J Clin Pathol 1984;37:233–254

Lymphoproliferations in the bone marrow: identification and evolution; classification and staging

R BARTL,*† B FRISCH,‡ R BURKHARDT,*† K JÄGER,† R PAPPENBERGER,§ G HOFFMANN-FEZER¶

*From the *Abteilung für Knochenmarksdiagnostik an der Medizinischen Klinik Innenstadt der Universität München, Munich, †Abteilung für Hämatomorphologie, Institut für Hämatologie der Gesellschaft für Strahlen- und Umweltforschung mbH, Munich, Federal Republic of Germany, ‡Institute of Haematology, Tel-Aviv Municipal-Governmental Medical Centre, Sackler School of Medicine, Tel-Aviv University/Israel, §Institut für Medizin. Informatik und Systemforschung (MEDIS) der Gesellschaft für Strahlen- und Umweltforschung mbH, Munich,¶ Abteilung für Immunologie, Institut für Hämatologie der Gesellschaft für Strahlen- und Umweltforschung mbH, Munich, Federal Republic of Germany*

SUMMARY Bone marrow biopsies from 3229 patients with lymphoproliferative disorders and 1156 patients with benign or reactive lymphoproliferations were investigated and criteria for distinguishing between them are given. Bone marrow involvement was found in 89% of multiple myeloma, 64% of non-Hodgkin's lymphomas and 8% of Hodgkin's disease. According to the predominant proliferative cell type there were five major entities in multiple myeloma and non-Hodgkin's lymphomas: (1) plasmacytic; (2) lymphocytic; (3) hairy cell; (4) immunocytic; (5) centrocytic. These were further classified into distinct subtypes each of which had independent prognostic significance. The mode of spread of the lymphoproliferative disorders in the bone marrow showed one of six architectural patterns, which together with the quantity of infiltration in the biopsy (reflecting the tumour cell burden) had significant predictive value. These results demonstrate the value of bone marrow biopsies in the identification, classification and staging of lymphoproliferative disorders, as well as in monitoring the course of disease and the response to therapy.

It is over a decade since the value of bone marrow biopsies in the staging of non-Hodgkin's lymphomas has been recognised.[1] They are now taken routinely in the initial investigation of patients with non-Hodgkin's lymphomas to estimate the progression of disease at time of presentation (staging) and to type the mode of proliferation (growth pattern) in the bone marrow.[2–8] A bone marrow biopsy may be diagnostic in patients without peripheral lymphadenopathy,[9] and may aid classification when inconclusive or divergent histologies are found at other sites. A bone marrow biopsy also provides information on the extent of tumour cell burden (volume percentage) and on the function and response to therapy of the residual marrow and of the neoplasia. A bone marrow biopsy thus offers insight into the biological behaviour of the disease process in the individual patient. The aim of this study was to provide a comprehensive survey of lymphoproliferations in the bone marrow based on a retrospective and prospective analysis of bone marrow biopsies in 4385 cases.

Accepted for publication 9 November 1983

Patients and methods

Initial pretreatment bone marrow biopsies were taken (after informed consent had been obtained) from 3213 patients diagnosed by the established criteria on lymph node biopsies as suffering from one of the lymphoproliferative disorders and from 512 patients during follow-up periods of one to 10 years. In 270 patients the diagnosis of non-Hodgkin's lymphoma (subsequently confirmed) was first made by bone marrow biopsy. In addition, 1156 biopsies from patients with reactive plasmacytosis, benign monoclonal gammopathies, benign lymphoid nodules, reactive lymphocytosis and non-specific granulomas, were also evaluated. Normal bone and bone marrow values were obtained from 160 biopsies of individuals without evidence of disease taken

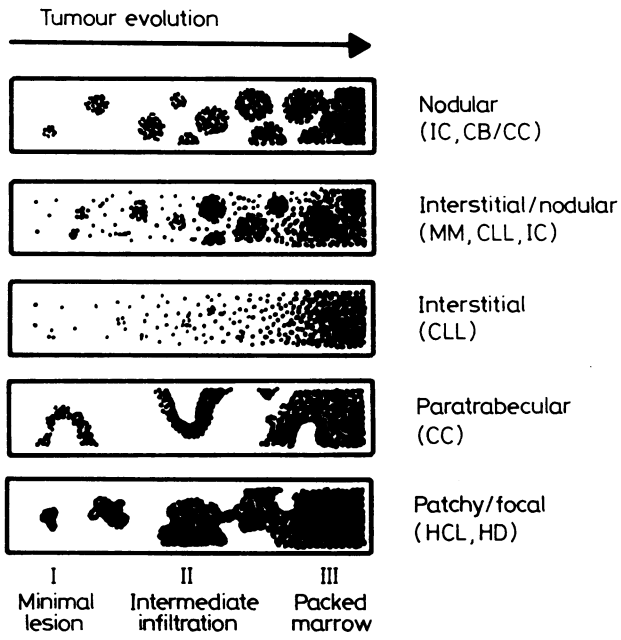


Fig. 1 *Tumour evolution of lymphoproliferative disorders in the bone marrow.*

during investigation for other conditions. All biopsies were obtained under local anaesthesia from the iliac crest, fixed, dehydrated, and embedded in methacrylate without decalcification, cut and stained as previously described.[10–12] In 186 cases immunohistological reactions (FITC and PAP) were performed on fresh frozen cryostat sections of one half of the longitudinally cut biopsy core.[13–15] The histological and morphometric variables investigated in 2186 biopsies were used for multivariate data analysis by selected BMDP programs.[16]

Results

RECOGNITION AND EVOLUTION OF LYMPHOPROLIFERATIONS IN THE BONE MARROW

The overall division of the biopsies into the different entities is shown in Table 1; the different types of lymphoid tumour evolution in the bone marrow are summarised in Fig. 1.

Plasmacytosis and multiple myeloma

To provide criteria for distinguishing between reactive and neoplastic plasmacytosis, bone marrow biopsies of the following clinical groups were analysed: patients with chronic inflammatory diseases (group RP, 210 cases), patients with benign monoclonal gammopathy (group BMG, 164 cases), and patients with early multiple myeloma (group EMM, 69 cases). In all three groups plasma cells, singly or in twos and threes, were located around capillaries (Fig. 2a) or loosely dispersed among haematopoietic and fat cells. In addition group EMM exhibited small, tight clusters of plasma cells in paratrabecular and periarterial regions which were not seen in groups RP and BMG (Fig. 2c). The histological growth pattern thus proved the most reliable characteristic for distinguishing reactive from malignant plasmacytosis. Only very early (albeit rare) cases of multiple myeloma required immunohistology for the identification (Fig. 2b). With progression of disease

Table 1 *Lymphoproliferations*

	No of patients
Benign	
Reactive plasmacytosis	210
Benign monoclonal gammopathy	164
Reactive lymphocytosis	110
Benign lymphoid nodules	566
Reactive granulomas	106
Malignant	
Multiple myeloma	813
Non-Hodgkin's lymphomas	1351
Hodgkin's disease	1011
Angioimmunoblastic lymphadenopathy	54

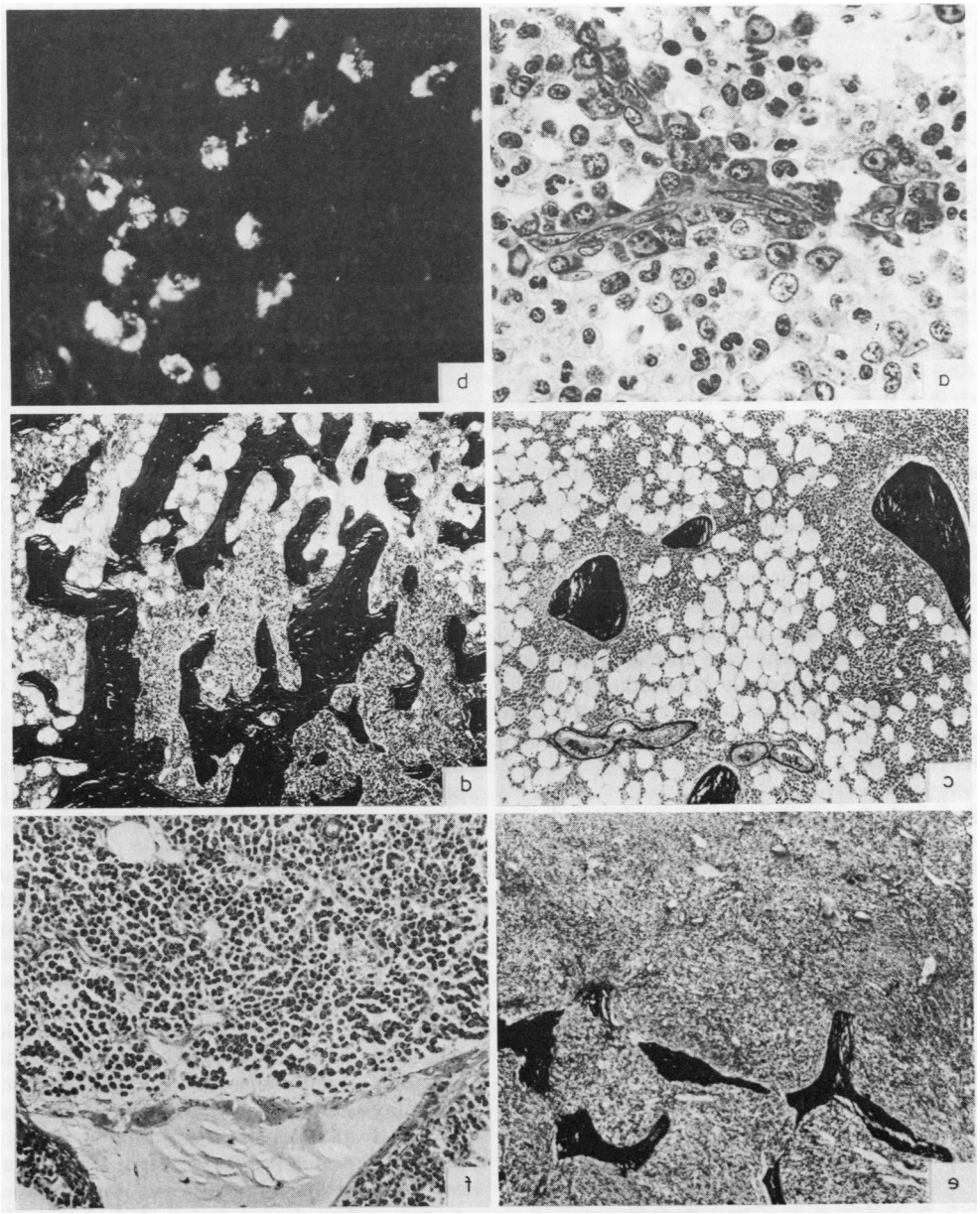


Fig. 2 *Plasmacytosis and multiple myeloma: (a) reactive plasmacytosis with plasma cells located around capillaries. Giemsa ×400; (b) early stage of an IgA kappa multiple myeloma, confirmed by follow-up studies. FITC, frozen section ×250; (c) interstitial pattern with endosteal aggregates of myeloma cells. Note the marked increase of fat cells. Gomori ×40; (d) interstitial/nodular pattern of multiple myeloma with osteosclerotic bone reaction. Gomori ×40; (e) packed marrow pattern of multiple myeloma with osteolytic bone reaction. Gomori ×40; (f) osteoclastic bone resorption in vicinity of dense myelomatous infiltration. Giemsa ×100.*

the small plasma cell aggregates expanded to form nodules, "multiple myelomas", both inter- and paratrabecular with a tendency to confluence into densely packed masses (myelomatous pattern) which progressed eventually to the "packed marrow" with virtual replacement of haematopoietic

