Bone Marrow Involvement in Hodgkin's Disease: The Significance of Non-Infiltrative Changes

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Abstract

We have tried to elucidate the significance of so called non- infiltrative changes in order to find their place in the staging procedure particularly in countries where facilities for elaborate clinical staging are not available. Seventy nine out of 88 patients were classified into 3 groups depending upon the histological findings in their bone marrow trephine biopsies. Bone marrow in Group-I (n=20) patients was essentially normal. The established criteria of bone marrow involvement were fulfilled in Group III patients (n=25); while bone marrow in Group-Il patients (n=34) showed non-infiltrative changes. The clinical presentalion, peripheral blood parameters and LDH levels of the 3 groups of patients were compared. There was progressive anaemia, neutrophilic leucocytosis and increase in ESR from Group I to Ill. The change was statistically significant when Group I was compared with Group II or Group III but non-significant when Group II was compared with Group III. It is, therefore, postulated that both Groups II and Ill reflect the bone marrow involvement although the changes observed in Group II do not satisfy the previously established criteria for this purpose (JPMA 47:110,1997).

Introduction

Hodgkin's disease (HD)is anunusual malignant disease because the malignant cells (Reed-Stemberg giant cells and mononuclear Hodgkin's cells) constitute only 1-2% of the total cells in the involved tissue, remaining being the reactive cells[1]. Probably for this reason it is a curable disease in upto 80% of the cases and cure should be the goal of treatment[2,3]. Careful staging and evaluation of prognostic features, however, determines the selection of treatment[2] and thus the disease free survival (DFS) of the patients[4]. Staging laparotomy with splenic examination was considered essential in the past but has now been given up because of its high mortality and morbidity and because of availability of non-invasive imaging techniques for estimation of the tumour mass. Bone marrow examination, inspite of some objections[5], is still camed out as a part of staging work-up to determine the pathological stage of the disease in patients with clinically advanced stage disease. To a large extent it replaces the laparotomy because bone marrow involvement has been found to be invariably associated with splenic invasion[6]. Definitive histopathologic criteria for the diagnosis of the bone marrow involvement in HD have been established7 and are strictly followed. Using these criteria bone marrow involvement in HD has been reported from <2% to as high as 45% of the patients depending upon the clinical stage. The reasons for this variability include the population affected, number of biopsies, methods of processing and even the type of needle used for obtaining the biopsy specimen[6-9]. In addition to the established criteria for diagnosing bone marrow involvement in HD, a variety of non-infiltrative changes have also been reported in the bone marrow[10,11]. Some of these bear strong correlation with the stage of the disease[12] but their significance in the staging of the disease process is yet to be determined. In this prospective study we have tried to determine the significance of such non- infiltrative changes as regards to their place in the staging procedure for HD.

Material and Methods

This study deals with 88 patielits seen over a period of three years. Inclusion criteria were Hodgkin's disease diagnosed on lymph node biopsy, detailed medical examination and staging and an adequate trephine biopsy was obtained and evaluated at AFIP.

Two ml blood sample was collected in EDTA from each patient. Peripheraiblood counts were performed onTechnicon H-ITM automated haematology analyzer which was calibrated daily with commercial standards obtained from TechniconTM. The quality control was maintained by running an in-house control after every 10 samples. Erythrocyte sedimentation rate (ESR) was determined on each sample as recommended by ICSH[13]. Peripheral blood smears were stained with Leishman stain. A differential leucocyte count (DLC) of 200 cells was petformed and absolute count of each .cell type was calculated. This was compared with DLC obtained from H-i autoanalyzer for accuracy. A 5.0 ml blood sample was collected in a plain tube for biochemical profile, particularly LDH estimation using kit manufactured by Boehringer Manheim (Germany). A 1.0 to 1.5 cm long core biopsy specimen was obtained from iliac crest of only one side under local anaesthesia using Islam biopsy needle, The specimen was fixed in buffered formal saline, decalcified and processed for paraffin embedding by. standard techniques. Five micron thick sections were cut and stained with Haematoxylin and Eosinand with silver impregnationmethod for assessment of reticulin. These sections were examined by three of the authors and only the consensus observations were recorded. Sections having atleast three intact inter-trabecular spaces were evaluated. On the basis of histological findings, patients were grouped into three categories.

Group I Essentially Normal: When no significant deviation from normal was noticed in all the material examined and there was no increase in the reticulin. Group! **Non-infiltrative changes:** When one or more of the following were observed without **RS cells or their mononuclear variants or other atypical mononuclear cells:**

a. Gross changes in cellularity e.g. marked hyperplasia or hypoplasiä (<50% of normal cellularity) with either inflammatoiy cells (eosinophils, lymphocytes, plasma cells) or increase in reticulin.

b. Predominance of inflammatory cells consistent with Hodgkin's environment (eosinophils, lymphocytes, plasma cells, histiocytes, fibroblasts).

c. Areas of necrosis with inflammatory cells or increased reticulin.

d. Stromal damage with increase in reticulin.

e. Disturbed haematopoiesis.

