Clinical Investigation

Peripheral Blood and Bone Marrow Abnormalities in the Acquired Immunodeficiency Syndrome

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In reviewing the peripheral hematologic manifestations, bone marrow changes and clinical course in 41 consecutive patients with acquired immunodeficiency syndrome (AIDS), frequent findings included anemia (95%), leukopenia (76%), bone marrow hypercellularity (73%) and pancytopenia (41%). These hematologic abnormalities were not clearly associated with specific clinical manifestations of AIDS, but support the conclusion that the hematopoietic system is a target organ in AIDS. The mechanisms of these abnormalities still need to be evaluated. Clinicians should be aware of these commonly encountered changes.

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Hematologic abnormalities are often encountered in patients with the acquired immunodeficiency syndrome (AIDS). The abnormalities vary but peripheral cytopenias are most commonly seen.[1-7] Myelodysplastic features have also been described[8] and correlations drawn between marrow cellularity and peripheral cytopenia.[9] We report a high incidence of peripheral cytopenia often with associated striking bone marrow hyperplasia. The bone marrow and peripheral blood findings of 41 patients with AIDS are described. Clinical characteristics of the patient population are reviewed and presented with the hematologic changes.

Patients and Methods

The medical records of 64 consecutively confirmed cases of AIDS at the University of Colorado Health Sciences Center and affiliated hospitals were reviewed. All the patients met the Centers for Disease Control criteria for the diagnosis of AIDS.[10] Most of these patients had been seen by one of us in a clinical setting. The diagnosis of AIDS was made in this cohort during a 39-month period from May 1982 through August 1985, with most of the cases being seen during the latter part of this time span.

Of the 64 patients, 41 had bone marrow examinations, which form the basis of this review. Clinical characteristics and course were determined by chart review. Bone marrow aspirations with biopsies and peripheral complete blood counts were done concurrently. After informed consent, bone marrow cores were taken from the posterior iliac crest using a Jamshidi needle. Specimens were first placed in B5 fixative for two hours and then in 10% buffered formalin until processed. The cores were decalcified in 1.35N hydrochloric acid for one hour and embedded in paraffin. Thin sections were stained with commercially available Wright's, Giemsa's, Silver methenamine, hematoxylin and eosin, Ziehl-Neelsen and Prussian blue. Reticular stains were not routinely done. Bone marrow cellularity was determined by two observers.

At the time of the bone marrow examination, all the patients had or were thought to have the diagnosis of AIDS. Most bone marrow examinations were done to investigate peripheral hematologic abnormalities, but five were done at autopsy. Other indications prompting bone marrow study were fever of unknown origin, culture for disseminated infectious diseases or staging for lymphoma. Circulating T-lymphocyte subsets were quantitated using commercially available monoclonal antibody (OK T4 for T-helper/inducer cells and OK T8 for T-suppressor/cytotoxic cells).

Results

All patients in the study group were men ranging in age from 23 to 62 years, with a median age of 39 years. All were admittedly homosexual except three. One of the three had hemophilia and had received factor VIII concentrate for longer than 10 years, the second patient had intraoperative blood transfusions three years previously and the third patient was a heterosexual man who had contacts with prostitutes. This last patient presented with a rapidly fatal illness and at autopsy was found to have disseminated *Pneumocystis carinii* pneumonia and cytomegalovirus infection. Of the 41 patients, 12 admitted to recreational intravenous drug use. *P carinii* pneumonia was documented in 27 patients, 14 of whom were treated with trimethoprim and sulfamethoxazole (TMP/SMX). In all, 25 patients had other opportunistic infections, either alone (6 patients) or in combination with *P carinii* pneumonia (19 patients).

All of the 41 patients have died of their disease. The median survival after the diagnosis of AIDS was 7.6 months. The causes of death were often combinations of advanced Kaposi's sarcoma, opportunistic infections and extreme inanition associated with advanced infection with the human immunodeficiency virus.