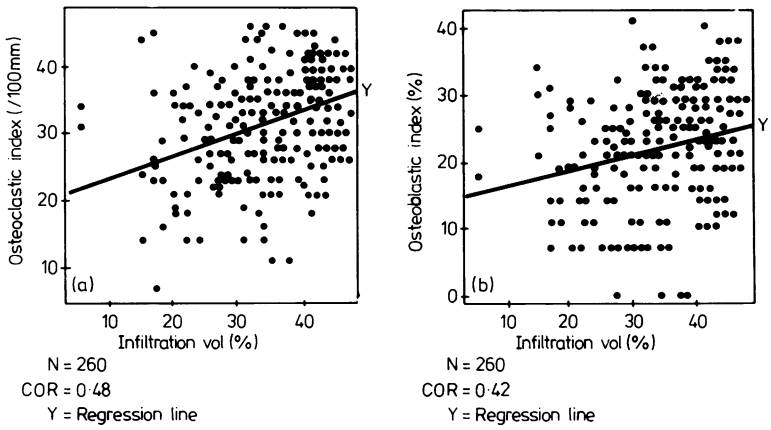


Fig. 3 *Correlation between myeloma cell mass and osseous remodelling in the bone marrow (pretreatment patients). Osteoclastic index (OC) = number of osteoclasts per 100 mm trabecular circumference. Osteoblastic index (OB) = percentage of trabecular circumference covered by cuboidal osteoblasts.*

and fat tissues and accompanied by osteoporotic-osteolytic bone lesions (Fig. 2e, f). Only four cases showed osteosclerotic bone lesions (Fig. 2d). The activity of the bone cells, particularly osteoclasts, correlated with the plasma cell burden (Fig. 3). There was also a statistically significant correlation between the volume ratio of fat/haematopoiesis and the plasma cell mass in the pre- and post-treatment biopsies (Fig. 4). Cytotoxic therapy reduced but did not eradicate the myeloma, as residual cells were always present in sequential biopsies of 112 treated patients (Fig. 4).

Lymphocytosis, benign lymphoid nodules, and malignant lymphomas

Lymphocytes normally may comprise up to 20–25% of the population of nucleated cells in the bone marrow, itself an organ of lymphopoiesis. Increases may occur either absolute, or relative due to reduction in haematopoietic elements. The distinction of these

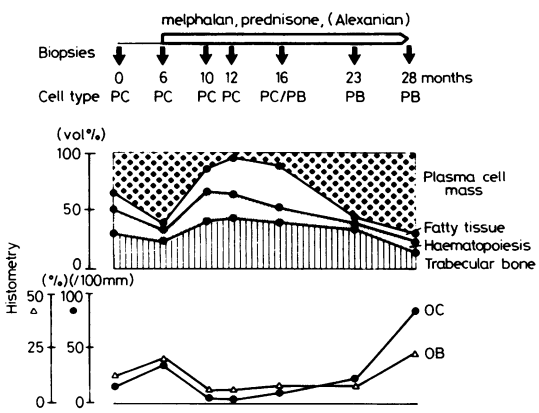
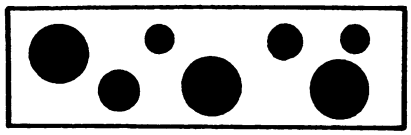
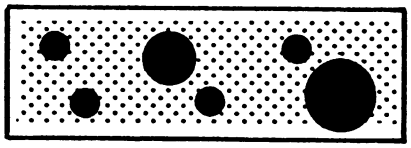
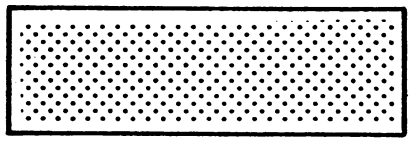
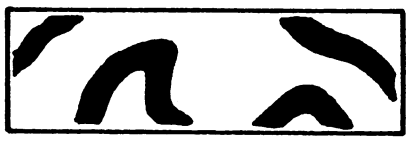
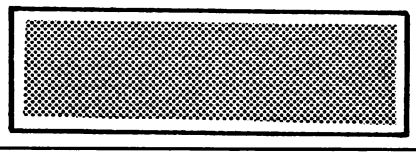


Fig. 4 *Quantitative evaluation of sequential biopsies in a patient with multiple myeloma (68 years old, male, IgD lambda, Bence-Jones negative).*

cases from early lymphocytic lymphomas with interstitial spread may not be possible by histology alone. Benign lymphoid nodules, found in 8% of 7080 bone marrow biopsies investigated, occur more frequently in the older age groups (36% in patients over 70 yr). They are classified into four types according to Hashimoto *et al.*:[17] type A = nodules with germinal centres 5%; type B = sharply demarcated nodules 30%; type C = well defined nodules 45%; type D = small aggregates of lymphocytes 22%. The size of the nodules varies from 0·1 to 2 mm (average 0·4 mm). They consist of small lymphocytes, some plasma cells and occasional histiocytes and capillaries within a reticular fibre network. Though usually single, multiple nodules occur in a quarter of the cases; and the question of recognition as benign or malignant arose when they were large and/or numerous. In these cases immunohistology was required and of 14 such cases investigated, six revealed a monoclonal cell population indicating non-Hodgkin's lymphoma immunocytic and the other eight cases were polyclonal (reactive lesions). All other bone marrows of patients with non-Hodgkin's lymphoma low grade malignancy were not equivocal and six bone marrow patterns were observed at presentation: nodular (151 cases), interstitial/nodular (466 cases), interstitial (359 cases), paratrabecular (39 cases), patchy/focal (165 cases), and packed marrow (455 cases) (Table 2, Fig. 5). In the first type, the nodules expanded as the disease progressed to form multinodular areas. In the interstitial type the lymphocytes, loosely dispersed among the haematopoietic and fat cells and around the blood vessels, may not initially be evident on low power examination. Subsequently they form a dense homogeneous infiltration, the packed marrow pattern. Infiltrates which initially appeared as paratrabecular foci extended to envelop the cancellous bone and inwards to replace the

Table 2 *Growth patterns of lymphoproliferative disorders in the bone marrow (at time of initial diagnosis)*

Growth patterns		*Median survivals* (months)*	*MM*	*CLL*	*HCL*	*IC*	*CC*	*CB/CC*	*HD*
Nodular n = 151, 9%		58	+	ϕ	ϕ	++	ϕ	+++	ϕ
Interstitial/ nodular n = 466, 29%		28	++	++	ϕ	++	ϕ	ϕ	ϕ
Interstitial n = 359, 22%		23	++	++	ϕ	ϕ	ϕ	ϕ	ϕ
Paratrabecular n = 39, 2%		20	ϕ	ϕ	ϕ	ϕ	+++	ϕ	ϕ
Patchy focal n = 165, 10%		20	ϕ	ϕ	+++	ϕ	ϕ	ϕ	+++
Packed marrow n = 455, 28%		16	++	++	++	++	++	+	++

*From time of biopsy to death or date of last contact.

MM = multiple myeloma
CLL = chronic lymphocytic leukaemia
HCL = hairy cell leukaemia
IC = immunocytoma
CC = centrocytic lymphoma
CB/CC = centroblastic/centrocytic lymphoma
HD = Hodgkin's disease

haematopoietic and fat tissues and thus eventually also presented a packed marrow. Finally the patchy growth pattern with a tendency to confluence also showed a packed marrow in the later stages. At presentation of patients with non-Hodgkin's lymphoma high grade malignancy only the focal (sarcomatous)

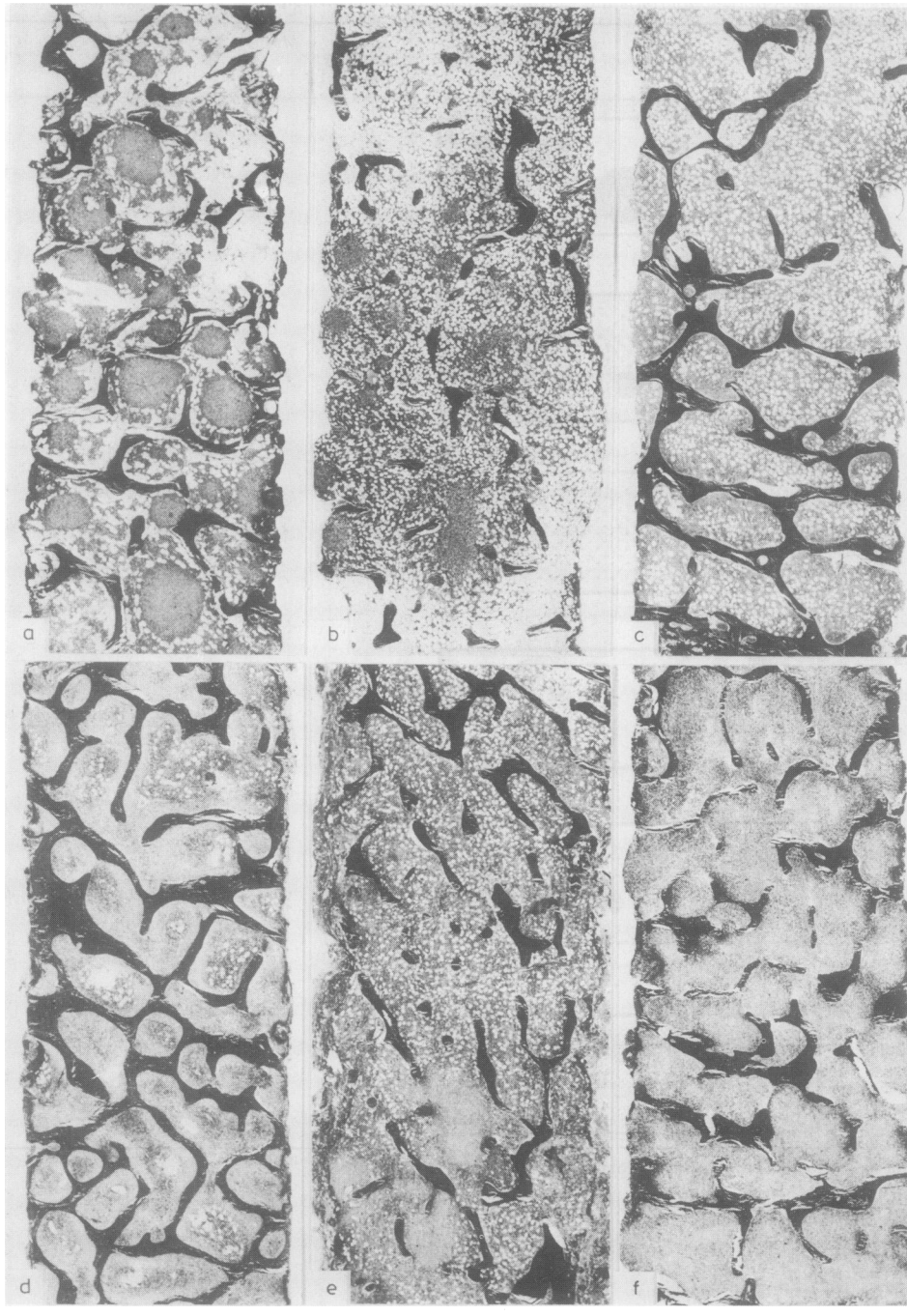


Fig. 5 *Growth patterns of non-Hodgkin's lymphomas in the bone marrow. Gomori ×10. (a) nodular pattern in immunocytic lymphoma, (b) interstitial/nodular pattern in immunocytic lymphoma, (c) interstitial pattern in chronic lymphocytic leukaemia, (d) paratrabecular pattern in centrocytic lymphoma, (e) patchy/focal pattern in hairy cell leukaemia, and (f) packed marrow pattern in non-Hodgkin's lymphoma high grade malignancy.*