Group III Infiltration with HD: When one of the following was seen[8]:

a. Typical RS cells and/or their mononuclear variant, Hodgkin's cells, in the characteristic cellular environment of HD.

b. Atypical mononuclear cells in the presence of characteristic cellular environment of HD.

c. Foci of marked fibrosis.

Results

The age of 88 patients ranged from 2 to 72 years (mean 25 years). Sixty-six patients were male and 22 female (M:F= 3:1). The disease predominantly affected young males (50 males as compared to 13 females of age <30 years). The age and sex distribution is shown in Table I.

Table I. Age and sex distribution.

Age group (years)	Males	Females	M:F ratio
1-10	14	3	4.7:1
11-20	14	7	2:1
21-30	22	3	7.3:1
31-40	5	2	2.5:1
41-50	7	5	1.4:1
51-60	2	1	2:1
>60	2	1	2:1
Total	66	22	3:1

Five patients had pancytopenia with mean values of Hb, total leucocyte count and platelet count of 8.9 g/dl, 2.6x109/l and 63x 10 9/l respectively, 4 patients had thrombocytopenia alone with mean platelet count of 74x109/l. These 9 patients were excluded from further analyses to avoid gross variations. Remaining 79 patients were assigned to three groups. Group! included 20 patients, group 1134 and group 11125 patients. The distribution of the clinical stage and the histological sub-types in all the three groups are shown in Table II.

Table II. Distribution of clinical stage and histological type.

	Total (n=79)		Group I (n=20)		Group II (n=34)		Group III (n=25)	
	n	%	n	%	n	%	n	%
Clinical stage								
I A	20	25.3	5	25	11	32.4	4	16
I B	12	15.2	4	20	6	17.6	2	8
I E	1	1.3	0	0	0	0	1	4
II A	9	11.3	3	15	3	8.8	3	12
Total early stage disease	42	53.1	12	60	20	58.8	10	40
II B	8	10.1	2	10	5	14.8	1	4
III A	7	8.9	3	15	1	2.9	3	12
III B	22	27.9	3	15	8	23.5	11	44
Total advanced stage disease	37	64.9	8	40	14	41.2	15	60
Histological types								
MC	58	73.4	17	85	23	67. 7	18	72
NS	15	19.0	2	10	7	20.6	6	24
LP	3	3.8	1	5	2	5.9	0	0
LD	2	2.5	0	0	1	2.9	1	4
Variants	1	1.3	0	0	1	2.9	0	0

Mixed cellularity (MC) was the most common histological type seen (73.4%). Clinically early stage disease (CS-IA, CS-IB, CS-IE and CS-IIA) was present in 42(53.1%) patients and the remaining 37 (46.9%) had clinically advanced stage disease (CS-IIB, CS- lilA, CS-IIIB). On the basis of the established criteria, 25 (31.6%) patients had bone marrow involvement. Of these, 15 patients already had clinically advanced stage disease. Therefore, the clinical stage of the disease was advanced to pathological stage-IV (advanced stage only in 10 out of all 79 (12.6%) and out of 42 (23.8%) clinically early stage disease patients. Clinically, B symptoms were present in 9 out of 20 (45%) of group Ipatients, 19 out of34 (56%) patients of group II and 14 out of 25(56%) patients of group III.
The peripheral blood parameters of the three groups are shown in Table III.

Table III. Peripheral blood parameters and their comparison.

Group	Hb	TLC	N	L	M	E	PLT	ESR
I	12.39±2.08	7.5±2.19	4.26±1.82	2.4±1.72	0.43±0.26	0.44±0.26	331±109	42±29
II	11.13±2.01	10.28±4.51	7.25±3.97	2.36±1.0	0.66±0.36	0.72±0.62	392±132	65±47
III	10.38±2.76	11.34±4.52	7.47±3.85	2.83±1.73	0.47±0.37	0.48±0.53	348±142	80±42
				Comparison (P value)				
I ---> III	0.006	<0.001	<0.001	0.208	0.335	0.420	0.332	0.001
I --->II	0.019	0.008	0.002	0.461	0.041	0.046	0.048	0.034
II -->III	0.127	0.196	0.419	0.103	0.106	0.063	0.125	0.111

Hb= Haemoglobin
N= Neutrophil count
M= Monocyte count
PLT= Platelet count
TLC=Total leucocyte count
L= Lymphocyte count
E= Eosinophil count

This table shows significant reduction in Hb level between group I and II and group I and Ill. The difference between group II and III was non-significant. Similarly there was a significant increase in neutrophils in group II and III than group I while the difference between group II and III was non-significant. There was significant increase in ESR in group II and III patients is compared to group I, while the difference inESR of patients of group II and III was non-significant. Monocytes, eosinophils and platelets were significantly increased in group II as compared to patients in group I. The significance of difference is lost when group I and II are compared with group III. The LDH levels (U/L)were 208±49 in group I, 249±45 in group II and 257±43 in group III patients. The differencebetween group land group II as well as group I and group III is st..tistically significant (p=0.O27 and 0.0 19 respectively) while between group II and group III it is not significant (p=0.459).

