

Isolated Bone Marrow Manifestation of HIV-Associated Hodgkin Lymphoma

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Human immunodeficiency virus-associated Hodgkin lymphoma frequently involves the bone marrow and is usually recognized at staging after Hodgkin lymphoma diagnosis on a lymph node or other tissue biopsies, but occasionally the marrow involvement is the only apparent manifestation of disease. In the latter setting, diagnosis can be problematic. From a total of 42 patients with newly diagnosed human immunodeficiency virus-associated Hodgkin lymphoma, 22 subjects had positive marrow involvement at diagnosis; 16 of them had additional substantial histological and/or clinical extramedullary Hodgkin lymphoma. In the remaining 6 patients the bone marrow was the only site of disease at diagnosis. In all six cases, bone marrow biopsy revealed obvious lymphomatous involvement. Reed-Sternberg cells were identified both morphologically and immunophenotypically in all cases. Spared marrow tissue consistently showed fibrosis. All patients were males with a median age of 35 years (range, 31–58 years). All presented with fever, blood cytopenias, and severe CD4+ lymphocyte depletion (median, 70 cells/mm³). After diagnosis, all staging procedures were negative, and all patients were treated with chemotherapy. Median survival was 4 months (range, 2–118 mo). Longer survival was achieved in the patients who completed chemotherapy regimens; three subjects, however, died shortly before the full completion of chemother-

apy, two of them from Hodgkin lymphoma. Isolated bone marrow HIV-associated Hodgkin lymphoma may be an underestimated condition in HIV-infected patients; in those individuals with unexplained fever and blood cytopenias, bone marrow biopsy should be performed with the aim of assessing for Hodgkin lymphoma, even in the absence of nodal and visceral lymphomatous involvement. A rapid diagnosis of isolated bone marrow HIV-associated Hodgkin lymphoma could expedite therapy.

KEY WORDS: AIDS, Biopsy, Bone marrow, HIV, Hodgkin lymphoma.

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The occurrence of Hodgkin lymphoma (HL; 1) in human immunodeficiency virus (HIV)-infected individuals is a well-known phenomenon (2), although its inclusion in acquired immunodeficiency syndrome (AIDS)-defining illnesses is still a matter of debate (3, 4). In contrast to the case of HL unrelated to HIV infection, HL in HIV patients most often shows an altered natural history (5) and is characterized by disseminated disease, with bone marrow involvement being a relatively common feature (6, 7). The bone marrow may be the primary manifestation of disease, although a subsequent nodal biopsy usually shows tumor localization, even in the absence of obvious lymphadenopathy or organomegaly (8). These aspects may hamper easy recognition of HIV-associated HL, especially in cases lacking typical clinical findings or significant lymphadenopathy.

We report 6 cases of HIV-associated HL presenting with fever and cytopenia and with isolated bone marrow involvement at diagnosis. Bone marrow persisted in being the unique site of disease in all patients but two, in one of whom extramedullary microscopic foci of lymphoma were confirmed at

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autopsy. The main pathologic and clinical characteristics of HIV-associated HL isolated to bone marrow, as well as the implications for clinical management are discussed.

MATERIALS AND METHODS

Selection Criteria

From a total of 42 HIV-positive patients with newly diagnosed HL at the “S.Luigi” Center for Infectious Diseases of San Raffaele Scientific Institute and at the Department of Hematology of Spedali Civili of Brescia, 22 patients (52% of entire series) were identified with bone marrow involvement on bone marrow biopsy at diagnosis; the latter was carried out in the majority of cases because of fever of unknown origin or peripheral blood cytopenia. Nine patients were excluded because marrow disease was discovered during usual staging procedures, after histologically proven lymph node involvement. An additional six patients were excluded because the bone marrow biopsy was positive for Hodgkin lymphoma in the context of substantial clinical and/or radiographic evidence of nodal, liver, or spleen involvement. One additional patient was also excluded because a suspicious lymphadenopathy was found by positron emission tomography (PET), although clinically inapparent and not evidenced by CT scans. A single patient (Case 2), despite the lack of clinically evident lymphadenopathy, underwent a lymph node biopsy but failed to show nodal HL.

The highly selective criteria adopted allowed the identification of six patients showing bone marrow HL involvement as the only site of disease at diagnosis.