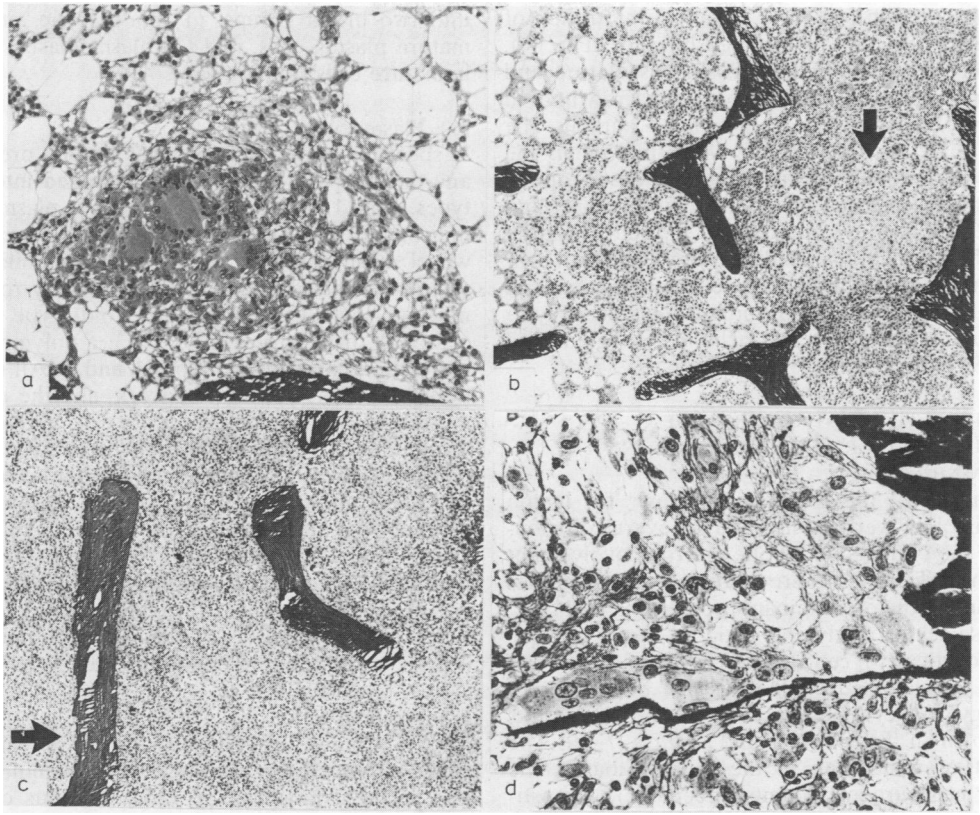


Fig. 6 *Granulomas and Hodgkin's disease: (a) large noncaseating epithelioid-cell granuloma with giant cells in a patient with Hodgkin's disease, nodular sclerosis, stage II. Gomori ×100; (b) small focus (arrow) of Hodgkin's disease in the bone marrow. Gomori ×40; (c) Hodgkin's disease with complete replacement of the bone marrow and osteoclastic osseous reaction (arrow). Gomori ×40; (d) osteoclastic resorption of a trabecula surrounded by lymphogranulomatous tissue. Gomori ×250.*

type and the packed marrow were observed. Histomorphometry of the bony trabeculae revealed normal volume percentages in about 60%, osteopenia in 35% and osteosclerosis in only 5% of the non-Hodgkin's lymphoma patients. Marked osteopenia or osteolytic lesions were predominantly found in patients with "packed marrow". Increased osseous remodelling was observed in 6% of the non-Hodgkin's lymphoma patients. One patient with hairy cell leukaemia showed multiple osteolytic lesions on *x*-ray of the skull; another one with immunocytic lymphoma had a coexisting Paget's disease.

Granulomas and Hodgkin's disease

Granulomas were encountered in 106 cases (4% of 2650 biopsies investigated). Single or multiple, they ranged in size from small to large (0·1 to 2·3 mm) usually with but occasionally without multinucleated giant cells, and consisted of epithelioid cells, histiocytes, small vessels, reticulin fibres, and variable numbers of lymphocytes as well as occasional macrophages and eosinophils (Fig. 6a). When large, the distinction between such non-specific granulomas, angioimmunoblastic lymphadenopathy, malignant histiocytosis, systemic mastocytosis and Hodgkin's disease in the bone marrow may not be possible on histology alone. Nevertheless for the initial diagnosis of Hodgkin's disease, Reed-Sternberg cells within a characteristic stromal environment are mandatory, while the presence of mononuclear Hodgkin cells within such a setting suffices for confirmation of bone marrow involvement, when Hodgkin's disease is already documented elsewhere (Ann Arbor). Minimal lesions of Hodgkin's disease were small, usually paratrabecular foci with Hodgkin or Reed-Sternberg cells (Fig. 6b). Large intertrabecular areas of lymphogranulomatous tissue

were observed in 51% and complete infiltration of the marrow in 31% of involved biopsies (Fig. 6c). There were variations in fibrosis, vasculature and cellular composition of the involved marrow both in the same biopsy and from patient to patient. The structure of the trabeculae was normal when the lesions were small, but varied from osteosclerotic to osteoporotic/osteolytic when the foci were large and confluent. Osseous remodelling showed primarily osteoblastic activity including primitive bone production in oedematous, hypocellular spaces, and marked osteoclastic resorption in hypercellular lymphogranulomatous areas, rich in Hodgkin and Reed-Sternberg cells (Fig. 6d).

CLASSIFICATION AND STAGING OF LYMPHOPROLIFERATIVE DISORDERS IN THE BONE MARROW

The criteria of the Kiel classification[31] for lymph node histology were applied and the overall distribution of the patients with bone marrow involvement is given in Table 3. There were five major entities according to the predominant proliferative cell type: (1) plasmacytic; (2) lymphocytic; (3) hairy cell; (4) immunocytic; (5) centrocytic. Each of these was further subclassified on the basis of bone marrow histology and cytological features, and the results are shown in Tables 4, 5 and Figures 7–10. The amount of infiltration in the biopsy, that is the tumour cell burden (volume percentage), was estimated in the initial bone marrow biopsy of all untreated patients. Three cut off points were utilised: less than 20 vol% of the bone marrow biopsy area occupied by the infiltration, 20–50 vol%, and more than 50 vol% as shown in Table 6.

Multiple myeloma

Multiple myeloma in the bone marrow was divided into two broad groups: (1) plasmacytic, primarily mature plasma cells, and (2) plasmablastic, mainly immature cells (Fig. 7, Table 4).

(1) Plasmacytic

A spectrum of plasma cells was usually present in any one biopsy. These were subdivided into three types. Type 1: mature Marschalko type plasma cells, indistinguishable from normals, with excentric cartwheel nuclei, perinuclear hof and basophilic cytoplasm; few had nucleoli. Type 2: mainly round or notched small (lymphoplasmacytoid) type plasma cells with little cytoplasm. Type 3: a polymorphous population consisting of types 1 and 2. All infiltrations had a fine reticulin network, and the residual marrow showed an increase in fat cells, reduction in haematopoiesis and maturation arrest of erythropoiesis. Increased remodelling of the cancellous bone was almost invariably present. Disease progression was accompanied by anaemia, infections and bone-related problems. The frequency of a leukaemic blood picture (plasma cell leukaemia) was highest in patients with type 2 and this had an unfavourable prognosis. The prognostic value of the initial plasma cell burden is shown in Table 6.

(2) Plasmablastic

The infiltration consisted of large, polymorphic, often multinuclear plasma cells with prominent central nucleoli, moderate to large amounts of cytoplasm and frequent mitotic figures. Dispersed among them were mature plasma cells, lymphocytes and immunoblasts. As the most frequent growth pattern was the packed marrow type, fat and haematopoiesis were scant. Increased osseous remodelling especially pronounced osteoclastic activity was frequent. The patients showed a rapid downhill course (Table 4), aggravated by hyper-

Table 3 *Frequency of bone marrow involvement in lymphoproliferative disorders (at time of initial diagnosis)*

Histological group	*Patients*	*Positive biopsies (%)*
Multiple myeloma	813	89
Plasmacytic	546	94
Plasmablastic	267	79
Non-Hodgkin's lymphomas	1351	64
Lymphocytic	286	99
Hairy cell	152	95
Immunocytic	253	85
Centrocytic	92	71
Centroblastic/cytic	260	20
"Blastic" (sarcomatous)	272	25
Unclassifiable	36	—
*Hodgkin's disease**	1011	8
Nodular sclerosis	363	3
Lymphocyte predominance	154	6
Mixed cellularity	346	9
Lymphocyte depletion	148	22

*Diagnosed only by lymph node histology.

Table 4 *Classification of lymphoproliferative disorders by bone marrow histology (at time of initial diagnosis)*

Histological groups		*Predominant cell type*	*Patients, % in each group*	*Median survivals* (months)*
Plasmacytic			546	36
Marschalko	(1)		70%	46
Small, round	(2)		10%	24
Small, notched	(3)		6%	19
Polymorphous	(1–4)		14%	16
Plasmablastic	(4)		267	9
Lymphocytic			283	43
Small, round	(1)		70%	48
Small, notched	(2)		5%	32
Large	(3)		20%	27
Prolymphocytic	(4)		5%	10
Lymphoblastic	(5)		13	6
Hairy cell			144	25
Ovoid	(1)		47%	57
Convoluted	(2)		37%	15
Indented	(3)		16%	5
?	(?)			
Immunocytic			215	46
Lymphoplasmacytoid	(1, 2)		49%	75
Lymphoplasmacytic	(1, 3)		46%	28
Polymorphous	(1–4)		5%	13
Immunoblastic	(4)		30	5
Centrocytic			65	25
Small, cleaved	(1)		60%	35
Large, cleaved	(2)		25%	16
Polymorphous	(1–3)		15%	12
Centroblastic/cytic	(3, 1)		52	50
Centroblastic	(3)		25	5
HD, low content of lymphocytes	(3, 4, 2, 1)		49	15
HD, high content of lymphocytes	(1, 2, 3, 4)		30	55
HD, high content of epithelioid cells	(2, 1, 3, 4)		5	62
AILD	(1–5)		38	28

*From the time of biopsy to death or date of last contact.
AILD = angioimmunoblastic lymphadenopathy.

