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HAEMATOLOGIC EVALUATION AFTER RADIATION THERAPY IN HODGKIN'S DISEASE

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Recent results of the treatment of malignant lymphoma have been promising. In particular, using modern irradiation techniques, a full and permanent cure seems to be possible, and up to 80 per cent of the patients with Hodgkin's disease survive for 5 years (KAPLAN 1972, AISENBERG & QAZI 1976). A total dose of 35 to 45 Gy (3 500–4 500 rad) given during a period of 4 to 7 weeks (KAPLAN & ROSENBERG 1975) has been found necessary to reduce the recurrences as much as possible. In advanced cases of Hodgkin's disease multiple cytostatic drug therapy is indicated (DEVITA et coll. 1970).

After the usual curative doses of irradiation, a local depression in the haematopoiesis follows. The dose response data for irradiation of human bone marrow are limited. SYKES et coll. (1964) performed sternal aspirations in patients with localized carcinoma of the breast following 17 to 60 Gy. Little or no marrow regeneration was found in 54 of 56 patients given 30 Gy or more up to 84 months after irradiation. RUBIN et coll. (1973) used $^{99}Tc^{m}$-S colloid scanning technique for analysing the condition of the bone marrow in 27 patients with Hodgkin's disease at various times before and after intensive irradiation. They observed a partial to complete bone marrow regeneration at 40 Gy in 85 per cent of the irradiated sites at two years. In 71 consecutive patients irradiated for Hodgkin's disease KUN & JOHNSON (1975) found

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Table 1

Clinical data of 30 patients treated for Hodgkin's disease

Case/Age/Sex	Stage Ann Arbor classification	Grade*	Radiation field	Calculated bone marrow dose (Gy)	Drug therapy	Recurrence at time of marrow biopsy
1/73/M	I A	NS(MC)	Mantle	40	No	No
2/32/F	II A	(NUD)	Mantle	40	Procarb. ad 2.5 g	No
3/31/M	III A	NS	Mantle Inv. Y	38 22	MOPP ×6 MVPP ×3	No
4/29/M	III B	NS	Mantle Inv. Y	42 24	No	No
5/22/F	II B	NS	Mantle	40	No	No
6/31/F	II A	MC	Mantle Inv. Y	40 24	CVPP ×12	No
7/23/M	I A	NS	Mantle	40	MOPP ×4 CVPP ×1 ABVD ×1	Yes
8/71/F	III A	NS	Mantle Inv. Y	40 24	No	No
9/27/M	II A	NS	Mantle	40	CVPP ×6 COPP ×3 Bleomyc. ad 0.3 g	Yes
10/29/F	II A	NS	Mantle	32.4	MOPP ×9 CAVe ×1	No
11/62/F	I B	LP	Mantle	38	No	No
12/20/M	III B	NS	Mantle Inv. Y	40 24	CVPP ×6	No
13/35/F	III B	MC	Mantle Inv. Y	40 24	CVPP ×6 MOPP ×3 MVPP ×7	Yes
14/50/F	II A	NS	Mantle	48	Procarb. ad 4 g	No
15/59/M	II A	(NUD)	Mantle	39	No	No
16/56/M	I A	NS	Mantle	40	No	No
17/65/M	II A	LP	Mantle	40	No	No
18/28/M	III B	(NUD)	Mantle Inv. Y	40 24	No	No
19/27/M	II B	LD	Mantle Inv. Y	40 14	CVPP ×8 ABVD ×1 MOPP ×7	Yes
20/24/M	II A	NS	Mantle	40	MOPP ×8	No
21/28/F	III B	LP	Mantle Para-a.	40 24	MOPP ×1	Yes

Table 1 (*cont.*)

Case/Age/Sex	Stage Ann Arbor classification	Grade*	Radiation field	Calculated bone marrow dose (Gy)	Drug therapy	Recurrence at time of marrow biopsy
22/40/F	II B	NS	Mantle	44	CVPP ×12 MOPP ×15	No
23/65/M	I B	LD	Mantle	30.5	VPP ×4 CVPP ×7	No
24/41/M	II A	LP	Mantle	40	CVPP ×4	No
25/69/F	I A	LP	Mantle	40	No	No
26/59/F	IV	(NUD)	Mantle	40	COPP ×12	No
27/25/M	I B	(NUD)	Mantle	40	No	No
28/70/F	I A	LP	Mantle	40	No	No
29/35/M	III A	LP	Mantle Inv. Y	42 25	COPP ×3 CVPP ×6	No
30/31/M	I A	(NUD)	Mantle	40	No	No