Discussion

The clonal origin of malignant cells of HD, the RS cell and the Hodgkin's cell, has been established beyond doubt[14,15] but controversy exists in the origin of these cells[1,3,13,14,16]. The bulk of evidence suggests their origin either from an immature lymphoid cell or from antigen presenting cell. The malignant change appears to occur because of a primary infection, or reactivation of a latent infection due to decrease in immunity with age[1,3,15,17,18]. The malignant clone originates in the lymphoid tissue. The disease can only spread to the bone marrow through the haematogenous mute, because bone marrow is lacking in lymphatics. The malignant cell can enter the blood streamonly at lymphovascularjunctions, e.g., thoracic duct and spleen[10]. The RS cell itself is too large to travel long distance through this route. It is probably the early transformed cell or the mononuclear Hodgkin's cell which enters the blood and metastasis to distant organs like bone marrow[14].

The early transformed cell and the Hodgkin's cell also have a replication advantage over the RS cell which cycles very slowly[14]. This maybe the reasonwhy the Hodgkin's cells are more frequent thanthe classic RS cells inthebone marrow. The malginant cell first establishes in foci, in the para-trabecular region of the bone marrow. These foci then become confluent to give a diffuse appearance[9]. The malignant cells of HD have been reported to produce several cytokines and factors with inflammatory and haemopoietic growth factors like activity[11,14]. Of these IL-i, G-CSF like activity and fibroblast

stimulating factor have their primary targets in the bone marrow. Through these factors they affect the local environment and systemic immune functions resulting in the polymorphic infiltrate[19]. The infiltrate constitutes almost 98% of the cells of the focus of invasion[1], in which the scanty actual culprit may not be morphologically identifiable in early stages. A gradual failure of the immune system, particularly of T-cell function, will eventually result in proliferation of the malignant cells making them more discemabie in a biopsy specimen. For this reason, the overall incidence of the bone marrow biopsies positive for HD, based on definitive criteria of Ann-Arbour is very low. But it is higher in patients with clinically advanced stage disease[5,9,11,16,19,20]. The advantage of bilateral biopsies is also not very significant. This is supported by a high rate of positive biopsies, i.e., 25/79 (31.6%) in our study, in which we performed only unilateral biopsies. On the other hand the reported incidence of so called non-specific changes in the bone marrow biopsy is significantly higher[6,10,11,21]. This is also supported by our finding of such changes in 34/79(43%) of patients.

It is hypothesised that early transfonued cell metastasise to the bone marrow. The secretion of various factors initially stimulate the production and recruitment of granulocytes and immune system, at the expense of erythropoiesis; this is reflected by gradual decrease inHb concentration and increase in ESR. These cells are spilled into the circulation to reach other affected sites and is seen as increased number of granulocytes in the peripheral blood. Later, polymorphic infiltrate in the bone marrow becomes compact around the malignant cells and the fibrous tissue content increases resulting in more generalised reduction in haemopoietic activity reflected in reduction of all haemopoietic parameters inthe peripheral blood. At this stage, the ESR remains the only parameter which is still on increase. Our study supports this hypothesis and shows a decline in Hb and increase in neutrophil count and ESR in group II patients with non-infiltrative changes in the bone marrow (significantly different from group I patients). But the difference is not obvious when compared with group III patients having infiltrative changes (Table III). There is a significant increase in peripheral blood eosinophils, monocytes and platelets in group II patients as compared to group I and this difference is lost as the disease progresses to that seen in group III patients (Table III). These observations suggest that the disease in group II patients is more advanced as compared to group I patients, no matter what is the apparent clinical stage. The inadequacy of clinical staging, in our set up, is suggested by the remarkably high frequency of B symptoms in otherwise clinical stage I disease (Table II). In fact, the disease intensity in this group is closer to that seen in group Ill patients. Similar observations have been reported previously[1,11,19], This conclusion is further supported by our observations onthe incidence of B symptoms and LDH levels. The incidence of B symptoms is similar in group II and group III patients (56%) which is comparatively lesser than the incidence in group I patients (45%). Similarly LDH levels in group I patients are significantly lower than group II and group III patients (p=0.027 and 0.0 19 respectively). It is therefore suggested that bone marrow examination should be an essential part of pre-treatnient work up in patients of Hodgkin's disease irrespective of clinical stage and the non-infiltrative changes in the bone marrow may also be considered as evidence of bone marrow involvement. In developing world, radiotherapy still remains the treatment of choice in early stage disease. This is partly because chemotherapy is expensive and not much expertise is available in its use. However, in advanced stage disease there is no controversy in the use of chemotherapy. The appreciation of these changes and their inclusion in the criteria of bone marrow involvement in HD will definitely alter the treatment ma significant number of cases. In this series, using definitive criteria of bone marrow involvement, the clinically early stage disease was advanced to pathologically advanced stage disease only in 10 out of 42 (23.8%) patients. However, if the non- infiltrative changes are also included in the criteria then the stage of the disease will be advanced in 30 out of 42 (71.4%) of cases (Table I). Keeping otherprognostic factors in mind, the use of aggressive therapy may further improve the outcome in these patients.