Table 5 *Frequency of bone marrow patterns and their prognostic relevance in lymphoproliferative disorders (at time of initial diagnosis)*

Histological type	*Patients*	*Nodular*	*Interstitial/ nodular*	*Interstitial*	*Para-trabecular*	*Patchy focal*	*Packed marrow*
Multiple myeloma							
Plasmacytic	513	4% (50)	39% (29)	36% (40)	—	—	21% (16)
Plasmablastic	211	—	50% (12)	20% (21)	—	—	30% (5)
Non-Hodgkin's lymphomas							
Lymphocytic	283	—	32% (107)	42% (36)	—	—	26% (25)
Hairy cell	144	—	—	—	—	75% (28)	25% (18)
Immunocytic	215	41% (74)	33% (56)	6% (34)	—	—	20% (17)
Centrocytic	65	—	—	—	60% (29)	—	40% (19)
Centroblastic/cytic	52	80%(56)	—	—	—	—	20% (12)
"Blastic" (sarcoma type)	68	—	—	—	—	—	100% (5)
Hodgkin's disease							
Low content of lymphocytes	49	—	—	—	—	62% (17)	38% (12)
High content of lymphocytes	30	—	—	—	—	80% (60)	20% (42)
High content of epithelioid cells	5	—	—	—	—	60% (63)	40% (52)
Angioimmunoblastic lymphadenopathy	38	—	—	—	—	76% (34)	24% (12)

% = percentage in each histological type.
( ) = median survival time (months) from time of biopsy to death or date of last contact.

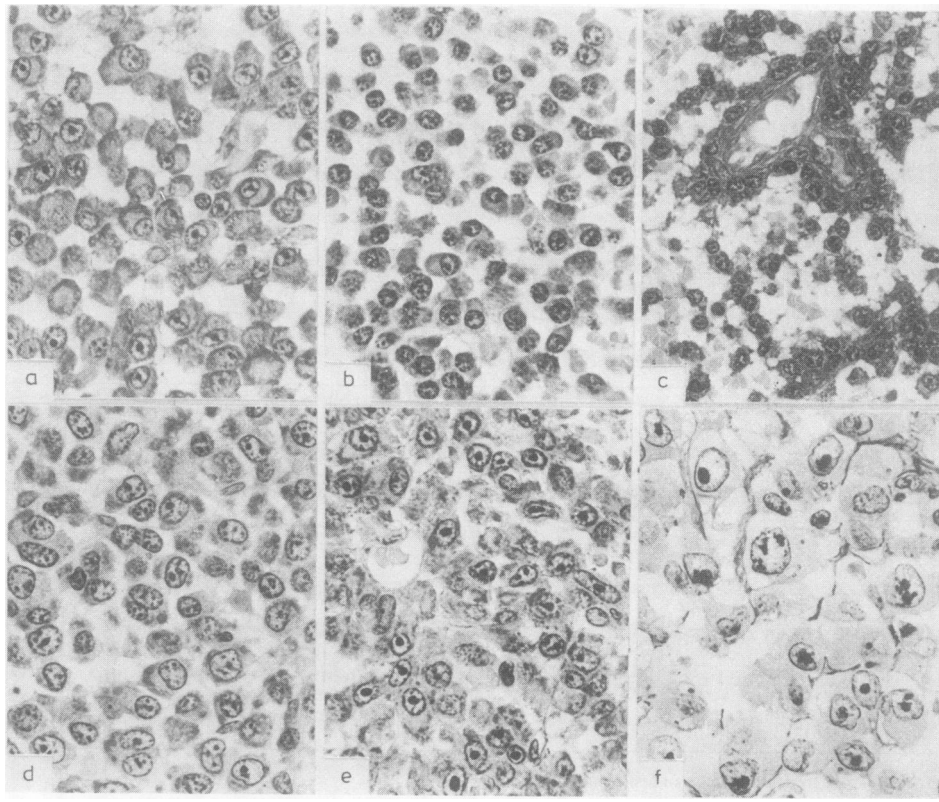


Fig. 7 *Classification of multiple myeloma based on bone marrow histology. Giemsa ×400: (a) multiple myeloma plasmacytic, Marschalko type; (b) multiple myeloma plasmacytic, small round type; (c) multiple myeloma plasmacytic, small notched type; (d) multiple myeloma plasmacytic, polymorphous type; (e) multiple myeloma plasmablastic; (f) multiple myeloma plasmablastic, pleomorphous type.*

calcaemia and renal insufficiency.

Non-Hodgkin's lymphomas

(1) Lymphocytic

The infiltrations consisted of typical small lymphocytes showing three growth patterns each with prognostic significance: (1) interstitial; (2) interstitial/nodular; (3) packed marrow (Table 5). Nodules with follicular centres were present in 25% of the biopsies. There was some variation in lymphocytic size and numbers of nucleated cells, a higher proportion of which indicated a poorer prognosis (Table 4). Fat and haematopoietic tissue were progressively reduced as the tumour cell burden increased and this constituted a reliable prognostic factor (Table 6). Six cases had somewhat larger lymphoid cells with nucleoli, moderate amounts of cytoplasm and a positive acid phosphatase reaction (prolymphocytic).[18] The bone marrow was extensively replaced with a corresponding reduction in normal elements and the prognosis was poor (Table 4). In 58 cases the lymphocytes had slight notches or indentations (Fig. 8b) corresponding to the B_2 lymphocyte.[31 47] These also had a more unfavourable prognosis.

(2) Hairy cell

There was a patchy to complete replacement of the marrow by hairy cells having abundant cytoplasm with lateral extensions, and rodlike inclusion bodies in 45% of the cases. The hairy cells were widely dispersed within a reticulin fibre network which also contained plasma cells, lymphocytes, mast cells and extravasated erythrocytes. The hairy cell nuclei displayed a wide morphological spectrum comprising three main configurations, one of which usually predominated in each biopsy and had predictive value. Type 1: in 47% of the cases small ovoid nuclei; type 2: in 37% of the cases medium sized convoluted nuclei; and type 3: in 16% of the cases large indented nuclei usually with a single prominent nucleolus (Table 4). Hairy cell involvement of the bone marrow occurred in three patterns: (1) multiple small patches; (2) large confluent areas; (3) complete replacement. The survival times correlated significantly with the amount of tumour cell burden (Table 6). A high incidence of inclusion bodies in any of the subtypes also indicated a poor prognosis. Splenectomy significantly prolonged survival in patients with both the ovoid and convoluted types.

(3) Immunocytic

The specific infiltration consisted mainly of small lymphocytes with variable numbers of mature plasma cells, plasmacytoid cells and mast cells in a hypocellular marrow. Most cases had some lymphoid cells with cytoplasmic or nuclear PAS-positive inclusions. As in the lymph nodes three prognostically different subtypes were distinguished: (1) the lymphoplasmacytoid in 49% showing mainly a nodular pattern and clinical splenomegaly; (2) the lymphoplasmacytic in 46% with numerous plasma and mast cells and a combined interstitial and nodular pattern, and clinical lymphadenopathy; (3) the polymorphous subtype in 5% consisting of lymphocytes, plasma cells, centrocytes, centroblasts and immunoblasts and exhibiting the packed marrow pattern with clinical lymphadenopathy, splenomegaly and pancytopenia. The infiltrated areas showed a fine reticulin fibrosis, and haematopoietic precursors were found within the infiltrations as well as in areas between them. The tumour burden in the initial biopsy correlated with the survival times (Table 6). Clinically 89% of the patients had IgM paraproteinaemia, the disease thus corresponding to Waldenström's macroglobulinaemia.

(4) Centrocytic

Most of the involved bone marrows showed paratrabecular infiltrations of small to medium sized lymphoid cells with cleaved nuclei and narrow rims of cytoplasm. Fibres radiating out from the trabeculae formed a reticulin network within the infiltrations. Between the paratrabecular seams the marrow spaces were occupied by fat cells and residual haematopoietic precursors. The size and nuclear morphology of the infiltrating cells were used for subtyping: small cleaved 46%, large cleaved 43%, and polymorphous 11%. The subtype classification and the extent of infiltration in the biopsy both showed significant correlations with survival (Tables 4, 6).

(5) Centroblastic/centrocytic

This lymphoma showed a strictly nodular pattern frequently of follicles with germinal centres consisting of lymphocytes, centrocytes and centroblasts within a fine reticular network. Between the nodules the marrow had a normal aspect both in structure and cellular composition. It was not possible to subtype this lymphoma which with 50 months had the longest median survival of all the malignant lymphomas with bone marrow involvement.

(6) Lymphoblastic (only sarcoma type, 16 cases), Centroblastic (8 cases), and Immunoblastic (5 cases) Each of these groups showed similar clinical and histological features. All involved marrows had a packed marrow pattern, with only isolated residual haematopoietic elements. All had a very unfavourable course with median survivals of about 5 months.