Pathologic Material

Bone marrow biopsies were fixed in Bouin’s solution (4 cases), B5 (1 case), and neutral buffered formalin (1 case); all were decalcified using an EDTA-based solution, paraffin embedded, and sectioned at 4 μ m. Sections were stained with hematoxylin and eosin, Giemsa, periodic acid–Schiff, Ziehl-Neelsen, and a reticulin stain. A careful search for opportunistic agents was performed on multiple sections from each case. The minimum requirement for HL diagnosis was the identification of classical Reed–Sternberg cells or their variants embedded in the adequate cellular background (9).

Immunohistochemistry and *In Situ* Hybridization

Immunohistochemical stains were carried out using a DAKO automatic immunostaining device. CD30 (1:300), CD15 (1:10), ALK-1 (1:50), CD45RB (1:100), CD20 (1:400), and monoclonal CD3 (1:10) antibodies, were obtained from DAKO (Denmark); with the exception of CD45, all the monoclonal antibodies underwent microwaving antigen exposure using EDTA buffer at pH 8. Oct-2 polyclonal antibody was purchased from Santa Cruz and applied (1:200) after microwave antigen retrieval in EDTA buffer. Epstein-Barr virus–encoded small RNA (EBER) oligonucleotides (DAKO) were used to test Epstein-Barr virus using *in situ* hybridization, according to manufacturer’s guidelines.

RESULTS

Clinical Findings

The main clinical data of the six patients are summarized in Table 1. All patients were males, with a median age of 35 years (range, 31–58 years).

TABLE 1. Clinical Characteristics

Case No.	Age (y)/Sex	Previous Drug Abuse	Previous OI	Previous HAART	Nadir CD4	CD4 at Diagnosis	Symptoms at Presentation	Symptoms to Diagnosis (mo)	Chemotherapy (cycles)	Survival from Diagnosis (mo)
1	58/M	No	No	No	20	20	Fever, cytopenia	4	ABVD (1)	2
2	36/M	Yes	No	No	31	31	Fever, cytopenia	3	ABVD (1)	4
3	31/M	Yes	No	Yes	159	549	Fever, cytopenia	5	ABVD (6)	18 (+)
4	49/M	No	PCP	No	54	54	Fever, cytopenia	NA	ABVD (6) + G-csf	114 (+)
5	33/M	Yes	EC	No	26	104	Fever, cytopenia, asthenia	1, 5	EBV(4)+ G-csf+AZT	4
6 ^a	34/M	No	No	Yes	64	86	Fever, cytopenia	1, 5	ABVD (2)	3 (+)

OI, opportunistic infections; HAART, highly antiretroviral therapy; ABVD, adriamycin, bleomycin, vinblastin, dacarbazine; EBV, epiadriamycin, bleomycin, vinblastin; AZT, zidovudine; G-csf, granulocyte colony-stimulating factor; PCP, *pneumocystis carinii* pneumonia; EC, esophageal candidiasis; NA, not available; +, alive.

^aThis patient is currently under treatment.

Previous drug abuse was registered in three of the six cases. Before diagnosis, AIDS-defining fulfilling criteria were present in two cases (*Pneumocystis carinii* pneumonia and esophageal candidiasis, respectively). Of note, compared with the whole series of HIV-associated HL cases referred to our institutions ($n = 35$) for which CD4 cell count was valuable at diagnosis, these six subjects had lower median CD4+ cell counts (70 cells/mm³; range, 20–549 versus 210 cells/mm³; range, 10–915). Fever and peripheral blood cytopenias were a constant finding. Total-body CT scans failed to demonstrate any superficial or deep lymphadenopathy as well as liver or spleen involvement. The interval between the onset of symptoms and diagnosis ranged from 1.5 to 5 months (mean, 3 mo) in the five valuable cases. After the diagnosis was established, five patients were treated with Adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD), and one, with epiadriamycin, bleomycin, and vinblastine (EBV) chemotherapy regimens with variable schedules (see Table 1); in addition, two patients received prophylactic granulocyte-colony stimulating factors (G-CSF). During the course of the disease, no evidence of HL outside the bone marrow was noted in Patients 3 and 4, who achieved complete remission. Patient 6 is currently under treatment and does not show extramedullary lymphoma 3 months from diagnosis and after the completion of two chemotherapy cycles.

