of Haematology and Transfusion Medicine, Medical College, Kolkata, India

***Corresponding author:** Uttam Kumar Nath, Institute of Haematology and Transfusion Medicine, Medical College, Kolkata, India, Tel: +919433982756; E-mail: nath.uttam@gmail.com

Rec Date: 02 Dec, 2014; **Acc date:** 25 Dec, 2014; **Pub date:** 27 Dec, 2014

Abstract

Background: Hemophagocytic lymphohistiocytosis (HLH) has been described in patients with human immunodeficiency virus (HIV) infection. However, limited data is available in the era of highly active antiretroviral therapy (HAART).

Study design and methods: We conducted this prospective study over a two year period on 30 HIV infected patients. Our aim was to assess the frequency of hemophagocytosis in HIV-infected patients. We also tried to identify the underlying etiology and assess specificity of different parameters for HLH.

Results: Hemophagocytosis was identified in 14(46%) patients. 10/14(71%) had pancytopenia while 4/14(29%) had bicytopenia. 6(43%) had elevated ferritin (>500 µg/l). Fever (5/14), splenomegaly (4/14) and raised triglyceride (2/14) were identified less frequently. Underlying etiology could be identified in 6/14(43%) cases - disseminated tuberculosis in 4/14(29%) and one case each of Japanese encephalitis and disseminated candidiasis. Median CD 4 count was 123/cu.mm. 12(40%) patients were on HAART. 4/30 (13%) patients fulfilled criteria for HLH. Bicytopenia was least specific (25%) for diagnosis of HLH. Morphological evidence of hemophagocytosis had low specificity for HLH (29%); however, moderate / severe hemophagocytosis had higher specificity (66%). Raised ferritin levels (>500 µg/l) had a specificity of 66% while ferritin levels (>800 µg/l) had 100% specificity. Increased triglyceride levels (>265 mg/dl) had 50% sensitivity and 100% specificity.

Conclusion: Opportunistic infections represent the most common identifiable cause of HLH in HIV patients. Bicytopenia has got low specificity while moderate/severe hemophagocytosis, increased ferritin levels (>800 µg/l) and raised triglyceride levels have got high specificity for HLH in this setting. Larger prospective studies would be helpful in identifying modified diagnostic criteria in this setting.

Keywords: HAART; Hemophagocytic lymphohistiocytosis (HLH); HIV/AIDS

Introduction

Hemophagocytic syndrome is a clinicopathological entity characterized by increased pro-liferation and activation of benign macrophages (histiocytes) with phagocytosis of hematopoietic cells throughout the reticuloendothelial system [1]. It is characterized by fever, hepatosplenomegaly, cytopenias, and hyperferritinemia. Hemophagocytic lymphohistiocytosis (HLH) comprises two different conditions that may be difficult to distinguish from one another: a primary and a secondary form. The primary form, Familial Hemophagocytic Lymphohistiocytosis (FHL) is a fatal disease with a median survival of less than 2 months. Secondary, or reactive, HS can be triggered by malignancy, infection, or autoimmune disease.

Diagnosis of secondary HS requires documentation of a fluctuating fever, hepatosplenomegaly, cytopenia (involving two or more cell lines), increased ferritin levels, hypofibrinogenemia and hypertriglyeridemia, as well as morphological evidence of hemophagocytosis principally in the bone marrow but also in the spleen, liver, lymph nodes, skin, or cerebrospinal fluid [2]. Low or absent NK cell activity and soluble IL-2 receptor levels are limited by their availability in the developing countries and are not used routinely for diagnosis. Hemophagocytosis syndrome carries a poor prognosis and early recognition is crucial for effective treatment.

HIV infection, alone or in association with other opportunistic infections, has been reported to be a cause of reactive hemophagocytosis [3]. HIV associated hemophagocytosis is triggered by number of factors. Increased cytokine levels triggered by malignancy or opportunistic infections and the generalized defects in natural killer (NK) and T-cell cytotoxicity associated with HIV infection could play a possible causative role. However, most of the data of HLH in HIV infected individuals is from the pre HAART era. Only few retrospective studies have assessed the prevalence of HLH in the HAART era [4]. The criteria for diagnosis of HLH are also often non specific and have never been validated in a prospective setting in HIV infected patients.