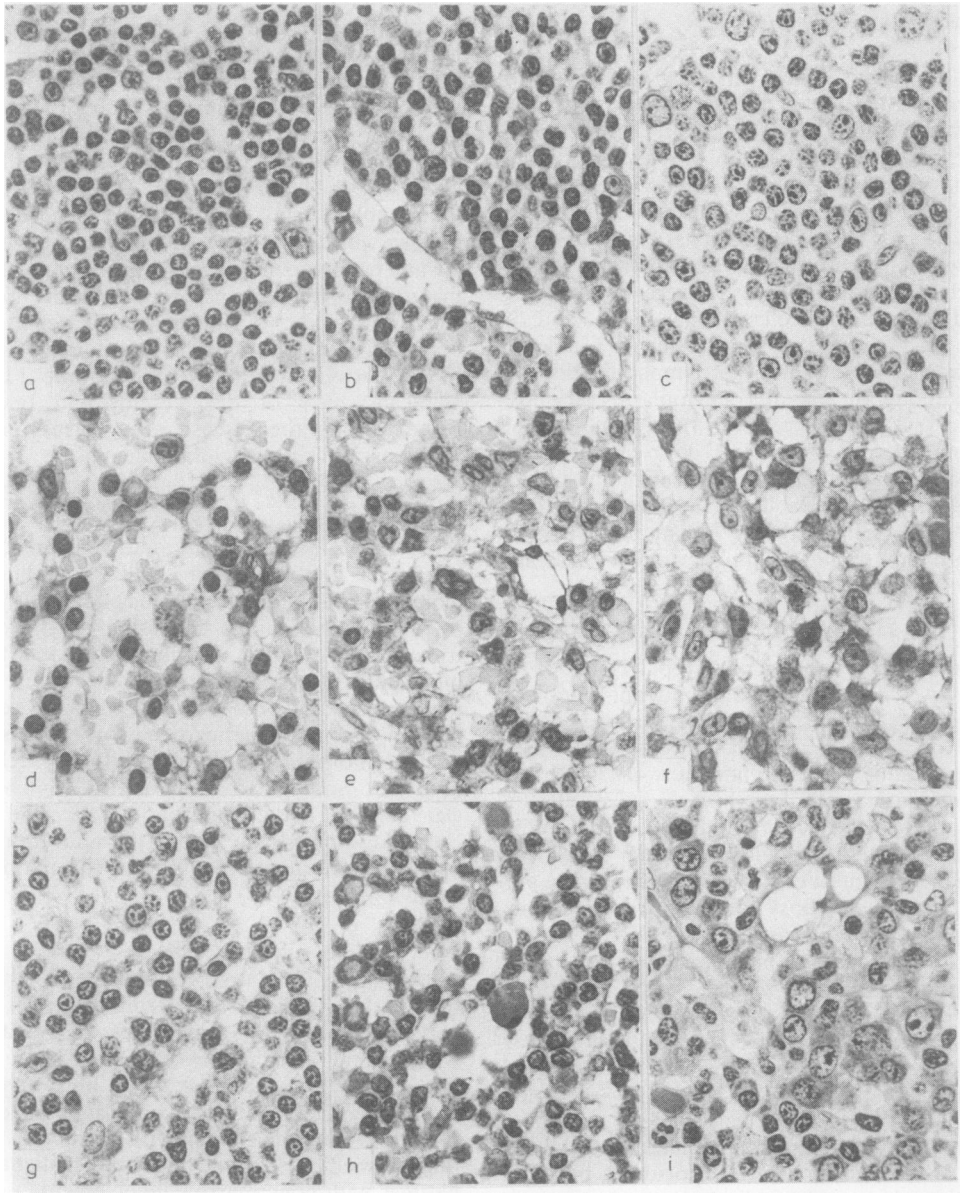


Fig. 8 *Classification of non-Hodgkin's lymphomas based on bone marrow histology (I). Giemsa ×400. (a) non-Hodgkin's lymphoma lymphocytic, small round type; (b) non-Hodgkin's lymphoma lymphocytic, small notched type; (c) non-Hodgkin's lymphoma lymphocytic, large type; (d) non-Hodgkin's lymphoma hairy cell, ovoid type; (e) non-Hodgkin's lymphoma hairy cell, convoluted type; (f) non-Hodgkin's lymphoma hairy cell, indented type; (g) non-Hodgkin's lymphoma immunocytic, lymphoplasmacytoid type; (h) non-Hodgkin's lymphoma immunocytic, lymphoplasmacytic type; (i) non-Hodgkin's lymphoma immunocytic, polymorphous type.*

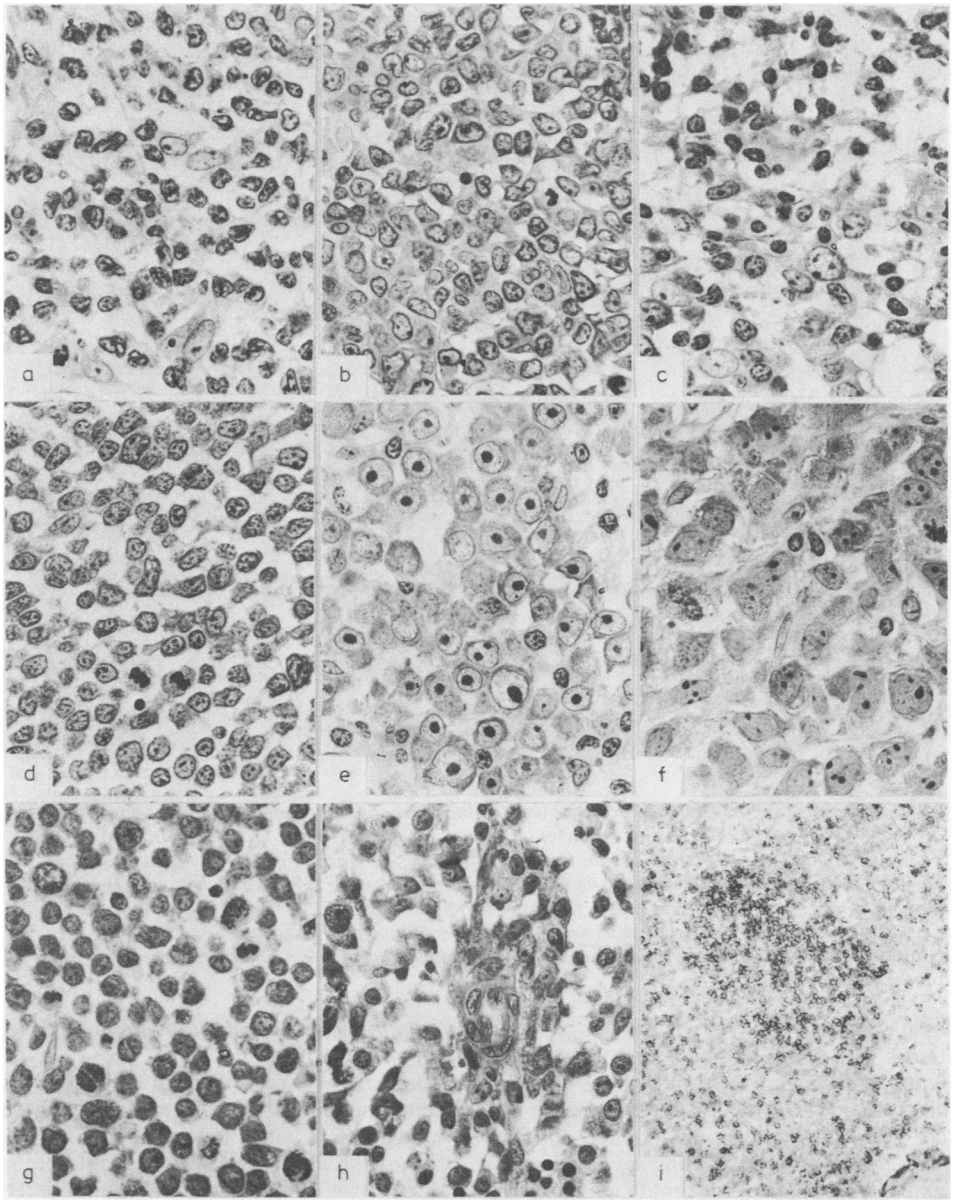


Fig. 9 *Classification of non-Hodgkin's lymphomas based on bone marrow histology (II). Giemsa ×400. (a) non-Hodgkin's lymphoma centrocytic, small type; (b) non-Hodgkin's lymphoma centrocytic, large type; (c) non-Hodgkin's lymphoma centroblastic/centrocytic; (d) non-Hodgkin's lymphoma lymphoblastic; (e) non-Hodgkin's lymphoma immunoblastic; (f) non-Hodgkin's lymphoma centroblastic; (g) non-Hodgkin's lymphoma prolymphocytic; (h) perivascular infiltration in a patient with mediastinal T cell lymphoma. Giemsa ×400; (i) nodular infiltration of T lymphocytes in immunocytoma. PAP ×100.*

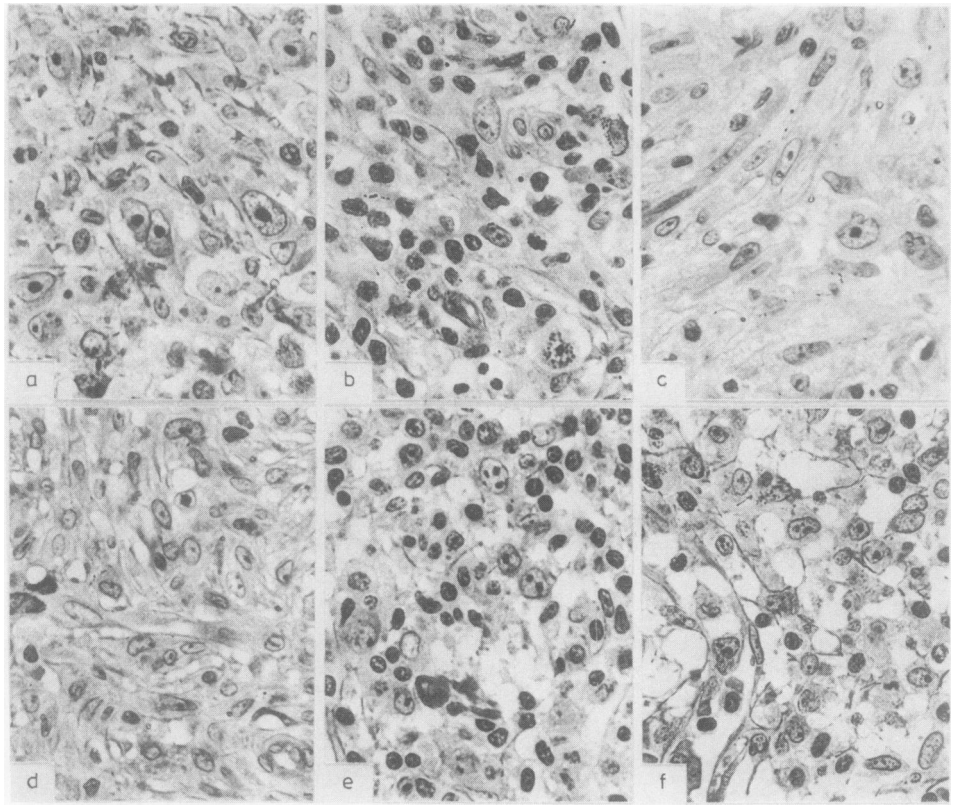


Fig. 10 *Classification of Hodgkin's disease and angioimmunoblastic lymphadenopathy based on bone marrow histology: (a) Hodgkin's disease with low content of lymphocytes. Giemsa ×400; (b) Hodgkin's disease with high content of lymphocytes. Giemsa ×400; (c) Hodgkin's disease with high content of epithelioid cells; (d) granulomatous tissue with atypical mononuclear cells in a patient with Hodgkin's disease, taken as evidence of involvement (criteria of Ann Arbor). Giemsa ×400; (e) angioimmunoblastic lymphadenopathy with a heterogeneous cell population and interstitial deposits. Giemsa ×400; (f) angioimmunoblastic lymphadenopathy with hyperplastic capillaries and a fine reticulin framework. Gomori ×400.*

(7) Unclassifiable
In 36 patients with non-Hodgkin's lymphomas we had problems classifying merely on the basis of bone marrow histology. However, 29 of them could be categorised as non-Hodgkin's lymphoma of high grade malignancy and the remaining seven cases as non-Hodgkin's lymphoma of low grade malignancy.