T-cell subset evaluations were completed in 35 patients. The OK T4-OK T8 ratios were less than 1.0 in 33 (94%) and less than 0.4 in 22 (63%) patients. This aberration in the ratio

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ABBREVIATIONS USED IN TEXT

AIDS = acquired immunodeficiency syndrome
TMP/SMX = trimethoprim/sulfamethoxazole

TABLE 1.—*Bone Marrow Cellularity Versus Peripheral Abnormalities**

Cellularity, %	Patients, number	Leukopenia and Anemia	Thrombocytopenia and Anemia	Pancytopenia
<25	2	2	2	2
>25 <50	13	7	7	5
≥50	26	20	15	10

*Leukopenia is defined as a leukocyte count of <4,000 per μl; thrombocytopenia is defined as a platelet count of <150,000 per μl, and anemia is defined as a hematocrit of <40%.

of helper cells to suppressor cells was due to a pronounced reduction in the number of helper cells and a significant increase in the number of suppressor cells, which is consistent with the diagnosis of AIDS.

Peripheral cytopenias were not directly predictive of the subsequent cellularities found on marrow biopsy. Cytopenic patients were more likely to have hypercellular marrows (Table 1). Of the 41 patients, 26 (63%) had marrow cellularities of 50% or more. Of these 26 patients, 10 had pancytopenia. Thirteen patients (32%) had normal cellularity defined as being greater than 25% and less than 50%; of these, 5 had pancytopenia. Only two patients had hypocellular marrows and both had pancytopenia. One of these patients had hemophilia and the other was a patient with advanced Kaposi's sarcoma treated with vinblastine sulfate.

The clinical manifestations of AIDS and its correlations with the peripheral hematologic abnormalities are presented in Table 2. Of the 27 patients with *P carinii* pneumonia, 14 had been treated with TMP/SMX before marrow biopsy. Most of these patients had significant peripheral cytopenias and yet had marrow hypercellularity. This was also seen in the patients with *P carinii* infection who had not received TMP/SMX at the time of biopsy. In fact, peripheral cytopenia associated with marrow hypercellularity was commonly observed also in patients with opportunistic infections other than *P carinii* pneumonia, Kaposi's sarcoma alone and lymphoma. This striking tendency toward peripheral cytopenia, often with marrow hypercellularity, occurred independent of the clinical manifestations of AIDS or exposure to TMP/SMX.

The patients' therapy included the use of a variety of other medications within one month of bone marrow examination. Fourteen patients had received various antibiotic combinations for treatment of disseminated *Mycobacterium avium-intracellulare* infection, *Mycobacterium tuberculosis* or other nontuberculous infections. Six patients had received ketoconazole for fungal disease. Five patients had received cytotoxic chemotherapy for Kaposi's sarcoma. Two patients were given vinblastine, two patients were treated with a combination of vincristine sulfate and methotrexate and one was given doxorubicin (Adriamycin) hydrochloride and bleomycin sulfate. One of the patients who had received vinblastine died two weeks after receiving the chemotherapy. This patient was one of the two patients with a hypocellular bone marrow and accompanying pancytopenia. In this case, the hematologic changes may have been associated with therapy. The other patients who received chemotherapy did not show any clear bone marrow effect to account for peripheral hematologic abnormalities. Leukopenia occurred in 79% of the patients treated with trimethoprim and sulfamethoxazole, while 67% of the remaining 27 patients had leukopenia. If the five patients who received cytotoxic chemotherapy are excluded from consideration, the incidence of leukopenia becomes 92% in TMP/SMX-treated patients while 75% of the remaining 24 patients had leukopenia. No clear cause and effect between TMP/SMX use can thus be established to explain the leukopenia, although there is a trend suggesting a higher proportion of neutropenic patients among those receiving TMP/SMX.