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References

1. Collin, RH. The pathogenesis of Hodgkin's disease.Blood Rev., 1990;4:61-68.
2. Hsu, SM. Never ending controversies in Hodgkin's disease. Blood, 1990:75:1742-1744.
3. Horwich, A. The management of early Hodgkin's disease. Blood Rev., 1990;4:181-186.
4. Bayle.Weisgerber, C., Lemercier, N., Teillet, F. et al. Hodgkin's disease in children. Results of therapy in amixed group of 178 clinically and pathologically staged patients over 13 years. Cancer. 1 984;54:21 5-222.
5. Macintyre. BA.. Haughan Hudson, B., Linch, D.C. eta!. The value of staging bone marrow trephine biopsy in Hodgkin's disease. Eur. J. Flaematol., 1987;39:66-70.
6. Rosenberg, S.A. Hodgkin's disease of the bone marrow. Cancer Res,, 1971;31:1733-1736.
7. Lukes, R.J. Criteria of involvement of lymph node, bone marrow, spleen and liver in Hodgkin's disease. Cancer Res.. 1971 ;3 1:1755- 1767.
8. Abrahamsen, A.F., Jakobsen, E., Langholm, R. et al. Bone marrow examination in Hodgkin's disease. Acts Onco!., 1992,31:41-2.
9. Bartle, R., Frisch, B., Burkhardt, R. et al. Assessment ofbone marrow histology in Hodgkin's disease: Correlation with clinical factors. Br. J. Haematol., 1982;5 1:345-360.
10. Wittles, B. Surgical pathology of bone marrow - Core biopsy diagnosis. In: Bennington J.L. Ed. Major problems in pathology. London, W.B. Saunders Company, 1985. pp. 17:91-97.
11. Te-Velde, J., Spaandet, PG., Van Den, B.C. et al. The bone marrow inHodgkin's disease: The non-involved marrow. Histopathology, 1 978;2:31 -46.
12. Stein, H., Mason, D. Y., Gerdes, J. et al. The expression ofthe Hodgkin's disease associated antigen Ki-l in reactive and neoplastic lymphoid tissue. Evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. Blood, I 985;66:848-858.
13. Herbst, H., Tippleman, G., Anagnostopoulos, I. etal. Immunoglobulin andTcell receptor gene rearrangements in Hodgkin's disease and Ki-1 positive large cell lymphoma: Disassociation between phenotype and genotype. Leukaemia Res., 1989;13:103-116.
14. Chopra, R. and Goldstone, A.H. Recent advances in Hodgkin's disease. Cun. Opinion Haematol., l994;1 :285-294.
IS. Gutensohn, N. and Cole, P. Childhood social environment and Hodgkin's disease. N. Engi. J. Med., 1981 ;304: 135-140.
16. Kaplan, H.S. Hodgkin's disease: Unfolding concepts concerning its nature, management and prognosis. Cancer, 1980;45 :2439-2474.
17. Anagnostopoulos, I., Herbst, H., Niedobitek, G. et al. Demonstration of monoclonal EBV genomes in Hodgkin's disease and Ki-1 positive large cell lymphoma by combined Southern blotting and in situ hybridisation. Blood, 1989;74:810-816.
18. Pallesen, G.,Hamilton-Dutoit, 5.J., Rowe, M et al Expression of Epstein-Barr virus latent gene products in tumour cells of Hodgkin's disease. Lancet, 1991 ;337:320-322.
19. Kaplan, H. S. Hodgkin's disease. Cambridge, Harvard University Press, 1980.
20. Ellis, ME., Diehi, L.F., Granger, E. et al. Trephine needle bone marrow biopsy in the initial staging ofHodgkin's disease: sensitivity and specificity of the Ann Arbor staging procedure criteria. Am. 3. Hematol., 1 989;30: 115-120.

21. O'Carroll, D.I, Mckenna, R.W. and Brunning, RD. Bone marrow manifestations ofHodgkin's disease. Cancer, 1976,38 :1717-1728.