

Hematological profile of HIV patients in relation to immune status - a hospital-based cohort from Varanasi, North India

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ABSTRACT

Objectives: To study the spectrum of hematological manifestations and evaluate the relationship between various hematological manifestations and CD4 cell counts in a hospital-based cohort of HIV-infected adults in and around Varanasi, North India.

Materials and Methods: The clinical and hematological profiles of the patients attending the Infectious Disease Clinic, Varanasi, India were recorded. The relationship between CD4 counts and various hematological manifestations was analyzed.

Results: A total of 470 HIV-infected individuals were followed for 830 person years of observation (PYO). Rate of hematological episodes was 1047 episodes per 1000 PYO. CD4 counts were significantly lower in individuals with severe anemia and neutropenia compared to those without. However, no relation could be established between thrombocytopenia and CD4 counts. In the above-mentioned population, CD4 levels were significantly lower in those with anemia/neutropenia harboring any particular disease compared to those who had the same disease without anemia/ neutropenia.

Conclusions: There is a strong negative association between CD4 counts and the severity of anemia and neutropenia in this population. They can be considered as good clinical indicators to predict and access the underlying immune status. Though fall in the CD4 levels during neutropenia is observed, it is difficult to comment since the estimations of CD4 rely on the total leukocyte counts. However, the relation between anemia and disease progression is straight forward and quite useful for the treating physician. (Turk J Hematol 2008; 25: XX)

Key words: HIV, hematological manifestations

ÖZET

HIV pozitif olan hastaların hematolojik özelliklerinin immün durumları ile ilişkisi: Kuzey Hindistan'da Varanasi şehrinde hastane temelli kohort

Amaç: Kuzey Hindistan'da Varanasi şehrinde bir hastanede HIV ile enfekte erişkin hastaların hematolojik özelliklerini ve bunların CD4 hücre sayıları ile ilişkilerini araştırma.

Metod: Hindistan'da Varanasi şehrindeki bir hastanenin enfeksiyon kliniğine baş vuran hastaların klinik ve hematolojik özellikleri kaydedildi. Çeşitli hematolojik bulgular ile CD34 sayısı arasındaki ilişki analiz edildi.

Sonuçlar: HIV ile enfekte olan toplam 470 birey toplam 830 hasta izlem yılı (HİY) takip edildi. Hematolojik epizod hızı 1000 HİY'nda 1047 idi. CD34 hücre sayısı ağır anemisi ve nötropenisi olanlarda olmayanlara göre belirgin olarak daha düşüktü. Buna karşın trombositopeni ile CD34 sayıları arasında bir ilişki saptanmadı. Yukarıda belirtilen hasta grubunda anemi veya nötropeni ile birlikte herhangi bir başka hastalığı olanların CD34 sayısı aynı hastalıkla birlikte anemi veya nötropenisi olmayanlara göre daha düşüktü.

Çıkarım: Bu hasta popülasyonunda anemi veya nötropeninin ağırlık derecesi ile CD34 sayısı arasında kuvvetli bir negatif ilişki vardır. Bu hastalarda anemi ve nötropeninin varlığı alttaki immün durumu tahmin etmekte yardımcı olabilecek önemli bir belirteç olarak kabul edilebilir. Nötropeni sürecinde CD34 sayılarının daha da düşüğü gözlemlendiği için hastaların lökosit sayılarına dayanarak CD34 sayısını tahmin edebilmek oldukça güçtür. Buna karşın anemi ile hastalığın progresyonu arasındaki ilişki daha doğrusal olup, tedavi eden hekimler açısından oldukça yardımcı olabilir.