Splenomegaly was documented clinically in eight patients. There was no correlation between thrombocytopenia and bone marrow megakaryocyte numbers seen in these patients. Lymphoma was identified in two patients. On histologic examination, both lymphomas were of high grade with an abdominal origin in one patient and a central nervous system origin in the other.

Peripheral hematologic abnormalities were extremely common. Anemia was the most common abnormality seen, with hematocrits of less than 40% seen in 39 of the 41 patients (95%). The average hemoglobin was 9.8 grams per dl. Reticulocyte counts, even in those patients with severe anemia, were greatly depressed. Moderately severe anemia with hematocrits less than 30% was noted in 56% of the patients.

Leukopenia, defined as a total leukocyte count of less than 4,000 per μl, was present in 76% of the patients. Only 12% of the patients had a leukocyte count of greater than 7,000 per μl. The peripheral leukocyte differentials commonly showed a leftward shift, with a moderate number of immature neutrophils present. A relative lymphopenia along with increased monocyte numbers was typically seen on the peripheral smears. Table 3 shows the absolute counts obtained in the ten patients with pancytopenia and concomitant bone marrow hy-

TABLE 2.—*Clinical Manifestations of AIDS Correlated With Hematologic Abnormalities**

Patient Group	Patients, number	Leukopenia and Anemia	Thrombocytopenia and Anemia	Pancytopenia	Increased Marrow Cellularity
Pneumocystis carinii pneumonia with TMP/SMX treatment	14	11	6	5	10
Pneumocystis carinii pneumonia without TMP/SMX treatment	13	7	10	5	8
Other opportunistic infections	6	5	2	2	5
Kaposi's sarcoma	6	5	6	5	5
Lymphoma	2	1	0	0	2

AIDS=acquired immunodeficiency syndrome, TMP/SMX = trimethoprim/sulfamethoxazole

*Leukopenia is defined as leukocyte count of <4,000 per μl; thrombocytopenia is defined as a platelet count of <150,000 per μl, and anemia is defined as a hematocrit of <40%.

percellularity. These differentials and clinical characteristics were similar to those seen in the entire group.

Thrombocytopenia was also common: 59% of the patients had platelet counts of less than 150,000 per μl. The range of thrombocytopenia seen in these patients was great. Only 12% of the patients had a platelet count greater than 250,000 per μl. None of the patients with normal or hypercellular marrow showed a decreased number of megakaryocytes.

Pancytopenia was present in 17 of the 41 patients studied. The clinical data of the ten pancytopenic patients with bone marrow hypercellularity are presented in Table 3. The clinical manifestations of AIDS, time since diagnosis (not shown in table) and treatment given were compared to the hematologic changes, and no clear correlations were found. The percentages of lymphocytes and plasma cells seen in these representative bone marrows are also shown.

The bone marrow biopsy of patient 1, shown in Figure 1, was representative of the hypercellularity and lymphocytic infiltrations seen. Although lymphocytic and plasmacytic marrow infiltration was prominent, the hypercellularity was accounted for primarily by a striking trilinear hyperplasia. There were no abnormalities in maturation noted in either erythroid or myeloid lines. In general, there was an elevated myeloid to erythroid ratio. Some marrow specimens also had areas of focal necrosis and serous fat atrophy.

Discussion

The incidence of AIDS is increasing rapidly worldwide. In the United States 13,000 cases were reported in 1986.[11] AIDS is a systemic disease with multiple complications. This report further confirms the hematopoietic system as another target organ in AIDS. The clinical and laboratory characteristics of our patients were typical of patients with AIDS. The most striking observation in our patients was the degree of marrow hyperplasia encountered, often with concomitant peripheral pancytopenia (Tables 1 and 2). Of 64 patients, 39 had anemia. The degree of anemia did not correlate with any clinical manifestation of AIDS. The reticulocyte counts were depressed, with only one patient having a mild elevation of the reticulocyte count in the face of anemia. The rest evidenced no peripheral responsiveness to their anemia while concurrent bone marrow studies commonly showed erythroid hyperplasia at all stages of maturation and increased iron stores. These changes suggest ineffective erythropoiesis, but

