

UNREMITTING EARLY STAGE HODGKIN'S DISEASE: REPORT OF 7 CASES AND BONE MARROW TISSUE IMMUNOHISTOCHEMICAL MARKER STUDY

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Abstract- Bone marrow is infrequently implicated in early stages of Hodgkin's disease. We studied the immunohistochemical bone marrow tissue of 7 out of 20 cases with early stage Hodgkin's disease of the mixed cellularity variant, diagnosed by lymph node biopsy at initial presentation, not responding to radiotherapy alone, in order to examine possible marrow attack. A statistically significant prevalence of CD45, CD45RO, and CD4 positive infiltrates, to the advantage of unremitting hosts, was found. The predominance of CD4-positive cells in the bone marrow space might be suggestive of involvement in the process and could explain the abnormal cytokine production leading to reduced T-cell immunity and inefficient antitumor response despite the existence of a vast majority of reactive infiltrating immune cells.

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INTRODUCTION

Bone marrow involvement in Hodgkin's disease (HD) is low at initial presentation (6%) in patients with stages I and II disease and in nodular sclerosis (1, 2 and 4%, respectively) (1). The highest incidence of bone marrow involvement is found with lymphocyte depletion and mixed cellularity HD (2, 3). However, since one of the criteria for systemic spread (stage IV) is involvement of the bone marrow, a bone marrow biopsy is still an integral part of the initial investigation of patients with HD.

It is of interest that CD4+ T lymphocytes with a T helper 2 (Th2)-like immunophenotype are the most abundant cell types in Hodgkin's lymphoma tissues (4). Th2 cells produce IL-5, which primes T cells and activates eosinophils (5). Moreover, interaction of these T cells with neoplastic cells and eosinophils also involves ligands of TNF (tumor necrosis factor) receptors (CD30L and CD40L) (6). T lymphocytes as well as eosinophils transmit via these ligands proliferate and antiapoptotic signals to Hodgkin and Reed-Sternberg (HRS) cells and thereby influence tumor biology (6, 7).

In our series, we studied the immunohistochemical profile of bone marrow infiltrates in early stages (I-II) of classical HD mixed cellularity variant treated with radiotherapy alone, in order to explain partial non-response to treatment, given that the reactive lymphocytes in HD involved marrow are predominantly CD4 positive T-cells.

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MATERIALS AND METHODS

Our study population included 20 patients (age range 32-72 years; 16 males and 4 females) with stage I-II Hodgkin's disease according to the Ann Arbor criteria.

All our selected cases included the classical HD mixed cellularity variety recovered from routine lymph node histological and immunohistochemical examination. The Regional Committees of Ethics approved the study. Written informed consent was obtained from all patients, and the procedures followed were in accordance with the institutional guidelines.

The genotypic profile of our HD cases (affected cervical lymph nodes) is shown in table 1. In brief, the HRS cells of 18 cases harbored highly mutated rearranged *IgH* genes, whereas the other 2 cases displayed HRS cells with clonal TCR rearrangements. Southern blot hybridization using a specific EBV Bam H1W fragment probe, showed the presence of EBV genomes in two of our cases (patient no. 5 and 8).

The possibility that the two Hodgkin lymphomas with rearranged TCR genes were confused with anaplastic large cell lymphomas, which may mimic HD, were excluded by their negative labeling for ALK-1 whose expression is restricted to anaplastic large-cell lymphoma (8, 9). We included in our study antibodies recognizing megakaryocytes CD61 (Y2/51) to be certain that the abnormal cells are truly derived from HD.

All patients were treated with radiotherapy alone. Seven of the patients showed no remission of the disease presenting the characteristic clinical symptoms of the disease (fever, night sweats and weight loss). An iliac crest trephine bone marrow biopsy was performed in all twenty subjects.

Immunohistochemistry

Bone marrow biopsy specimens following a short decalcification time were processed for routine and immunohistochemical examination by a panel of monoclonal antibodies against CD30 (Ber-H2), CD15 (C3D1), EMA, CD45 (LCA), CD20 (L26), CD4, CD8, ALK-1, CD45RO (UCHL1), CD56 (NK cells), CD61 (Y2/51) and CD68 (PG-M1).