Materials and Methods

We performed bone marrow evaluation on 30 adult HIV infected patients over a 2 year period (August 2012 – July 2014). Informed consent was obtained in all the patients. Relevant clinical and laboratory data were obtained in all these patients for evaluation of

HLH. Microbiological cultures for tuberculosis and fungi were sent as relevant. The bone marrow slides were evaluated independently by two pathologists and morphologically hemophagocytosis was graded on aspirate smears as-(0)-Absent; (1+)(Mild)-<2 histiocytes with HPS/Slide; (2+)(Moderate)-2-5 histiocytes with HPS/Slide; (3+)(Severe)->5 histiocytes with HPS/Slide [5]. Ferritin was measured by ELISA, CD 4 Count was enumerated by single platform BD FACS Calibur™ machine and triglyceride assay was done using Glycerol-3-phosphate oxidase-peroxidase (GPO-PAP) method. The patients were classified as having HLH on fulfilling 5/8 criteria for HLH [2]. The sensitivity and specificity of each criteria for HLH was subsequently assayed taking into consideration presence or absence of each criteria and correlating them with the diagnosis of HLH.

Results

A total of 30 HIV infected patients were enrolled in the present study. The age of the patients ranged from 17-57 years (Mean -34years). Their baseline characteristics are summarized in Table 1. The indications of bone marrow evaluation were unexplained bicytopenia (16,54%); pancytopenia (12,40%); organomegaly (12,40%); lymphadenopathy (9, 30%), pyrexia of unknown origin {PUO} (7, 27%) and hematological malignancy (4, 14%) 18 patients had more than one indication for bone marrow.

Characteristics		No. of patients	%
Sex	Male	18	60
	Female	12	40
Indication for bone marrow examination	Cytopenias	16	54
	Organomegaly	12	40
	Lymphadenopathy	9	30
	PUO	7	27
	Hematological Malignancy	4	14
CD 4 Count(cells/cu.mm)	>200	11	37
	≤200	19	63
HAART Therapy	Yes	12	40
	No	18	60
Hemophagocytosis on BMA	Present	14	46
	Absent	16	54

Table 1: Baseline patient characteristics (n=30)

The median CD 4 count was 123/cu.mm. (range 2-366/cu.mm). 12(40%) patients were on HAART. However, the median duration of HAART therapy was less than six months. Of the four patients who had hematological malignancy, three patients had Non Hodgkin's Lymphoma (NHL) and one patient had Hodgkin's lymphoma. Histological classification of the three cases of NHL revealed diffuse large B Cell Lymphoma in two patients and plasmablastic lymphoma in one.

Morphologically, hemophagocytosis on bone marrow aspirate (BMA) was identified in 14(46%) patients- mild in 8/14(58%), moderate in 3/14(21%) and severe in 3/14(21%). 10/14(71%) had pancytopenia while 4/14(29%) had bicytopenia. 6(43%) had elevated ferritin (>500 µg/l). Fever (5/14), splenomegaly (4/14) and raised triglyceride (2/14) were identified less frequently. Underlying etiology for hemophagocytosis could be identified in 6/14(43%) cases - disseminated tuberculosis in 4/14(29%) and one case each of Japanese encephalitis and disseminated candidiasis. These are summarized in Table 2.

Characteristics		No. of patients	%
Hemophagocytosis	Mild	8	58
	Moderate	3	21
	Severe	3	21
Fever	Present	5	38
	Absent	9	62
Cytopenias	Bicytopenia	4	29
	Pancytopenia	10	71
Splenomegaly	Yes	4	29
	No	10	71
Ferritin levels (µg/L)	<500	8	58
	≥500	6	42
Triglyceride levels (mg/dl)	<265	12	86
	≥265	2	14

Table 2: Association of Hemophagocytosis with other parameters of HLH (n = 14)

Photomicrographs from a case of hemophagocytosis in a case of disseminated tuberculosis (documented on culture) are shown in Figures 1a and 1b. 4/30(13%) patients fulfilled criteria for HLH.

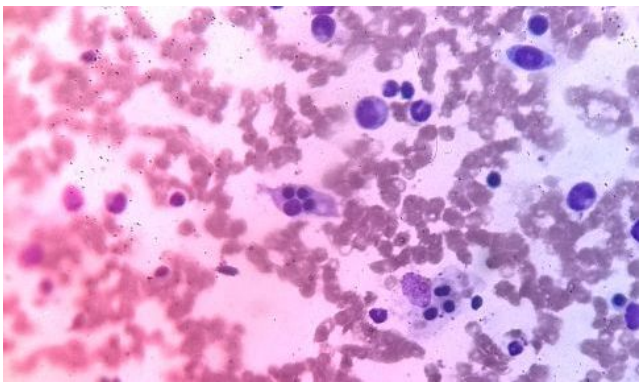

Figure 1a: Hemophagocytosis in a case of HIV with tuberculosis. 1a) Phagocytosis of erythroid precursors (1000x).