TABLE 3.—*Patients With Pancytopenia and Bone Marrow Hypercellularity*

Patient	Age, yr	Clinical Status	Marrow Cellularity, %	Myeloid: Erythroid Ratio	Lymphocytes and Plasma Cells, %	Total Neutrophils, /μl	Total Lymphocytes, /μl	Hct, %	Platelets*	T-cell Helper-Suppressor Ratio	Iron Concentration	Medication Within 30 d
1 . . .	32	KS	90	1:2	<10	1,925	1,400	31.2	110	0.67	↑	Triazolam
2 . . .	36	KS	90	3:1	18	2,457	1,092	32.4	141	0.29	N	Phenytoin
3 . . .	36	PCP, Crypto	85	3:1	<10	790	190	18.0	64	0.60	N	TMP/SMX
4 . . .	28	KS	80	1:2	15	720	800	27.5	94	0.08	↑	Vitamins
5 . . .	44	KS, MAI and CMV infection	70	7:1	<10	1,634	38	19.7	42	. . .	↑	TMP/SMX
6 . . .	36	KS, PCP	65	10:1	10	1,360	255	31.8	120	0.39	N	TMP/SMX
7 . . .	53	PCP	60	3:1	<10	1,584	90	33.0	140	0.08	↑	Cefazolin
8 . . .	45	PCP	50	2:1	<10	1,586	832	30.7	125	0.04	↑	TMP/SMX
9 . . .	31	KS, PCP, MAI infection	50	4:1	20	2,100	150	36.1	86	0.20	↑	None
10 . . .	28	KS	50	3:1	10	3,120	780	24.9	23	0.75	↑	TMP/SMX

CMV=cytomegalovirus, Crypto=cryptococcosis, Hct=hematocrit, KS=Kaposi's sacroma, MAI=*Mycobacterium avium-intracellulare*, N=normal, PCP=*Pneumocystis carinii* pneumonia, TMP/SMX=trimethoprim/sulfamethoxazole, ↑=increased

*$\times 10^3$ per μl.