Table 1. Genotype of the classical Hodgkin's disease mixed cellularity variety (affected cervical lymph nodes)

Patient no	Age/Sex	Genotype
1	55/M	B
2	71/M	B
3	60/F	T
4	34/M	B
5	36/M	B
6	32/F	T
7	38/M	B
8	42/F	B
9	43/M	B
10	35/M	B
11	53/M	B
12	47/M	B
13	38/F	B
14	49/M	B
15	51/M	B
16	40/M	B
17	56/M	B
18	64/M	B
19	39/M	B
20	72/M	B

Abbreviations: M, male; F, female.

Immunostaining was performed on formalin-fixed, paraffin-embedded sections employing the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique (10). Paraffin-embedded sections were pre-treated by pressure-cooking as previously described (11).

The immunostained sections were examined with a $\times 40$ objective and the distribution of the above antibodies within the cell was recorded. Every stained cell was scored as positive regardless of staining intensity. To count the number of positive cells, a 10×10 square calibrated grid was inserted into the eyepiece of an Olympus B $\times 40$ binocular microscope.

Five-to-ten high power fields were examined for each section and at least 1000 cells were scored, depending on cellularity. The number of positive cells was recorded per high power field.

RESULTS

Histology

The bone marrow appearance was that of a hypercellular one in eight cases, a hypocellular in seven, and a normocellular in the remaining ones. In all twenty patients no replacement of the bone marrow space by fibrous or granulomatous tissue was observed.

Immunohistochemistry

The sections were examined independently by two observers, and positive cellular staining for the relevant antibodies were manifested as yellow cytoplasmic granularity and/or surface membrane expression. There was: 1) No evidence of lymphocyte-rich HD (L & H cells), mononuclear HD, binuclear HD, or nodular sclerosis variant (lacunar cells): CD30-, CD15-, EMA-, 2) Evidence for the presence of megakaryocytes: CD61+, 3) A mild increase in the number of the lymphoid cells constituting up to 20-25% of the nucleated cell population in the bone marrow: CD45+. The T-cell to B-cell ratio had been estimated as 81 to 91. Lymphocytes of small-cell type, preferentially located in the center of marrow space, were arranged either in small clusters of 2-10 lymphocytes resembling minute bunches of grapes, or in 1-2 cell thickness layers distributed in a way that we have called "streamlet like" pattern or "Indian" like. A paratrabecular pattern of localization has not been considered, while randomly reactive lymphoid

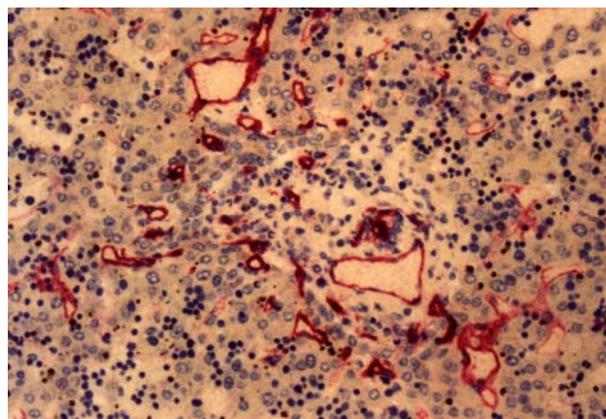


Fig. 1. Immunohistochemical expression of CD4 in the bone marrow space in patients with untreated stage I - II Hodgkin's disease (strong reactivity). Immunostaining with CD4 Mab, APAAP technique, original magnification $\times 400$.

follicles were observed. On immunohistochemical grounds the lymphocytes were identified as of T-cell origin. Such a cellular arrangement has been considered as a piece of evidence against lymphomatous lesion. A predominance of CD4 expression in the bone marrow stroma was found in the seven non-responded patient-cases (Fig. 1) over the thirteen cases with a favorable outcome (Fig. 2). The difference was statistically significant ($P = 0.015$, Chi square test) (Table 2). A contralateral iliac crest trephine was obtained from all seven unfavorable hosts, which showed a polymorphous cellular composition of lymphocytes, plasma cells, histiocytes, neutrophils, and granulocytes. On multiple sections rare abnormal CD30 positive cells were disclosed and BM involvement was established. Chemotherapy was employed.