* See FRANSSILA et coll. 1967.

no evidence for residual haematologic depression after 5 years of disease-free survival, even in patients treated initially with total nodal irradiation. However, the local marrow effects of the irradiation were not recorded. Recently KNOSPE et coll. (1976) used ^{52}Fe bone marrow scanning from 1 to 73 months after radical irradiation of patients with malignant lymphoma. Marrow regeneration was observed in most patients after an interval of 12 months or longer. The recovery did not seem to be dose-related and the dose of marrow ablation was not defined with doses of 40 to 50 Gy. The authors concluded that the erythropoietic function recovered in the irradiated marrow in most patients 1 to 2 years after about 40 Gy.

In most cases of effective irradiation the dose reaches the tolerance limit of the bone marrow. Thus, the exact knowledge of the bone marrow effects of irradiation would be of great importance. Furthermore, a careful characterization of these effects might bring new light on the understanding of the basic mechanisms of haematopoiesis.

The haematologic data obtained 2 to 7 years after irradiation of 30 patients with Hodgkin's disease are now reported with particular emphasis on the local bone marrow effects.

Material and Methods

The 30 patients included in the material represent 32 survivors of the total number of 56 consecutive cases irradiated for Hodgkin's disease in this hospital from 1969 to 1973. The megavoltage treatment was delivered with an 8 MeV linear accelerator to mantle fields or with a cobalt equipment according to an individual treatment plan.

Lymphography was performed in 27 patients, and laparotomy including splenectomy in 20 patients. In 7 patients the disease progressed after the primary treatment. Two of them have no further signs of disease at the present time, and the total number of obviously disease-free cases was 25. The detailed clinical data of the patients are presented in Table 1.

Bone marrow aspirations were performed with a Klima-type 1.5 mm (18-gauge) needle and 20 ml syringe after local anesthesia. Samples were taken both from the sternal manubrium and the posterior iliac spine. If the sternal aspiration did not give any visible cellular particles, the top of the needle was rotated inside the sternum in an orbit of 1 to $1\frac{1}{2}$ cm before a new aspiration. Both smears and pressing preparations were made for May-Grünwald-Giemsa (MGG). The amounts of marrow fat, reticular tissue (=cellular components of reticuloendothelial system), erythropoietic, granulopoietic, and megakaryopoietic tissue were evaluated semiquantitatively in coded preparations at microscopy.

The haemoglobin concentration, white cell count, and red cell count were measured with the Coulter Counter S. The platelet count was measured from the same EDTA-anticoagulated venous sample with the Thrombo counter C. Differential counts of white cells were made by one experienced laboratory nurse, and one of the authors examined the red cell morphology from MGG-stained films. The relative number of peripheral blood reticulocytes was calculated after methylene blue staining from 9 randomly chosen patients.

Results

The mean blood cell values of the 30 patients are within normal limits (Table 2).

In the 5 cases with recurrence the haemoglobin concentration was 106 g/l (male), 131 g/l (female), the white cell count $7\,300 \times 10^6$/l and the platelet count 350×10^9/l (mean values). Only one of the recurrence-free patients was anemic (Hb 114 g/l). His iron storage was normal. Seven cases had thrombocytosis (platelet values mean 470, range 410–560 $\times 10^9$/l), and 3 slight leukocytosis (10 500, 10 700 and 11 500 $\times$ 10^6/l). All of them were splenectomized.

The red cell morphology was characteristic in splenectomized cases with numerous Howell-Jolly bodies, target cells, and irregularly furrowed red cells.

All bone marrow samples were rich in blood and marrow fat. Iliac bone marrow aspirations were normal except one (No. 20), in which no haemopoietic cells were found. Instead, the appearance of the sternal marrow varied widely. A detailed analysis of the haematopoietic cells and marrow reticular cells is presented in Table 3.

Haematopoietic or reticular cells were found in 27 preparations. A complete regeneration of the sternal bone marrow was evident in 5 cases: one exposed with 30.5 Gy and 4 with 40 Gy. In addition regeneration of the three haematopoietic cell lineages occurred in one marrow after 40 Gy in 5 to 7 weeks, but the cell numbers were low.