Anahtar kelimeler: HIV, hematolojik bulgular

INTRODUCTION

A variety of hematological manifestations are seen at every stage of human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) and often pose a great challenge in the comprehensive management. These manifestations also reflect the underlying immune status if interpreted cautiously, especially if the patient is in regular follow-up. They may cause symptoms that are life-threatening and impair the quality of life of these patients [1]. The most important of them are cytopenias. Anemia and neutropenia are generally caused by inadequate production (because of suppression of the bone marrow by the HIV infection through abnormal cytokine expression and alteration of bone marrow microenvironment) [2]. Thrombocytopenia is caused by immune-mediated destruction of the platelets, in addition to inadequate platelet production. The incidence and severity of cytopenia are generally correlated to the stage of the disease. Other causes of cytopenia in these patients include treatment-related adverse events or secondary to the opportunistic infections or malignancies, or other preexisting or coe-

xisting medical problems. Optimal management of the underlying HIV infection is essential, and mild cytopenia in asymptomatic patients may need no specific management [2-4].

This study was conducted since there is no information from northern India regarding the hematological manifestations and the probable interpretation of the same in this population, using one of the most easily available investigations (complete hemogram). Therefore, this study presents preliminary data regarding hematological manifestations in this area. We also tried to evaluate the relationship between various hematological manifestations and CD4 cell counts in a hospital-based cohort of HIV-infected adults attending our clinic.

MATERIALS AND METHODS

All HIV-infected patients attending the Infectious Disease (ID) clinic, Sir Sunderlal Hospital, Varanasi, between January 2001 and December 2003 were included in the study. This is a tertiary care teaching hospital with catchment area of five states (UP, MP, Bihar, Jharkhand, Chattisgarh) of India. The annual attendance of

new HIV cases is approximately 150. Patients underwent thorough clinical examination and the results were noted in the records. The hematological profiles of the patients including hemogram, cell indices and bone marrow (wherever necessary) were recorded at presentation and during the follow-up. Whenever the patient presented with any features suggestive of involvement of the hematological system, or with laboratory features suggesting involvement of the system, we recorded it as an episode. We also recorded the episodes as new if it was noted at presentation. However, the subsequent episodes were recorded as new only if the patient had complete resolution from the previous episode. An informed consent was obtained from all the subjects of the study as a routine workup, which was already an existing practice in the ID clinic.

In this population, HIV infection is transmitted mainly by heterosexual contact. The clinical diagnosis of AIDS was made using Centers for Disease Control (CDC) criteria. The male to female ratio was 3.7:1 with a median age of 34 years. Anemia was labeled as drug-induced if there were no other identifiable causes after the work-up, if a temporal relation was determined with the introduction of the drug, and if recovery was obtained following cessation of the drug. Iron deficiency anemia was diagnosed based on the serum iron levels.

HIV serology: HIV status was confirmed by antibody testing using ELISA with two different antigens [5].

CD4 assay: CD4 count was performed by using FACS count (Becton Dickson, Singapore) as per the protocol given by the manufacturer. CD4 counts were measured routinely in the first visit and during the follow-up visits. For this study, each time a participant presented with any hematological manifestation, their most recent CD4 count was used for the analysis. Patients in whom no CD4 count was available were excluded from the analysis. The relationship between CD4 counts and various hematological manifestations was analyzed.

Definitions: All the opportunistic infections were diagnosed as per the standard case definitions [5]. Stratification of the various hematological manifestations was done as per the table given below (Table 1).

Statistics: The association between CD4 counts and different hematological presentations was compared between different grades of anemia, neutropenia and thrombocytopenia using the Kruskal-Wallis test on actual CD4 levels. Leukocyte values, hemoglobin levels and platelet counts were taken as continuous variables and correlated with CD4 levels. A p value of less than 0.05 was considered to indicate statistical significance. Medicalc 7.5 version was used for the calculation purposes.

Table 1. Definition of mild, moderate and severe hematological manifestations

	Hemoglobin (g/dl)	WBC ($\times 10^6$)	Platelets ($\times 10^6$)
Mild	10-12	2000-4000	50,000-100,000
Moderate	8-10	1000-2000	20,000-50,000
Severe	<8	<1000	<20,000

Table 2. Immunological status of patients with anemia (numbers indicate episodes rather than number of patients)

Anemia	CD4 <200 (n=304)	CD4 200-500* (n=197)	CD4 >500 * (n=102)	Total (n=603)
Mild	39	44	55	139
Moderate	100	118	32	250
Severe	165	35	15	215