(8) Mediastinal lymphomas
Bone marrow biopsies taken in a few cases of mediastinal lymphomas (subsequently shown to be of T cell type) had a sparse interstitial and a somewhat more dense perivascular infiltration with preservation of haematopoietic tissue (Fig. 9h). This type of spread did not correspond to any of the distinct patterns described above, though it could well represent an early phase of the packed marrow type.

Hodgkin's disease and angioimmunoblastic lymphadenopathy
Bone marrow involvement in Hodgkin's disease has been described above. Reed-Sternberg cells were found in 62% and mononuclear Hodgkin cells in 95% of the involved cases. It should be noted that the extent of the infiltration (volume percentage or tumour cell burden) as used above does not apply to Hodgkin's disease in which the actual amount of tumour cell burden or putative malignant cells may be very low if by malignant cells is meant the number of Reed-Sternberg or Hodgkin cells. The rest of the infiltration consists of stromal elements contributed by the host. Nevertheless the degree of lymphocytic infiltration constituted a significant predictive factor. A low content of lymphocytes indicated a shorter life expectancy than a high con-

Table 6 *Staging of cumulative lymphoproliferative disorders by bone marrow histology (at time of initial diagnosis)*

Histological type	*Patients*	*Infiltration volume in the biopsy (vol%)**			*Survival statistics†*
		Stage I (<20)	*Stage II (20–50)*	*Stage III (>50)*	
Multiple myeloma					
Plasmacytic	546	40% (48)	40% (29)	20% (14)	++
Non-Hodgkin's lymphomas					
Lymphocytic	283	17% (78)	44% (38)	39% (31)	++
Hairy cell	144	7% (30)	44% (20)	49% (15)	+
Immunocytic	215	43% (75)	30% (57)	27% (18)	++
Centrocytic	65	25% (42)	40% (30)	35% (15)	+

*Volume percentage of the whole biopsy core.
†Breslow- and Mantel-Cox test; + = < 0·05, ++ = < 0·01.
% = Percentage in each histological type.
( ) = Median survival time (months) from time of biopsy to death or date of last contact.

tent—median survival of 15 and 55 months respectively (Table 4, Fig. 10a, b). Five patients had bone marrow involvement with a high degree of epithelioid cells correlating with a more favourable prognosis (Fig. 10c).

In our material, of 40 cases with angioimmunoblastic lymphadenopathy diagnosed by lymph node histology, bone marrow involvement was found in 24 cases. However, in another 14 patients a positive bone marrow was the only detected manifestation of angioimmunoblastic lymphadenopathy, without lymphadenopathy even during the course of disease. Involvement of the bone marrow was characterised by multiple, partly confluent foci with a heterogeneous cell population consisting of immunoblasts, lymphocytes, centrocytes, plasma cells and eosinophils. There were hyperplastic, occasionally arborising capillaries within a reticulin framework and interstitial deposition of PAS-positive material (Fig. 10e, f). Patients with angioimmunoblastic lymphadenopathy and bone marrow involvement had systemic symptoms in 85%, hepatosplenomegaly in 80% and a relatively short life expectancy with a median survival of 28 months. Conversion to haematological neoplasms was observed in only two cases (immunoblastoma, acute myeloid leukaemia).

HISTOLOGICAL VARIATION OF LYMPHOPROLIFERATIVE DISORDERS IN THE BONE MARROW

Two types of histological variations were observed: (1) simultaneous detection of different cell types and/or growth patterns within the same biopsy (coexistence) and (2) subsequent detection of different types and/or architectural patterns in follow-up biopsies (conversion, metamorphosis, transformation, progression). Furthermore myeloproliferative disorders or acute leukaemias developed in 62 cases as shown in Table 7.

Coexistence of lymphoproliferative disorders in the bone marrow

Double lymphoproliferative disorders at initial biopsy were observed in 16 cases (multiple myeloma 4, non-Hodgkin's lymphomas 10, Hodgkin's disease 2), and the combinations are given in Table 7. Co-

Table 7 *Histological variation of lymphoproliferative disorders in the bone marrow*

	Multiple myeloma	*Lymphocytic*	*Hairy cell*	*Immunocytic*	*Centrocytic*	*Centroblastic/ centrocytic*	*Non-Hodgkin's lymphoma†*	*Hodgkin's disease*	*Myeloproliferative disorders*	*Acute myeloid leukaemia*
Multiple myeloma	—	2 (2)*	— (1)	2 (2)	—	—	— (3)	—	9 (10)	— (7)
Lymphocytic	2 (2)	—	—	2 (2)	—	—	— (6)	1	1 (5)	—
Hairy cell	—	—	—	—	—	—	—	—	2 (1)	—
Immunocytic	2 (3)	2 (1)	—	—	—	—	— (7)	1	11 (12)	— (1)
Centrocytic	—	—	—	—	—	—	—	—	—	—
Centroblastic/ centrocytic	—	—	—	—	—	—	1 (2)	—	—	—
Non-Hodgkin's lymphoma	—	—	—	—	—	1	—	—	2 (1)	—
Hodgkin's disease	—	1	—	1	—	—	—	—	—	—

*Simultaneous detection at initial biopsy; in brackets subsequent detection at follow-up biopsies.
†Of high grade malignancy.

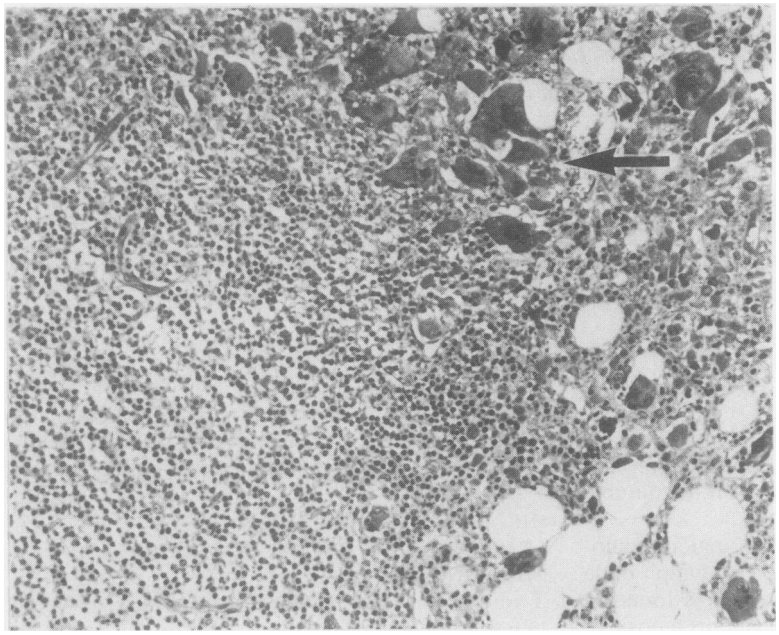


Fig. 11 *Concurrent immunocytoma and polycythaemia vera in the bone marrow. Note the polymorphous megakaryocytes at arrow. Giemsa ×100.*

existing lymphoproliferative and myeloproliferative disorders in the same bone marrow biopsy (Fig. 11) were detected in 25 cases; in 8 of these polycythaemia vera was found together with multiple myeloma. Differences in architectural pattern were occasionally observed in a single biopsy, in which case the predominant pattern was used for categorising.

Conversion of lymphoproliferative disorders in the bone marrow

Five hundred and twelve sequential biopsies were used to monitor the effects of therapy both on the infiltrations and on the haematopoietic tissues. Biopsies were also taken for restaging and for reclassification if clinical conversion was suspected. These serial biopsies demonstrated a change in lymphoid cell type in 31 cases, and conversion to a myeloid neoplasia in 37 cases (myeloproliferative disorders 29, acute myeloid leukaemia 8). Two patients with polycythaemia vera developed chronic lymphocytic leukaemia, 4 and 5 years respectively after the onset of disease. The growth patterns in the lymphoproliferative disorder remained stable in follow-up biopsies in most cases, thus reflecting the basic proliferative cell system as well as the tumour cell burden. In chronic lymphocytic leukaemia, nodularity correlated with a favourable prognosis, and transformation to a diffuse pattern signalled a faster disease progression. On the other hand, a switch from a diffuse to a nodular pattern was not observed in our series.

Discussion

Recognition of lymphoproliferations in the bone marrow

With increasing use of bone marrow biopsies as a diagnostic tool in internal medicine and oncology,[10 14 19–22] lymphocytes and lymphoid nodules are more frequently encountered, as their presence in the bone marrow may be reactive to a variety of non-haematological and haematological conditions.[23 24] Their recognition as benign or malignant is of considerable clinical importance especially as the benign infiltrates are more common, though prolonged follow-up is required for their recognition.[2 25–27] Moreover the number of plasma cells[28] as well as that of benign lymphoid nodules[18 24 29 30] increases in the older age groups while the overall cellularity in the iliac crest, from which bone biopsies are usually taken, tends to decrease with age. Coincidentally the peak incidence of the non-Hodgkin's lymphomas also occurs in the higher age groups.[30 31] In addition normal lymphocytes may be present together with neoplastic lymphoma cells, for example T lymphocytes in a B cell neoplasm such as follicle centre cell lymphoma or B-CLL, or the lymphocytes in the cellular infiltrates in Hodgkin's disease. The distinction between minimal benign and

neoplastic accumulations of lymphocytes and plasma cells[32] can best be made by means of immunological markers[14 33] and clearly benign lymphoid hyperplasia must be excluded when involvement of the bone marrow is suspected in an elderly patient with a lymphoproliferative disorder.[34] These observations support the hypothesis that the presence of a lymphoid neoplasm may be symptomatic of a widespread disturbance affecting the immune system as a whole and indicating defective immuneregulation, rather than being the expression of a single malignant cell clone.[35] The wide range of detection of bone marrow involvement reported in the literature[14 36] over the past decade is probably due to inclusion of unequal proportions of patients with early and advanced disease, as well as to technical aspects such as differences in biopsy size and preparation. Plastic embedded large single biopsies have proved their value in detection of minimal and focal lesions in the bone marrow.[10]

Growth patterns of lymphoproliferations in the bone marrow

The results presented here have confirmed and extended previous observations on the mode of spread of the lymphoproliferative disorders in the bone marrow as well as on the prognostic significance of the different patterns.[3 7 14 22 37–46] The more favourable course of a nodular as opposed to a diffuse spread was first recognised by Rappaport[66] for lymph nodes and recently confirmed by Damber *et al*[48] and by Straus *et al.*[49] It is not known what factors influence lymphoid cells to assume certain architectural arrangements in the bone marrow though these might include an inherent behavioural tendency to aggregate; a propensity to form architectural structures as in the lymph nodes; some local topographic influence or a chemotactic attraction to specific areas in the bone marrow. Why the centrocytic lymphomas should show a paratrabecular predilection is unclear, perhaps an association with the paratrabecular sinus is involved. It might be significant that whereas red and white cell and platelet precursors have preferred topographic localisations in the bone marrow none is known for lymphopoiesis. Nevertheless the chronic lymphoproliferative disorders exhibit characteristic modes of spread which provide independent information and should therefore be included in any classification system of lymphoproliferative disorders in the bone marrow.