Three subjects died during treatment, after one, one and four cycles, respectively; the median survival was 4 months (range, 2–118 mo). Two of these patients (Patients 2 and 5) died of disseminated HL, and the other (Patient 1) for disseminated cytomegalovirus infection. Patients 1 and 2 underwent autopsy (see below).

Pathologic Findings

All cases satisfied the minimum requirements for the morphologic diagnosis of HL. The degree of

involvement, as well as the salient morphologic characteristics of uninvolved marrow is summarized in Table 2. The marrow involvement was focal in four cases and extensive in two cases (Fig. 1A–B). The number of Reed-Sternberg cells varied from case to case but was never prominent (Fig. 1C). Within the background, the relative amount of fibroblasts was remarkable, whereas eosinophils were usually rare. In all cases, immunostains demonstrated the classical phenotype on Reed-Sternberg cells, namely reactivity for CD15 and CD30 (Fig. 1D) and lack of CD45RB, ALK-1, CD20, and CD3 (data not shown). In addition, as recently shown in classical HL in not-immunocompromised patients, Reed-Sternberg cells did not react for Oct2 antibody (10), whereas some of the surrounding lymphocytes were positive (Fig. 1D). Epstein-Barr virus was demonstrated by *in situ* hybridization through EBER in all three cases tested.

In all cases, residual bone marrow showed HIV-related myelopathy, mainly affecting erythroid and megakaryocytic lineages and, in most instances, pronounced fibrosis (see Table 2). In addition, appreciable sinusoidal dilatation was observed in three cases and was associated with focal marrow necrosis in one; in Case 3, extensive vascular thrombosis were observed in areas adjacent to lymphoma. In each case, the absence of opportunistic agents was confirmed through stains for microorganisms.

Autopsy was performed in Patients 1 and 2 and showed disseminated HL, involving bone marrow, lymph nodes, spleen and liver, only in Patient 2.

DISCUSSION

Hodgkin lymphoma represents the most common type of non-AIDS defining tumors that occur in HIV populations (11). When compared with its counterpart arising in immunocompetent individuals, HIV-associated HL is characterized by fre-

TABLE 2. Morphologic Characteristics

Case No.	1	2	3	4	5	6
Degree of involvement (%)	40	90	30	70	80	30
Pattern of involvement	Nodular	Diffuse	Nodular	Nodular	Diffuse	Nodular
Amount of Reed-Sternberg cells	Rare	Discrete	Rare	Discrete	Discrete	Rare
Prevalent cells in the background	Fibroblasts	Fibroblasts	Small lymphocytes	Fibroblasts and small lymphocytes	Histiocytes	Fibroblasts; small lymphocytes
Main features in residual marrow ^a	Polyclonal plasmacytosis sinusoidal dilatation	Focal necrosis; sinusoidal dilatation	Prominent necrosis; vascular thrombosis	Polyclonal plasmacytosis sinusoidal dilatation		
Amount of fibrosis ^b	3+	3+	4+	2+	2+	1+

^a In all cases, residual bone marrow showed HIV-related myelopathy.

^b The amount of fibrosis was estimated in areas not involved by HIV-HL.