In many samples the cellular differentiation was abnormal ($\pm$ in Table 3). In these

Table 2

Mean blood cell values

Parameter	Mean ± SD	N	Reference values
Haemoglobin	138 ± 19	17	130–165* (M)
(g/l)	134 ± 9	13	115–150 (F)
Erythrocytes	4.6 ± 0.6	15	4.3–5.6* (M)
(10^{12}/l)	4.4 ± 0.3	12	3.8–5.0 (F)
Reticulocytes** (10^9/l)	57 ± 24	9	18–158***
White blood cells (10^6/l)	7 200 ± 2 200	30	3 000–10 000*
Neutrophils**** (10^6/l)	4 500 ± 1 700	30	1 830–7 250***
Monocytes**** (10^6/l)	390 ± 180	30	200–950***
Lymphocytes**** (10^6/l)	2 300 ± 1 100	30	1 500–4 000***
Platelets (10^9/l)	340 ± 110	30	150–400*

* 95 % range for healthy adults in this laboratory.
** Reticulocytes = erythrocytes × per cent of reticulocytes.
*** 95 % range for normal adults.
**** Neutrophils (monocytes, lymphocytes) = WBC × per cent of neutrophils (monocytes, lymphocytes).

cases the continuance of maturation was defective and many of the haematopoietic cells were morphologically abnormal. Nuclear-cytoplasmic desynchronization and poor cytoplasmic maturation were observed. Erythropoietic and granulopoietic cells were irregularly bordered, and the nuclear-cytoplasmic ratio was higher than in their counterparts in normal iliac marrow. In granulopoietic series the cytoplasmic maturation was delayed as compared with the stage of the maturation of the nucleus and cytoplasmic basophilia deeper than expected. Similarly, the haemoglobinization stage was low in erythropoietic precursor cells, although the nucleus represented a differentiation stage of a normal polychromatophilic or orthochromatophilic normoblast.

In 7 of the 21 reticular cell positive cases no haematophoiesis was seen after 38 to 42 Gy, and in additional 9 cases the haematophoiesis was defective as in the cases without reticular cells. In two of them abnormal haematopoietic cells were observed.

Discussion

The normal blood cell values in the 30 patients indicate that the number and function of haematopeoietic precursor cells is normal, but it should be remarked that the distribution of these cells may be quite abnormal.

Table 3

Semiquantitative estimates of the haematopoietic precursor cells and reticular cells in the sternal marrow

Case	Sternal dose (Gy)	Interval (years)	Erythro-poiesis	Granulo-poiesis	Megakar-yocytes	Reticular cells
1	40	5	−	+	−	+ +
2	40	7	−	−	−	−
3	38	6	−	±	−	+
4	42	6	±	±	−	−
5	40	3	−	−	−	−
6	40	3	+	+	−	+ +
7	40	5	+ +	+ +	+ +	+ +
8	40	5	+	+	+	+
9	40	6	−	−	−	+
10	32.4	7	±	±	−	−
11	38	4	±	±	−	+ +
12	40	3	−	−	−	+ +
13	40	6	+ +	+ +	+ +	+ +
14	48	5	−	−	−	+ +
15	39	4	−	−	−	+ +
16	40	5	±	±	−	+ +
17	40	4	−	−	−	−
18	40	5	±	−	−	+ +
19	40	5	−	−	−	+ +
20	40	4	−	−	−	+ +
21	40	3	−	−	−	+ +
22	44	3	−	−	−	+ +
23	30.5	5	+ +	+ +	+ +	+ +
24	40	5	±	±	−	+ +
25	40	2	±	−	−	+
26	40	3	+ +	+ +	+ +	+ +
27	40	3	+	+	−	+
28	40	4	−	−	−	−
29	42	6	−	−	−	+ +
30	40	4	+ +	+ +	+ +	+ +

+ + Normal number of cells.
+ Decreased number of cells. All maturation stages present.
± Not all maturation stages present or abnormal morphology.
− No (precursor) cells present.

Platelet values higher than normal were observed in 7 patients and a slight leukocytosis in 3. These patients were splenectomized, which is likely to be the cause of the thrombocytosis and of the leukocytosis (McBride et coll. 1968, Singer et coll. 1941). In other recurrence-free cases all blood values were within the normal 95 per cent range. The results are in good agreement with those published by Kun & Johnson.