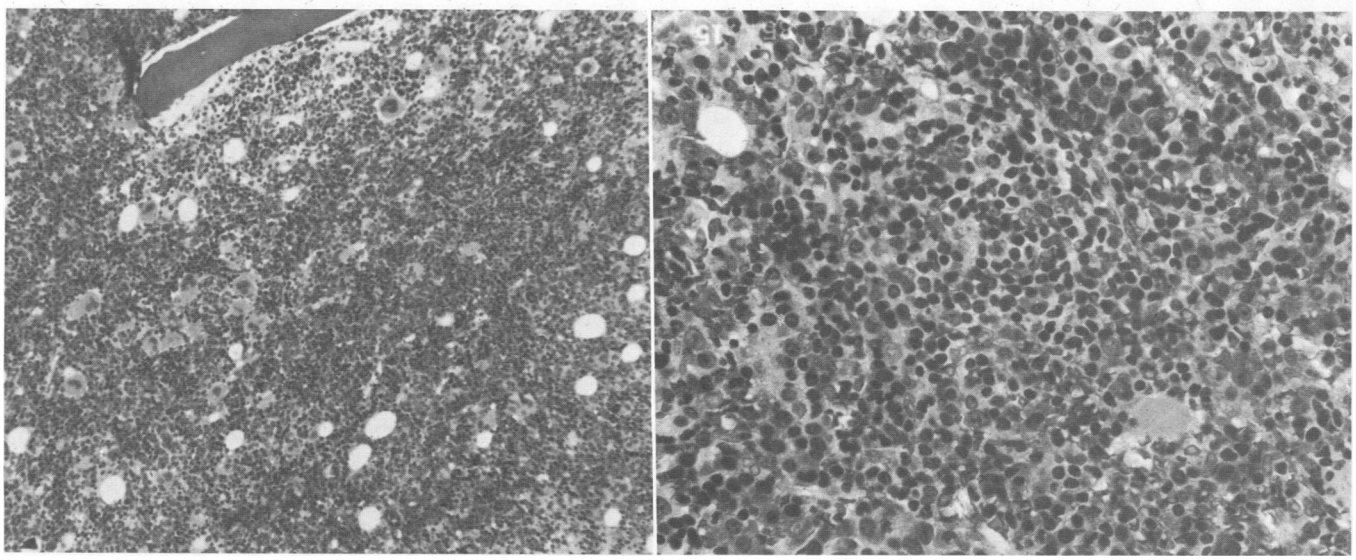

Figure 1.—Bone marrow biopsy from patient 1. **Left,** Light microscopy shows trilinear hyperplasia and hypercellularity (hematoxylin and eosin, original magnification × 100). **Right,** A higher magnification shows an increased number of lymphocytes and plasma cells (hematoxylin and eosin, original magnification × 400).

no mechanism of action was apparent, although the findings were most consistent with anemia of chronic disease. Infection, Kaposi's sarcoma or constitutional abnormalities associated with this ill patient population could be contributing factors. Another possible explanation for the anemia could be the potentially marrow-toxic drugs administered to some of the patients. Bone marrow hypercellularity and peripheral cytopenia occurred to an equal degree regardless of medication received in treatment. Marrow erythrophagocytosis, which has been reported by other observers,[1] was not seen in our patients. Alternatively, one could speculate about abnormal release mechanisms, changes in cell-cell interactions or autoimmune inhibition to explain the ineffective hematopoiesis.

Peripheral leukopenia, in addition to the usual lymphopenia, was also seen associated with bone marrow granulocytic hyperplasia at all stages of maturation. This finding suggests either increased turnover with accelerated peripheral utilization or ineffective granulopoiesis. There were no maturational abnormalities seen. Specifically, there were no megaloblastic features, as might have been encountered, since the use of trimethoprim/sulfamethoxazole has been associated with these findings. In addition, while the use of TMP/SMX has been associated with a striking tendency to neutropenia in AIDS patients,[12] patients in our study consistently had neutropenia, even when not receiving a sulfa-based compound.

Thrombocytopenia was also commonly encountered, as has been described in AIDS patients.[13] All bone marrow specimens reviewed showed normal or increased megakaryocyte numbers suggesting either ineffective thrombopoiesis or increased peripheral destruction as opposed to a hypoproliferative process. There are data reported by others consistent with an immune-mediated mechanism involving the deposition of immune complexes on platelet surfaces.[14] Assays were done on two of our thrombocytopenic patients (platelet count $< 50{,}000$ per μl) and showed an immunoglobulin antiplatelet antibody present, which is a nonspecific finding. Splenomegaly was present in eight patients and, in these cases, platelet sequestration may have contributed to the thrombocytopenia.

Lymphoid aggregates or an increased number of lymphocytes distributed throughout the marrow population of cells was seen in most of our patients. An increased number of plasma cells was also noted. This is a common finding in the marrows of AIDS patients as described by others.[1-9]

In summary, the often striking marrow hyperplasia with concomitant peripheral cytopenia identifies the bone marrow as another frequently targeted organ system in the acquired immunodeficiency syndrome. The disparity between peripheral cytopenia and marrow hyperplasia suggests mechanisms of either ineffective hematopoiesis, abnormal release or increased peripheral destruction. The clinical situation in patients with AIDS is often complex, and the cause of the hematologic abnormalities encountered may also be associated with infections, malignancy and drug treatments. It is quite possible, however, that these abnormalities are primary to the immune disturbance that characterizes this syndrome as suggested in part by the presence of increased numbers of marrow lymphocytes and plasma cells seen in the bone marrow. These mechanisms need to be further evaluated.

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