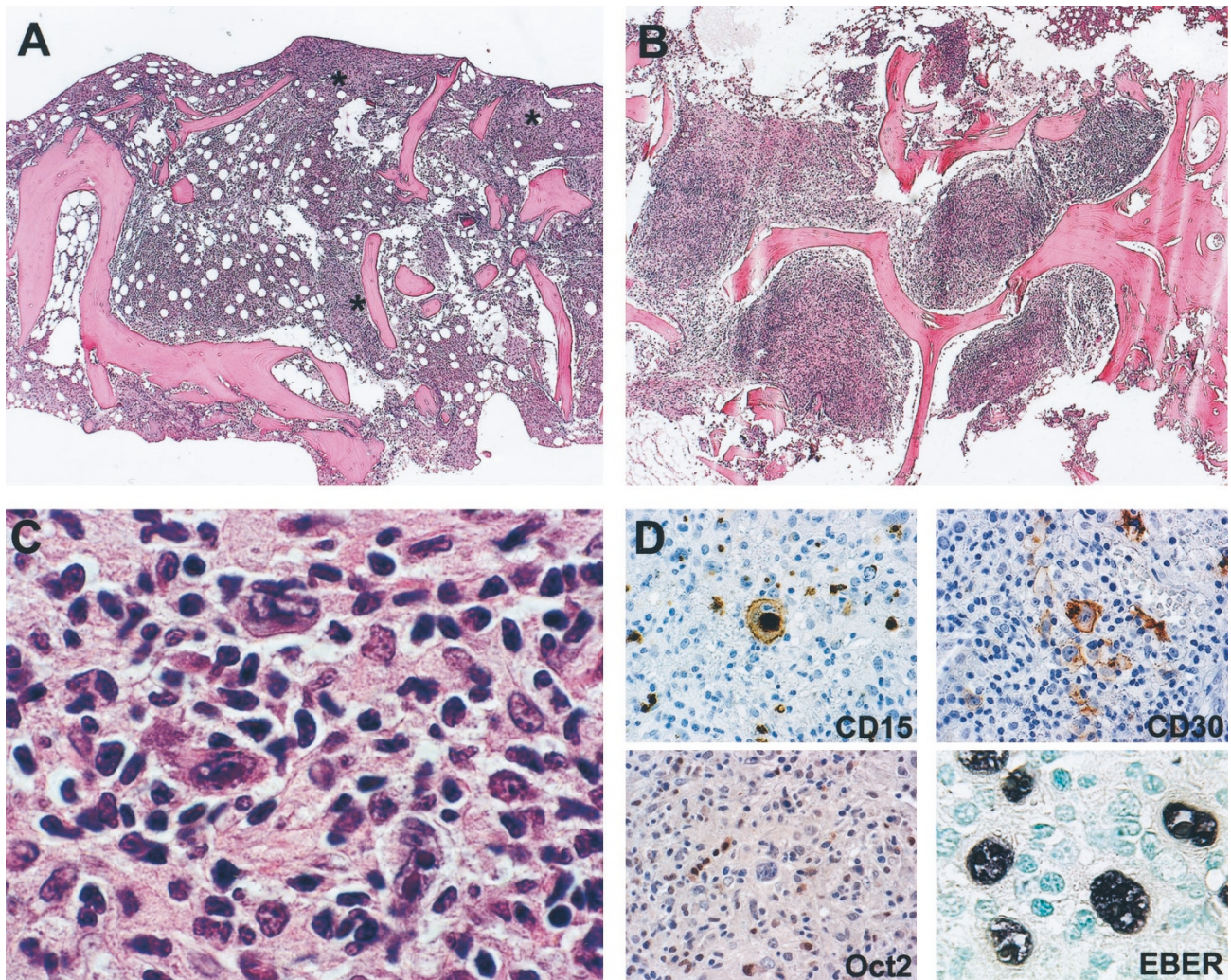


FIGURE 1. Different degrees of bone marrow involvement by HL in HIV are shown in **A** (focal deposits are indicated by asterisks) and **B** (diffuse involvement). Reed-Sternberg cells are identified in their proper background (**C**) and display a classic phenotype (**D**), with expression of CD15, CD30, lack of Oct2, and positivity for EBER.

quent occurrence at advanced stages, poor response to conventional therapy, and ominous outcome (11). In this study, we report the clinical, laboratory, and histopathological findings of six HIV-infected patients characterized by HL with exclusive bone marrow localization. Four of the six subjects failed to show extramarrow lymphomatous involvement during the course of the disease; of the other two, one died 4 months after diagnosis and showed disseminated HL at postmortem examination, whereas the other developed clinically disseminated disease 2 months after diagnosis. We defined this group of patients as having “isolated bone marrow HIV-associated Hodgkin lymphoma” (IBM-HIV-HL). The above-mentioned clinical courses suggest that additional investigations are needed to ascertain whether IBM-HIV-HL originates directly within the bone marrow or whether Reed-Sternberg cells in these cases are characterized by a peculiar tropism for bone marrow.

Our series encompasses a large group of HIV-associated HL. It appears that IBM-HIV-HL is far from being anecdotal, accounting indeed for 14% of HIV-HL patients who were diagnosed between 1985 and 2000 in two reference centers for HIV-related diseases.