Table 2. Immunophenotype of the classical Hodgkin's disease mixed cellularity variety (bone marrow biopsy)

Immunohistochemistry	Untreated cases N°7	Treated cases N°13	Statistic analysis
CD45 (+) cells/mm ²	68.1 \pm 20.8	45.7 \pm 15.2	$P = 0.021$
CD45 (+) cells/mm ²	70.4 \pm 32.7	32.5 \pm 17.8	$P = 0.019$
CD4 (+) cells/mm ²	38.20 \pm 6.4	28.90 \pm 7.60	$P = 0.015$
CD61 (+) cells/mm ²	2.65 \pm 0.73	2.35 \pm 0.81	Non significant
CD20 (+) cells/mm ²	9.1 \pm 2.7	8.9 \pm 1.8	Non significant
CD56 (+) cells/mm ²	-	-	-
CD30 (+) cells/mm ²	-	-	-
CD15 (+) cells/mm ²	-	-	-
EMA (+) cells/mm ²	-	-	-
CD68 (+) cells/mm ²	3.12 \pm 0.38	3.1 \pm 0.79	Non significant
CD8 (+) cells/mm ²	5.68 \pm 0.96	5.85 \pm 1.08	Non significant
ALK (+) cells/mm ²	-	-	-

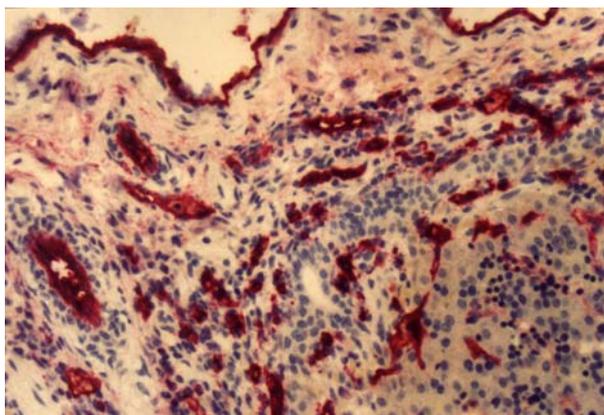


Fig. 2. Immunohistochemical expression of CD4 in the bone marrow space in patients with treated stage I - II Hodgkin's disease (mild reactivity). Immunostaining with CD4 Mab, APAAP technique, original magnification $\times 400$.

DISCUSSION

The diagnostic criteria of HD in bone marrow have essentially not changed since the now historic clinical pathologic studies of Lukes and Bartl (12, 13). The criteria differ somewhat depending on whether a primary diagnosis of HD is being established or whether the patient already has a diagnosis of HD established at another site.

1) Diagnostic: typical Reed-Sternberg cells or their variants in a proper background. Mononuclear variants in a proper background are diagnostic only in patients with a previously established diagnosis.

2) Strongly suggestive, but not diagnostic: abnormal large cells that lack the nuclear features of R-S-cells, but which are present in a cellular and/or fibrotic background that is characteristic of HD.

3) Suggestive: focal or diffuse fibrosis or necrosis only in a previously diagnosed patient. Various combinations of these patterns may be seen in the same biopsy specimen or in different biopsy specimens from the same patient. In addition, amorphous eosinophilic background material may be apparent. Necrosis is occasionally detected prior to treatment (14), but is more common in treated patients. Granulomas are sometimes associated with infiltration, but also occur in the absence of bone marrow infiltration. Reticulin is increased in areas of infiltration and collagen is often present. There is sometimes osteolysis or osteosclerosis; increased bone remodelling is usual (13). Preferentially the diagnosis of HD should be confirmed by

immunohistochemistry (CD3, 20, 15, 45, 30 and EMA). When the first diagnosis of HL is made on a trephine, a biopsy of lymph node should always be performed before treatment, both for confirmation of the diagnosis and for subclassification. One should not attempt to classify HD on the basis of the bone marrow biopsy. The histology of HD in bone marrow and lymph node may differ significantly, and all types of HD may produce the appearance of lymphocyte depletion and fibrosis in the BM. In our settings we found a statistically significant ($P = 0.015$) higher CD4 positive bone marrow infiltrate in unremitting HD over favorable disease, and we imply this increase might indicate bone marrow involvement. On the other hand, our findings could explain that the locally produced chemokines by T-lymphocytes are implicated in the accumulation of the abundant reactive infiltrates (*e.g.* eosinophils) in classical HD and thereby influence tumor biology. Confirmation of this indication needs large groups of patients and longer observation time together with adverse outcome of illness.

Conflict of interests

We have no conflict of interests.

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