The presence of a lymphoproliferative disorder in the bone marrow almost invariably has some effect on the bone. The most extensive and serious complications are generally seen in multiple myeloma, though osteolytic bone lesions with accompanying hypercalcaemia may occur in any lymphoproliferative disorder.[50–52] An early bone biopsy will give warning of increased activity so that measures may be taken to avoid osteolysis and hypercalcaemia.[53]

Histological classification of lymphoproliferative disorders in the bone marrow

General agreement on classification of the non-Hodgkin's lymphomas has not been achieved in spite of publication of the International Working Formulation.[54] In the United States the Lukes-Collins classification is widely used;[55–58] while in Europe there is a tendency for the Kiel classification to be employed.[31 59–61] One explanation is that though the modern trend is towards an immune based functional classification morphology remains the foundation of histopathology. In many cases the differentiation between B and T lymphomas may be made on that basis alone as their morphological features have now been well defined and in most instances are characteristic.[47 61–66] Moreover, in recent prospective studies[67] the histopathological grading of malignancy (low or high according to the Kiel classification) emerged as a powerful independent prognostic factor. The results of this study utilising the Kiel criteria show that classification by bone marrow histology is feasible, reproducible and has prognostic significance.

In the B cell lymphomas, histological assessment also requires estimation of the cellular composition which reflects the diverse structural forms of the neoplastic clone, as cells in each of the stages of the developmental pathway (lymphocytes, follicle centre cells, immunoblasts and plasma cells) are represented though their relative proportions vary in the different entities.[68 69] This explains the mixed populations of cells seen in the involved bone marrows which enabled subtype recognition.[34 70] Moreover recent work indicates that the different entities within the lymphoproliferative disorders are not sharply separated as shown for example by circulating lymphocytes bearing the same idiotype determinants as the plasma cells in patients with multiple myeloma[71 72] or cytoplasmic inclusions containing immunoglobulins in chronic lymphocytic leukaemia cells.[73–75] Likewise a strict categorisation to specific steps in the maturation pathway itself has not been confirmed by electron microscopic observation of lymphocytes during transformation,[76] by studies of immunoglobulin secretion showing that centrocytes may precede or follow centroblasts,[77] and by demonstration of acid hydrolases[78] in immature as well as in mature lymphocytes. Thus the presence of variable populations may help to account for unequal responses to therapy of patients with a superficially similar chronic lymphoproliferative

disorder and this may apply to pattern as well as to cell type.[79-81]

Staging and evolution of lymphoproliferative disorders in the bone marrow

Clinical staging procedures[82-87] attempt to estimate the spread and quantity of the tumour cell burden which in some cases is reflected in the volume percentage in the biopsy. But there are differences between the entities which may be roughly divided into three groups:[2] (1) the cumulative lymphomas such as chronic lymphocytic leukaemia, immunocytoma and hairy cell leukaemia with a systemic presentation in which stage is indicated by the amount of infiltration in the bone biopsy, (2) the primarily regional lymphomas with a centrifugal spread in which bone marrow involvement indicates stage IV, and (3) lymphomas with a sarcomatous (metastatic) growth indicating systemic disease, though not necessarily wide spread.

Recent reports have confirmed earlier ones on the occurrence of histological progression, conversion and transformation in the chronic lymphoproliferative disorders.[38 88-99] Since blasts, as shown in this survey, are normally present in the chronic lymphoproliferative disorders in the bone marrow, a transformation such as Richter's in chronic lymphocytic leukaemia[100] or to immunoblastic sarcoma in multiple myeloma or immunocytoma[101-103] or diffuse large cell from nodular lymphocytic lymphoma[104] may point to a shift in proliferation advantage of a cell type already present over another. Alternatively, the ability to mature is steadily decreased. Whether this is analogous to the blastic transformation as seen in chronic myeloid leukaemia or represents an additional mutation, or is treatment-induced, cannot be decided at present. However, reports of acute leukaemia supervening in untreated cases of lymphatic malignancies are extremely rare[105] in contrast to plasma cell neoplasias.[106] Zalcberg and coworkers[107] have shown that chronic lymphocytic leukaemia may arise after multiple myeloma and that two different clones are involved. On the other hand evolution of one lymphoma from another—for example, Sézary's syndrome in the course of hairy cell leukaemia—has also been described.[108] Moreover, coexistent lymphoproliferative and myeloproliferative disorders have been documented suggesting an expansion from a common abnormal pluripotent stem cell.[109] The high frequency of lympho/myeloproliferative disorders diagnosed either simultaneously or subsequently, found in this study, supports this unifying concept of haematological neoplasias. We have demonstrated by means of serial biopsies taken during extended follow-up periods of both treated and untreated patients that the bone marrow was not entirely cleared of lymphoma. Other long term follow-up studies have emphasised the fatal outcome of patients treated conservatively.[49] This underlines the question put by Longo *et al*[110] "What is so good about the good prognosis lymphomas?" since they cannot be cured. Indeed a chance for eradication might arise when conversion to a more malignant histology occurs—provided the cells had not previously been made resistant to therapy. This idea has led, in some centres, to the "watch and wait" approach.[94] Different aspects have been stressed in recent critical studies of staging and prognostic factors in the multiple myelomas and lymphomas. Some have emphasised the importance of the plasma cell mass in the bone marrow and its maturity as indicated by labelling indices.[111-116] These correspond to the infiltration volume and subtypes with nucleolated cells as shown in this study. Moreover cases with immature cells were prone to develop an aggressive terminal phase with emergence of a blastic type of multiple myeloma.[102 103] Other investigators have stressed clinical parameters such as haemoglobin, blood urea and β_2-microglobulin levels;[114 117] and the predictive value of some was confirmed in this study also. Nevertheless in a recent survey of patients surviving for more than 10 years,[118] the response to therapy was the most important factor in recognising long term survivors though this was not mentioned in the recent results of the Medical Research Council's Working Party on long term survival in myelomatosis (patients followed for up to 12 years).[119] Our own study shows that estimation of the tumour cell burden and of the cytological subtypes in the bone marrow biopsy are reliable prognostic indicators in multiple myeloma. Likewise in the cumulative variants of non-Hodgkin's lymphomas (chronic lymphocytic leukaemia, hairy cell leukaemia, immunocytic and centrocytic lymphomas) the infiltration volume in the biopsy reflects the tumour size and proved to be a reliable parameter for histological staging.[34 38 70] In chronic lymphocytic leukaemia, Rozman *et al* interpreted the different architectural patterns as variations in the amount of lymphoid accumulation during the natural course of disease.[85] However, we have shown that nodularity in the bone marrow in chronic lymphocytic leukaemia indicates a favourable prognosis and thereby reflects the intrinsic "malignancy" rather than the "stage" of the tumour.[38]

Bone marrow biopsy is now an integral part of staging in Hodgkin's disease since bone marrow involvement is one of the criteria for systemic disease, i.e. stage IV, indicating haematogenous spread

(as the bone marrow has no lymphatics) and an unfavourable prognosis.[44 120–122]. Furthermore the degree of Hodgkin's disease infiltration assessed in large scale biopsies proved to be a prognostic factor though not so significant as in the cumulative, primarily systemic lymphoproliferative disorders.[123] Angioimmunoblastic lymphadenopathy is regarded as a "hyperimmune entity resembling Hodgkin's disease"[124] or a "defectively regulated immune response to an unidentified antigen(s)".[125 126] The high frequency of bone marrow involvement previously reported[127 128] has been confirmed in this study, suggesting a primarily systemic onset. However, in about a third of the cases exhibiting the histologic picture of angioimmunoblastic lymphadenopathy in the bone marrow, no lymphadenopathy was evident during the course of disease: these cases were called "angioimmunoblastic myelopathy". The differentiation of these granulomatous reactions from those occurring in Hodgkin's disease (without Hodgkin or Reed-Sternberg cells) and in rheumatic or allergic conditions is not always possible.[20]

In conclusion, bone marrow biopsy has proved its value not only for identifying, classifying and staging of lymphoproliferative disorders at presentation, but also for monitoring and restaging during the course of disease. Progression, metamorphosis as well as consequences of therapy—success or failure—may be quantitatively and qualitatively evaluated by serial biopsies. Thus, in the bone marrow biopsy the clinician has a tool at his disposal which supplies decisive information on the diagnosis and therapy of any given patient with lymphoproliferative disorders.

The authors would like to express their gratitude to all colleagues who referred patients or sent biopsies. This work was supported by the Gesellschaft für Strahlen- und Umweltforschung mbH, Munich.

References

[1] Jones SE, Rosenberg SA, Kaplan HS. Non-Hodgkin's lymphomas. I. Bone marrow involvement. *Cancer* 1972;**29**:954–60.

[2] Bartl R, Frisch B, Burkhardt R, Kettner G, Mahl G, Fateh-Moghadam A, Sund M. Assessment of bone marrow histology in the malignant lymphomas (non-Hodgkin's): correlation with clinical factors for diagnosis, prognosis, classification and staging. *Br J Haematol* 1982;**51**:511–30.

[3] Coller BS, Chabner BA, Gralnick HR. Frequencies and patterns of bone marrow involvement in non-Hodgkin's lymphomas: observations of the value of bilateral biopsies. *Am J Hematol* 1977;**3**:115–9.

[4] Foucar K, McKenna RW, Frizzera G, Brunning RD. Incidence and patterns of bone marrow and blood involvement by lymphoma in relationship to the Lukes-Collins classification. *Blood* 1979;**14**:1417–22.

[5] Foucar K, McKenna RW, Frizzera G, Brunning RD. Bone marrow and blood involvement by lymphoma in relationship to the Lukes-Collins classification. *Cancer* 1982;**49**:888–97.

[6] Georgii A. Classification of non-Hodgkin lymphomas from biopsies of the bone marrow with special emphasis to their spread. In: Growther DG, ed. *Advances in medical oncology, research and education*, vol. VII. *Leukemia and non-Hodgkin lymphoma.* Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1979:179–88.

[7] Rosenberg SA. Hodgkin's disease of the bone marrow. *Cancer Res* 1971;**31**:1733–6.

[8] Rosenberg SA. Bone marrow involvement in the non-Hodgkin's lymphomata. *Br J Cancer* 1975;**31** suppl II:261–4.

[9] Burkhardt R. Bone marrow biopsy in malignant lymphoma. *Verh Dt Ges Pathol* Luzern 67. Tagung. Stuttgart, New York: Gustav Fischer Verlag, 1983:522–47.

[10] Burkhardt R. *Bone marrow and bone tissue: colour atlas of clinical histopathology.* Berlin: Springer, 1971.

[11] Burkhardt R. Bone marrow histology. In: Catovsky ed. *Methods in hematology. The leukemic cell.* Edinburgh: Churchill Livingstone, 1981:49–86.

[12] Jamshidi K, Swaim WR. Bone marrow biopsy with unaltered architecture—a new biopsy device. *J Lab Clin Med* 1971;**77**:335–42.