All patients with IBM-HIV-HL shared several clinical and pathological features. All were males who presented with persistent high fever (usually $>38^{\circ}\text{C}$) and peripheral blood cytopenia with marked decrease of circulating CD4⁺ lymphocytes; 50% of patients died, and the median survival was short. Furthermore, association with Epstein-Barr virus infection in Reed-Sternberg cells or their variants was demonstrated in all three patients tested.

Both pathologically and clinically relevant considerations can be drawn from the features shown by patients with IBM-HIV-HL. The median age of our patients is more advanced than the one reported in HIV-associated HL cases (35 years versus

29 to 31 years, respectively; 11, 12). Moreover, peripheral blood cytopenia is found in all six patients, whereas this feature seems to occur less frequently in HIV-related HL patients with bone marrow involvement (7). Furthermore, CD4 count evaluation at diagnosis showed a median value of 70 cells/mm³, which is lower than the median values found in HIV patients with HL who were evaluated at our institutions (210 cells/mm³) or in reported Italian (231 cells/mm³), French (306 ± 168 cells/mm³), and United States series (160 cells/mm³; 11, 13, 14).

Finally, median survival of our IBM-HIV-HL patients was very short (4 mo), compared with usual HIV-HL Italian population (15 mo) or when the analysis was restricted to Italian HIV-HL patients with CD4 count of <200 cells/mm³ (11 months; 12). Two patients, however, (Cases 3 and 4) are alive at 18 and 114 months from diagnosis, suggesting that completion of a chemotherapy regimen can result in complete remission and prolonged survival.

The occurrence of isolated bone marrow disease is a rare phenomenon known to occur mainly in non-Hodgkin's lymphomas and in immunocompetent individuals (15). Bone marrow involvement by HL occurs in 5–15% of patients without immunodeficiency (16). Data from the literature indicate that HIV-related HL can involve bone marrow secondarily during the natural course of the disease or as the first manifestation in the context of suspicious or overt lymphadenopathy.

Karcher (8) reported three HIV-seropositive patients with apparently isolated bone marrow involvement by HL at presentation; in all these cases, however, the marrow biopsy showed "atypical lymphohistiocytic lesions," and the definite diagnosis was obtained only on lymph nodes.

In addition, single cases from small series showing features potentially similar to IBM-HIV-HL as defined in the present study have been reported (17, 18), although selection criteria were not clearly specified. All cases hereby reported were diagnosed on the basis of detection of classical Reed-Sternberg cells with typical immunophenotype in the bone marrow; furthermore, strict selection criteria for isolated bone marrow involvement were adopted, and the single patient (Case 2) who, despite the lack of clinically evident lymphadenopathy, underwent a lymph node biopsy failed to show nodal HL.

The nodular pattern of involvement in some patients in our series raised differential diagnoses either with reactive polymorphous lymphohistiocytic lesions, a common feature in patients with immune disorders including HIV infection (18), and with other lymphoid neoplasms: the identification of classic Reed-Sternberg cells with the appropriate phenotype appeared critical in reaching a correct diagnosis and in excluding peripheral T-cell lymphomas,

T-cell-rich large B-cell lymphomas, and lymphocytic-predominance HL. The lack of immunoreactivity of Reed-Sternberg cells for the immunoglobulin gene transcription regulator molecule Oct2 is similar to that observed in the majority of classical HIV-unrelated HL (10). It should be noted that Oct2 negativity was confirmed in other cases of HIV-associated HL from our series, whereas HIV-associated large B-cell lymphomas were found to strongly express the antigen (data not shown).

Another interesting feature characterizing our IMB-HIV-HL patients was the stromal changes encountered on bone marrow biopsy, particularly fibrosis, necrosis, and sinusoidal dilatation. Pronounced (3+ or 4+) fibrosis of uninvolved areas was an almost constant finding in our patients (see Table 2). It is possible that the increase in reticulin fibers may contribute to cytopenias in addition to the other well-known causes of cytopenias in HIV-positive subjects. Identification of significant fibrosis in bone marrow biopsies from HIV patients should prompt for careful search of HL deposits.

In conclusion, any HIV-positive patient with unexplained fever and cytopenia should undergo bone marrow biopsy as part of the clinical work-up. The diagnosis of HL on bone marrow biopsy should stimulate a careful search for extramedullary localizations. However, the occurrence of isolated bone marrow disease must be taken into consideration, thus allowing both pathologists and clinicians to reach a rapid definite diagnosis and expedite therapeutic decisions.

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