Kruskal-Wallis P=0.001

RESULTS

A total of 470 HIV-infected individuals were followed for 830 person years of observation (PYO), and 869 distinct hematological episodes were observed during this period. Rate of hematological episodes was 1047 episodes per 1000 PYO. The results are shown in Tables 2, 3 and 4 for anemia, neutropenia and thrombocytopenia, respectively. We found that there was a strong negative association between CD4 counts and the severity of anemia and neutropenia in the HIV/AIDS patients. CD4 counts were significantly lower in individuals with severe anemia and neutropenia compared to those without ($p < 0.001$). However, no relation could be established between thrombocytopenia and CD4 counts ($p > 0.05$) (Table 5). Anemia was present in 74.6% of patients during the follow-up period. Similarly, neutropenia was encountered in 22.7% of patients. Very few patients (only 4.8%) had thrombocytopenia.

DISCUSSION

HIV already has 60 million victims globally, and in India, this figure is around 6 million patients. National AIDS Control Organisation (NACO) reported more than 1,400,000 full-blown AIDS cases by the end of January 2005 [5]. The

clinical spectrum of HIV infection in India is different from that in the rest of the world. There is also great variability within India as well. Hematological manifestations are the second commonest cause of morbidity and one of the common causes of mortality in HIV patients [2-5]. Though many studies have been conducted in India, in most of them, various aspects were addressed and the focus on the hematological manifestations was limited [6-9]. Most of the available data is from the west, which might not be directly applicable to the Indian subcontinent [10-14].

Anemia: In different study settings, the prevalence of anemia in persons with AIDS has been estimated at 63% to 95% [15], making it more common than thrombocytopenia or leukopenia in patients with AIDS. This high prevalence of anemia may be due to the high incidence of anemia, a long duration of anemia, or a combination of both. The incidence of 74.6 observed in the present study is in agreement with most of the available literature. HIV infection may lead to anemia in many ways: the important causes include

- Defective iron metabolism and reutilization [16,17]
- Opportunistic infections [18]
- Administration of chemotherapeutic agents [19]
- Vitamin B12 deficiency [20]

Table 3. Immunological status of patients with neutropenia (numbers indicate episodes rather than number of patients)

Neutropenia	CD4 <200 (n=150)	CD4 200-500* (n=45)	CD4 >500 * (n=31)	Total (n=226)
Mild	10	18	20	48
Moderate	25	17	7	49
Severe	115	10	4	129

Kruskal-Wallis $P < 0.001$

Table 4. Immunological status of patients with thrombocytopenia (numbers indicate episodes rather than number of patients)

Thrombocytopenia	CD4 <200 (n=15)	CD4 200-500* (n=12)	CD4 >500* (n=13)	Total (n=40)
Mild	6	3	4	13
Moderate	5	3	5	13
Severe	4	6	4	14

Kruskal-Wallis $P = 0.41$

In the present study, we found that in most of the cases (>80%), the cause was either drug-induced (commonest are zidovudine (AZT) and cotrimoxazole) or iron deficiency. Clinically significant anemia requiring blood transfusion was observed in as many as 42% of patients receiving AZT after 6 weeks of therapy, requiring red cell transfusion. Stage of illness also had direct effect on the drug-induced anemia, wherein severity of anemia is directly proportional to stage (only 3% of asymptomatic individuals develop significant reduction in hemoglobin). Treatment of anemia associated with cotrimoxazole (which was used in 65% of the individuals for *Pneumocystis carinii* pneumonia (PCP) prophylaxis) was very rewarding and almost all patients responded to therapy with the exception of 2 (by supplementation of folate). However, the patients did not experience any fall in other cell lines. We did not estimate the survival in patients with and without anemia, as the duration of follow-up was short, though the literature clearly indicates that it does affect the survival [13].

Neutropenia: Neutropenia is common and its incidence rises from 13% to 44% with disease progression from HIV to AIDS [21,22]. Taking into account the patient's CD4 cell count, it was shown that the incidence of neutropenia was 0.8% in HIV-positive patients with CD4 count >700 cells/ml, and rose to 13.4% in those with

CD4 count <249 cells/ml. We observed that 22.7% of patients had neutropenia. The correlation between the level of absolute neutrophil count (ANC) and the need for hospitalization for bacterial infections was proven in a few trials [23]. Though a fall in the CD4 levels during neutropenia is observed, it is difficult to comment as the estimations of CD4 rely on the total leucocyte counts (TLC).