Bicytopenia was least specific (25%). Morphological evidence of hemophagocytosis had low specificity for HLH (29%); however,

moderate/severe hemophagocytosis had higher specificity (66%). Raised ferritin levels (>500 µg/l) had a specificity of 66% while ferritin levels (>800 ng/ml) had 100% specificity. Increased triglyceride levels (>265 mg/dl) had 50% sensitivity and 100% specificity.

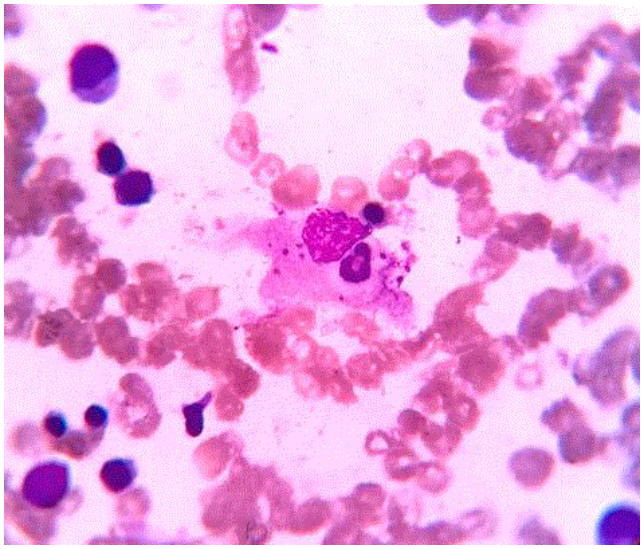

Figure 1b: Hemophagocytosis in a case of HIV with tuberculosis. 1b) Phagocytosis of neutrophils and platelets (1000x).

Discussion

This is the first prospective study looking at different aspects of hemophagocytosis in HIV infected patients in the HAART era. In this study patients with a wide range of clinicopathological features were included (Table 1). 18(60%) of the patients had more than one overlapping indications for bone marrow evaluation. 19(63%) of patients were having low CD4 count (≤ 200) while 12(40%) were on HAART. Previously, a single retrospective study had been published in the HAART era [4]. In that study, involving three tertiary care centers in France 58 cases of HLH in HIV patients were studied. The median CD4 count was 91 cells/mm^3 while 57% were receiving ART. This compares well with the median CD4 count of 123/cu.mm in our study with 40% of the patients on HAART.

The etiological triggers for HLH in HIV patients were primarily infectious agents in the pre HAART era [6]. During the pre-HAART period, HLH was mainly reported in patients with HIV end-stage disease and opportunistic infections were the most frequently reported triggers. In the HAART era, hematological malignancies are postulated to be the main etiological factors for RHS. Fardet, et al. identified an underlying cause of RHS in 56 out of the 58 patients [4]. Malignancy or a hematologic disorder was the most common etiological factor for HLH in 31(53%), followed by infections in 23(40%) and an autoimmune disorder in 2(7%). This could be due to increasing prevalence of malignancy in HIV-infected patients, corresponding to increased survival and decrease incidence of opportunistic infections related to the use of HAART.

On the contrary, in the present study opportunistic infections (mainly disseminated tuberculosis) were the most important cause of RHS in our patients. This could be related to very high prevalence rates of tuberculosis in India. Besides, poor access to health care facilities and delayed starting of HAART could also be playing a role in high incidence of opportunistic infections and related secondary RHS.

HLH in the setting of HIV infection has got a very high mortality rate ranging from 50% to 100% [6-8]. The mortality rates have declined to 31% in the HAART era. However, this could be confounded by several other factors -Eg., Changes in the underlying etiology of HLH(as described previously), improvement of supportive care, early diagnosis and treatment. In the present study, four patients fulfilled 5/8 criteria for diagnosis of HLH and all four of them expired within one week of diagnosis.