KNOSPE et coll. (1966, 1968) have elucidated the basic mechanisms operating after a local irradiation of the marrow. The late aplasia is correlated with radiation induced loss of sinusoidal structures, and the haemopoietic recovery depends upon sinusoidal regeneration. In the present material full haemotopoietic regeneration was observed in 5 out of 21 patients given 40 Gy in 5 to 7 weeks. Additionally, in 7 cases after a dose of 40 Gy some haematopoiesis was observed. In 9 sternal aspirations no haematopoiesis was found, although these samples are to be considered as representative ones.

From the present results it may be concluded that the bone marrow regeneration after irradiation is not an all or none phenomenon. The patchy irregular marrow regeneration observed in patients and in experimental animals by KNOSPE et coll. (1966, 1976) may be analogic to the deficient cell appearance in the bone marrow aspirations of the present patients. However, another explanation of the existence of morphologically or numerically abnormal haematopoiesis in bone marrow is also possible. Animal experiments suggest that the recovery of radiation injured bone marrow may be initiated by stem cells originating from unirradiated bone marrow regions after the stromal regeneration has taken place (CARSTEN & NOONAN 1959, DE VRIES & VOS 1966). In human peripheral blood stem cells usually circulate (CHERVENICK & BOGGS 1971). The seeding of these cells is possible, and these haematopoietic precursor cells may be temporarily located in the irradiated marrow. Without fully regenerated stroma the marrow does not function in the normal way to maintain morphologically normal haematopoietic colonies or numerically normal haematopoiesis (KABAKOV et coll. 1968). The constant injury of the marrow after radical irradiation is apparently caused by the deterioration of the microcirculation, and endothelial in nature (RUBIN & CASARETT 1968, ZOLLINGER 1970). The stem cells migrated from unirradiated regions are not able to repair this lesion (KABAKOV et coll. 1968, KNOSPE et coll. 1966, 1968).

KUN & JOHNSON, using $^{99}Tc^{m}$-S colloid, and KNOSPE et coll. (1976), using ^{52}Fe bone marrow scanning, noted a higher rate of regenerations in patients following irradiation with 40 Gy than noted in the present material. Instead, if the abnormal haematopoiesis is not considered a sign of a real marrow regeneration, the present results are similar to those published by SYKES et coll. (1964). They did not observe sternal marrow regeneration in 96.5 per cent of cases after 30 Gy or more given for mammary carcinoma. In the present material the percentage of full regeneration after 38 to 48 Gy was 18 (5/28). No clear correlation between the local marrow regeneration and irradiation of other fields, or cytostatic drug therapy was found.

A reliable quantitative examination of human bone marrow after irradiation is possible in only a few clinical situations. Quantitative evaluation ought to be performed by histologic methods, but the trepanation of the sternum and aspiration of marrow samples is difficult. Unfortunately, neither the nuclide scanning methods nor the direct marrow aspiration biopsy provides a quantitative measure of the cellularity in bone marrow.

SUMMARY

Haematologic evaluation of 30 patients 2 to 7 years after radiation therapy for Hodgkin's disease was made. A complete general recovery of haematopoiesis occurred as concluded from the normal or even supranormal blood cell values. However, in only 5 of 28 patients was a complete local regeneration of haematopoiesis observed in the sternal marrow biopsies after 38 to 48 Gy. In the other cases, haematopoiesis was numerically or morphologically abnormal or totally absent.

ZUSAMMENFASSUNG

Eine hämatologische Untersuchung von 30 Patienten wurde 2 bis 7 Jahre nach Bestrahlung wegen Hodgkinscher Erkrankung vorgenommen. Eine vollständige Regeneration der Hämatopoese wurde erreicht, wie aus den normalen und auch supranormalen Blutzellwerten beurteilt werden konnte. Jedoch wurde nur bei 5 von 28 Patienten eine komplette lokale Regeneration der Hämatopoese in den Biopsien des Knochenmarks des Sternums nach 38 bis 48 Gy beobachtet. In den anderen Fällen war die Hämatopoese numerisch oder morphologisch abnormal oder total abwesend.

RÉSUMÉ

Les auteurs ont fait une étude hématologique de 30 malades de 2 à 7 ans après un traitement par les radiations pour une maladie de Hodgkin. La numération des cellules sanguines qui est normale ou même supérieure à la normale, fait conclure à une restauration complète et générale de l'hématopoïèse. Cependant, c'est seulement chez 5 malades sur 28 qu'on a observé une régénération locale complète de l'hématopoïèse sur des biopsies de la moelle sternale après une irradiation allant de 38 à 48 Gy. Dans les autres cas, l'hématopoïèse était numériquement ou morphologiquement anormale ou totalement absente.

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