Thrombocytopenia in HIV Infection: Association of HIV infection with thrombocytopenia was long ago recognized [24]. References of a few cases and their description in the literature signal that patients might have AIDS rather than simple HIV infection, though more speculations would be unscientific. Incidence of thrombocytopenia is around 40% of HIV-infected persons, and in approximately 10% of the patients, it may be the first sign of AIDS [25]. Surprisingly, however, we found only 4.8% of HIV patients had thrombocytopenia, whose platelet counts improved after anti-retroviral therapy. We failed to find any reasons for it. In none of our patients was it the presenting feature and none of them had significant bleeding despite severe thrombocytopenia.

Coagulation Abnormalities in HIV Infection: Though an increased risk of venous thromboses is known, other coagulation abnormalities in the setting of HIV-infection are not well established. In a CDC study, it was found that the overall incidence of thrombotic episodes was 2.6 per 1000 HIV-infected persons [26]. In the present study, not even a single case of thrombosis was found. Moreover, with larger follow-up, we expect only a few cases as the reported incidence itself is very low.

Conclusion: There is a strong negative association between CD4 counts and the severity of anemia and neutropenia in HIV/AIDS patients. Anemia and neutropenia in HIV patients can be considered as good clinical indicators to predict and assess the underlying immune status. Though a fall in the CD4 levels during neutropenia is observed, it is difficult to comment as the esti-

Table 5. Definition of mild, moderate and severe hematological manifestations

Anemia	Mean±SD	Range
Hb 10-12 g%	380±120	180-670
Hb 8-10 g%	265±122	103-540
Hb <8 g%	131±87	30-256
TLC 2000-4000/mm ³	336±124	208-512
TLC 1000-2000/mm ³	194±124	50-482
TLC <1000/mm ³	147±67	48-237
PLC 50000-100000	490±140	350-790
PLC 20000-50000	379±192	182-428
PLC <20000	298±169	160-449

mations of CD4 rely on the TLCs. However, the relation between anemia and disease progression is straightforward and quite useful for the treating physician. In view of the high incidence of hematological disorders in clinically silent HIV infection and the progressive increase in the incidence of HIV in our population, it is strongly recommended that in all cases of unexplained blood dyscrasias, the possibility of 'silent' HIV infection must be seriously entertained.