We also tried to assess the sensitivity and specificity of the various commonly used parameters used to diagnose HLH. In this study, we found that bicytopenia was present in 54% of the patients and had 25% specificity for diagnosis of HLH. High incidence of cytopenias in the present study could be related to late stages of presentation of the patients in the present study. Choi et al reported that the most important factors for cytopenia were Initial AIDS status at presentation and HIV Viral load [9]. A limitation of the present study is that data on HIV Viral load was not available. Another important finding from the present study was that grading of hemophagocytosis on BMA slides improved the specificity for HLH. This has been reported previously by various authors including us [5,10]. Hence, we recommend that hemophagocytosis should be graded whenever possible. Mild hemophagocytosis could be non specific and be the manifestation of HIV infection itself [11]. This could also explain the fact that secondary etiological agent for hemophagocytosis could be identified in only 43% of our patients with probably the remainder of the patients manifesting hemophagocytosis as a result of HIV infection itself. Increased ferritin levels have got a high sensitivity and specificity for diagnosing HLH. In the present study, the specificity of ferritin levels for HLH was 100% taking a cut off of 800 µg/l. Higher cut off values for ferritin as a criteria for HLH has been proposed recently as cut off values of 500 µg/l was not determined based on evidence [2,12]. Very few studies have addressed the sensitivity and specificity of hypertriglyceridemia in HLH. In a study by Okamato et al., increased triglyceride levels had 68% sensitivity in diagnosing HLH [13]. In the present study, raised triglyceride levels had a 50% sensitivity for detecting cases of HLH. However, specificity was 100%. Thus, serum triglyceride levels represent a simple, inexpensive and specific marker for HLH in HIV positive patients.

Conclusions

In the present study, we found that opportunistic infections (mainly disseminated tuberculosis) represent the most common cause of reactive HLH in HIV infected patients. To the best of our knowledge, this is the first prospective study to assess the sensitivity and specificity of various criteria for HLH in HIV infected patients. Bicytopenia has got low specificity while moderate/severe hemophagocytosis, increased ferritin levels (>800 µg/l) and raised triglyceride levels have got high specificity for HLH in this setting. A limitation of the present study is the small number of patients with a confirmed diagnosis of HLH. Therefore, larger multicentric prospective studies should be conducted to devise effective scoring strategy for HLH in HIV infected patients. This can go a long way in helping us out of this HIV associated 'quagmire' [14].

References

1. Larroche C, Mouthon L (2004) Pathogenesis of hemophagocytic syndrome (HPS). Autoimmun Rev 3: 69-75.
2. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, et al. (2007) HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 48: 124-131.
3. Grateau G, Bachmeyer C, Blanche P, Jouanne M, Tulliez M, et al. (1997) Haemophagocytic syndrome in patients infected with the human immunodeficiency virus: nine cases and a review. J Infect 34: 219-225.
4. Fardet L, Lambotte O, Meynard JL, Kamouh W, Galicier L, et al. (2010) Reactive haemophagocytic syndrome in 58 HIV-1-infected patients: clinical features, underlying diseases and prognosis. AIDS 24: 1299-1306.
5. Singh ZN, Rakheja D, Yadav TP, Shome DK (2005) Infection-associated haemophagocytosis: the tropical spectrum. Clin Lab Haematol 27: 312-315.
6. Grateau G, Bachmeyer C, Blanche P, Jouanne M, Tulliez M, et al. (1997) Haemophagocytic syndrome in patients infected with the human immunodeficiency virus: nine cases and a review. J Infect 34: 219-225.
7. Sailler L, Duchayne E, Marchou B, Brousset P, Pris J, et al. (1997) [Etiological aspects of reactive hemophagocytoses: retrospective study in 99 patients]. Rev Med Interne 18: 855-864.
8. Bourquelot P, Oksenhendler E, Wolff M, Fegueux S, Piketty C, et al. (1993) [Hemophagocytic syndrome in HIV infection]. Presse Med 22: 1217-1220.
9. Choi SY, Kim I, Kim NJ, Lee SA,Choi YA, et al. (2011) Hematological manifestations of human immunodeficiency virus infection and the effect of highly active anti-retroviral therapy on cytopenia. Korean J Hematol 46: 253–257.
10. Bhatia P, Haldar D, Varma N, Marwaha R, Varma S (2011) A Case Series Highlighting the Relative Frequencies of the Common, Uncommon and Atypical/Unusual Hematological Findings on Bone Marrow Examination in Cases of Visceral Leishmaniasis. Mediterranean Journal of Hematology and Infectious Diseases 3: e2011035.
11. Evangelia Douka, Foteini Economidou, Serafim Nanas (2012) Infections Associated With the Hemophagocytic Syndrome. Hospital chronicles 7: 16–24.
12. Lehmberg K, McClain KL, Janka GE, Allen CE (2014) Determination of an appropriate cut-off value for ferritin in the diagnosis of hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 61: 2101-2103.
13. Okamoto M, Yamaguchi H, Isobe Y, Yokose N, Mizuki T, et al. (2009) Analysis of triglyceride value in the diagnosis and treatment response of secondary hemophagocytic syndrome. Intern Med 48: 775-781.
14. Pantanowitz L, Dezube BJ (2007) Editorial comment: hemophagocytic syndrome--an HIV-associated quagmire. AIDS Read 17: 500-502.