REFERENCES

1. Volberding PA, Baker KR, Levine AM. Human immunodeficiency virus hematology. *Hematology Am Soc Hematol Educ Program* 2003;:294-313.
2. Coyle TE. Hematologic complications of human immunodeficiency virus infection and the acquired immunodeficiency syndrome. *Med Clin North Am* 1997;81:449-70.
3. Salond E. Hematologic complications of HIV infection. *AIDS Rev* 2005;7:187-96.
4. Cosby CD. Hematologic disorders associated with human immunodeficiency virus and AIDS. *J Infus Nurs* 2007;30:22-32.
5. NACO HIV report in India 2005 accessed at <http://www.nacoonline.org/>
6. Kaur A, Babu PG, Jacob M, Narasimhan C, Ganesh A, Sarawathi NK, Mathai D, Pulimood BM, John TJ. Clinical and laboratory profile of AIDS in India. *J Acquir Defic Syndr* 1992;5:883-9.
7. Kothari K, Goyal S. Clinical profile of AIDS. *J Assoc Physicians India* 2001;49:435-8.
8. Kumaraswamy N, Solomon S. Spectrum of opportunistic infections among AIDS patients in Tamil Nadu, India. *Int J STD AIDS* 1995;6:447-9.
9. Shankarnarayan S, Gagate A, Samani PH, et al. Analytical Study of Clinical Case Profile of Opportunistic Infections in People Living with HIV/AIDS in Mumbai (abstract No. PeB-8215), XIV International AIDS conference, July 7-12, 2002.
10. Zon LI, Groopman JE. Hematological manifestations of the human immunodeficiency virus (HIV). *Semin Hematol* 1988;25:208-18.
11. Kreuzer KA, Rockstroh JK. Pathogenesis and pathophysiology of anemia in HIV infection. *Ann Hematol* 1997;75:179-87.
12. Sullivan PS, Hanson DL, Chu SY, Jones JL et al. Epidemiology of anemia in human immunodeficiency virus (HIV)-infected persons: results from the multistate Adult and Adolescent Spectrum of HIV Disease Surveillance Project. *Blood* 1998;91:Nov.1 (January 1). Centers for Disease Control and Prevention.
13. Saah AJ, Hoover DR, He Y, Kingsley LA, Phair JP. Factors influencing survival after AIDS: report from the Multicenter AIDS Cohort Study (MACS). *J Acquir Immune Defic Syndr* 1994;7:287-95.
14. Kaslow RA, Phair JP, Friedman HB, Lyter D, Solomon RE, Dudley J, Polk BF, Blackwelder W. Infection with human immunodeficiency virus: clinical manifestations and their relationship to immune deficiency. A report from the Multicenter AIDS Cohort Study. *Ann Intern Med* 1987;107:474-80.
15. Hillman RS. Anemia. In: Fauci AS, Martin JB, Braunwald E, et al., editors. *Harrison's Principles of Internal Medicine*. 14th ed. New York: McGraw-Hill, 1998: 334-9.
16. Bain BJ. Pathogenesis and pathophysiology of anemia in HIV infection. *Curr Opin Hematol* 1999;6:8993.
17. Fuchs D, Zangerle R, Astuer-Dworzak E, Weiss G, Fritsch P, Tilz GP, Dierich MP, Wachter H. Association between immune activation, changes of iron metabolism and anemia in patients with HIV infection. *Eur J Hematol* 1993;50:90-4.
18. Claster C. Biology of anemia, differential diagnosis, and treatment options in human immunodeficiency virus infection. *J Infectious Dis* 2002;85:S105-9.
19. Volberding PA, Lagakos SW, Koch MA, Pettinelli C, Myers MW, Booth DK, Balfour HH Jr, Reichman RC, Bartlett JA, Hirsch MS, et al. Zidovudine in asymptomatic human immunodeficiency virus infection. A controlled trial in persons with fewer than 500 CD4-positive cells per cubic millimeter. The AIDS Clinical Trial Group of the National Institute of Allergy and Infectious Diseases. *N Engl J Med* 1990;322:941-9.
20. Levine AM, Scadden DT, Zaia JA, Krishnan A. Hematological Aspects of HIV/AIDS. *Hematology Am Soc Hematol Educ Program* 2001:463-78.
21. Kaslow RA, Phair JP, Friedman HB, Lyter D, Solomon RE, Dudley J, Polk BF, Blackwelder W. Infection with human immunodeficiency virus:

- clinical manifestations and their relationship to immune deficiency. A report from the Multicenter AIDS Cohort Study. *Ann Intern Med* 1987;107:474-80.
22. Henry DH. Experience with epoetin alfa and acquired immunodeficiency syndrome anemia. *Semin Oncol* 1998;25:648.
 23. Jacobson MA, Liu RC, Davies D, Cohen PT. Human immunodeficiency virus disease-related neutropenia and the risk of hospitalization for bacterial infection. *Arch Intern Med* 1997;157:1825-31.
 24. Sullivan PS, Hanson DL, Chu SY, Jones JL, Ciesielski CA. Surveillance for thrombocytopenia in persons infected with HIV: results from the multistate Adult and Adolescent Spectrum of Disease Project. *J Acquir Immune Defic Syndr Hum Retrovirol.* 1997;14:374-9.
 25. Pechere M, Samii K, Hirschel B. HIV related thrombocytopenia. *N Engl J Med* 1993;328:1785-6.
 26. Sullivan PS, Dworkin MS, Jones JL, Hooper CW. Epidemiology of thrombosis in HIV-infected individuals. *AIDS* 2000;14:321-4.