[13] Bartl R, Burkhardt R, Vondracek H, Sommerfeld W, Hagemeister E. Rationelle Beckenkamm-Biopsie. Längsteilung der Proben zur Anwendung von mehreren Präparationsverfahren ohne Materialverlust. *Klin Wochenschr* 1978;**56**:545–50.

[14] Bartl R, Frisch B, Burkhardt R. *Bone marrow biopsies revisited. A new dimension for haematological malignancies.* Basel: Karger, 1982.

[15] Hoffmann-Fezer G, Thierfelder S, Pielsticker K, Rodt H. Immunohistochemical demonstration of cell surface antigens on tissue sections of lymphomas. *Leuk Res* 1979;**3**:297–304.

[16] Dixon WJ, Brown MB. BMDP-79 Biomedical computer programs p-series. System, program and statistical development. Berkeley: University of California Press, 1979.

[17] Hashimoto M, Masanori H, Tsukasa S. Lymphoid nodules in human bone marrow. *Acta Pathol Jpn* 1957;**7**:33–52.

[18] Galton DAG, Goldman JM, Wiltshaw E, Catovsky D, Henry K, Goldenberg GJ. Prolymphocytic leukaemia. *Br J Haematol* 1974;**27**:7–23.

[19] Burkhardt R, Frisch B, Bartl R. Bone biopsy in haematological disorders. *J Clin Pathol* 1982;**35**:257–84.

[20] Frisch B, Bartl R, Burkhardt R. Bone marrow biopsy in clinical medicine: an overview. *Haematologia* 1982;**3**:245–85.

[21] Krause JR. Lymphoproliferative disorders. In: Krause JR, ed. *Bone marrow biopsy.* New York, Edinburgh, London, Melbourne: Churchill Livingstone, 1981.

[22] Rywlin AM. *Histopathology of the bone marrow.* Boston: Little, Brown and Co, 1976.

[23] Jäger K, Burkhardt R, Bartl R, Frisch B, Mahl G. Benign lymphoid nodules in chronic myeloproliferative disorders. *Verh Dt Ges Pathol* Luzern 67. Tagung. Stuttgart, New York: Gustav Fischer Verlag, 1983:239–42.

[24] Rywlin AM, Ortega RS, Dominguez GJ. Lymphoid nodules of bone marrow, normal and abnormal. *Blood* 1974;**43**:389–400.

[25] Fine JM, Lambin P, Massari M, Leroux P. Malignant evolution of asymptomatic monoclonal IgM after seven and fifteen years in two siblings of a patient with Waldenström's macroglobulinemia. *Acta Med Scand* 1982;**211**:237–9.

[26] Gordon DS, Jones BM, Browning SW, Spira TJ, Lawrence DN. Persistent polyclonal lymphocytosis of B lymphocytes. *N Engl J Med* 1982;**307**:232–6.

[27] Waldenström JG. The benign monoclonal gammapathies: a study of monoclonal antibodies. In: Frick P *et al*, eds. *Advances in internal medicine and pediatrics*, vol 50. Berlin: Springer Verlag, 1982:31–77.

[28] Bartl R, Frisch B, Burkhardt R, *et al.* Bone marrow histology in myeloma: its importance in diagnosis, prognosis, classification

and staging. *Br J Haematol* 1982;**51**:511–30.

[29] Bartl R, Burkhardt R, Schlag R, Hill W. Nodular lymphoid hyperplasia and malignant lymphoma in the bone marrow biopsy in advanced age. 6th Meeting Internation Society of Haematology, European Division, Athens, 1981.

[30] Burkhardt R, Bartl R, Jäger K, Ehret W, Schlag R, Demmler K. Histobioptische Knochenmarksbeurteilung im Senium. In: Böhnel J, Heinz R, Stacher A, eds. *Hämatologie im Alter.* München, Wien, Baltimore: Urban & Schwarzenberg, 1982:20–28.

[31] Lennert K, Stein H. *Histopathology of non-Hodgkin's lymphomas* (based on the Kiel classification). Berlin: Springer-Verlag, 1981.

[32] Canioni D, Kermarec J. Interprétation des plasmocytoses médullaires minimes dans les dysglobulinémies monoclonales. Intérêt de l'étude de biopsies ostéomédullaires sur coupes semi-fines et par marquage immunologique. *Ann Pathol* 1982;**2**:279–92.

[33] Pizzolo G, Chilosi M, Cetto GL, Fiore-Donati L, Janossy G. Immuno-histological analysis of bone marrow involvement in lymphoproliferative disorders. *Br J Haematol* 1982;**50**:95–100.

[34] Bartl R, Frisch B, Mahl G, Burkhardt R, Fateh-Moghadam A, Pappenberger R, Sommerfeld W, Hoffmann-Fezer G. Bone marrow histology in Waldenstroem's Macroglobulinaemia. Clinical relevance of subtype recognition. *Scand J Haematol* 1983;**31**:359–75.

[35] Habesaw JA, Bailey D, Stansfeld AG, Greaves MF. The cellular content of non Hodgkin lymphomas: A comprehensive analysis using monoclonal antibodies and other surface marker techniques. *Br J Cancer* 1983;**47**:327–51.

[36] Brunning RD, McKenna RW. Bone marrow manifestations of malignant lymphoma and lymphoma-like conditions. In: Sommers SC, Rosen PP, eds. *Pathology annual*, part I. New York: Appleton-Century-Crofts, 1979:1–59.

[37] Bartl R, Burkhardt R, Gierster P, Sandel P, Fateh-Moghadam A. Significance of bone marrow biopsy in the multiple myeloma. In: Ulutin ON. Recent Progress in cell biology: leukocytes and platelets. *Bibl Haematol* 1978;**45**:81–6.

[38] Bartl R, Frisch B, Burkhardt R, Hoffmann-Fezer G, Demmler K, Sund M. Assessment of marrow trephine in relation to staging in chronic lymphocytic leukaemia. *Br J Haematol* 1982;**51**:1–15.

[39] Carbone A, Santoro A, Pilotti S, Rilke F. Bone marrow patterns and clinical staging in chronic lymphocytic leukaemia. *Lancet* 1978;i:606.

[40] O'Carroll DJ, McKenna RW, Brunning MD. Bone marrow manifestations of Hodgkin's disease. *Cancer* 1976;**38**:1717–28.

[41] Charron D, Dighiero G, Raphael M, Binet JL. Bone-marrow patterns and clinical staging in chronic lymphocytic leukaemia. *Lancet* 1977;ii:819.

[42] Hernandez-Nieto L, Montserrat-Costa E, Muncunill J, Rozman C. Bone-marrow patterns and clinical staging in chronic lymphocytic leukaemia. *Lancet* 1977;ii:1269.

[43] Lipshutz MD, Mir R, Rai KR, Sawitzsky A. Bone marrow biopsy and clinical staging in chronic lymphocytic leukemia. *Cancer* 1980;**46**:1422–7.

[44] Rilke F, Pilotti S, Carbone A, Lombardi L. Morphology of lymphatic cells and of their derived tumours. *J Clin Pathol* 1978;**31**:1009–56.

[45] Rowan RM. Multiple myeloma: some recent developments. *Clin Lab Haematol* 1982;**4**:211–30.

[46] Gray JL, Jacobs A, Block M. Bone marrow and peripheral blood lymphocytosis in the prognosis of chronic lymphocytic leukaemia. *Cancer* 1974;**33**:1169–78.

[47] Ralfkiaer E, Geisler C, Hansen MM, Hou-Jensen K. Nuclear clefts in chronic lymphocytic leukaemia. A light microscopic and ultrastructural study of a new prognostic parameter. *Scand J Haematol* 1983;**30**:5–12.

[48] Damber L, Lenner P, Lundgren E. The impact of growth pattern on survival in non-Hodgkin's lymphomas classified according to Lukes and Collins. *Pathol Res Pract* 1982;**174**:42–52.

[49] Straus DJ, Filippa DA, Lieberman PH, *et al.* The non-Hodgkin's lymphomas. I. A retrospective clinical and pathologic analysis of 499 cases diagnosed between 1958 and 1969. *Cancer* 1983;**51**:101–9.

[50] Valentin-Opran A, Charhon SA, Meunier PJ, Edouard CM, Arlot ME. Quantitative histology of myeloma-induced bone changes. *Br J Haematol* 1982;**52**:601–10.

[51] Demanes DJ, Lane N, Beckstead JH. Bone involvement in hairy-cell leukemia. *Cancer* 1982;**49**:1697–1701.

[52] Redmond J, Stites DP, Beckstead JH, George CB, Casavant CH, Grandara DR. Chronic lymphocytic leukemia with osteolytic bone lesions, hypercalcemia, and monoclonal protein. *Am J Clin Pathol* 1983;**79**:616–20.

[53] Paterson AD, Kanis JA, Cameron EC, *et al.* The use of dichloromethylene diphosphonate for the management of hypercalcaemia in multiple myeloma. *Br J Haematol* 1983;**54**:121–32.

[54] Dorfman RF, Burke JS, Berard CW. A new working formulation of non-Hodgkin's lymphomas: background, recommendations, histological criteria, and relationship to other classifications. In: *Advances in malignant lymphomas.* Proc Third Ann Bristol Meyers Symposium on Cancer Research. New York: Academic Press, 1981.

[55] Aisenberg AC. Cell lineage in lymphoproliferative disease. *Am J Med* 1983;**74**:679–85.

[56] Herrmann R, Barcos M, Stutzman L, Walsh D, Freeman A, Sokal J, Henderson ES. The influence of histologic type on the incidence and duration of response in non-Hodgkin's lymphoma. *Cancer* 1982;**49**:314–22.

[57] Lukes RJ, Parker JW, Taylor CR, Tindle BH, Cramer AD, Lincoln TL. Immunologic approach to non-Hodgkin's lymphomas and related leukemias. Analysis of multiparameter studies of 425 cases. In: Freireich EJ, ed. *Leukemia and lymphoma.* New York: Grune & Stratton, 1978:65–94.

[58] Nathwani BU, Kim H, Rappaport H, Solomon J, Fox M. Non-Hodgkin's lymphomas. A clinicopathologic study comparing two classifications. *Cancer* 1978;**41**:303–25.

[59] Bettini R, Chelazzi G. Prognostic value of the Kiel classification of malignant non-Hodgkin's lymphomas. *Tumori* 1979; **65**:207–13.

[60] Glimelius B, Hagberg H, Sundström C. Morphological classification of non-Hodgkin malignant lymphoma. II. Comparison between Rappaport's classification and the Kiel classification. *Scand J Haematol* 1983;**30**:13–24.

[61] Wright DH. The identification and classification of non-Hodgkin's lymphoma: a review. *Diagn Histopathol* 1982; **5**:73–111.

[62] Bain GO. Non-Hodgkin's lymphomas. Analysis of 92 cases using the "international" classification. *Arch Pathol Lab Med* 1983;**107**:64–9.

[63] Barton JC, Conrad ME, Vogler LB, Parmley RT. Isolated marrow lymphoma: an entity of possible T-cell derivation. *Cancer* 1980;**46**:1767–74.

[64] Dosoretz DE, Raymond A, Murphy GGF, Doppke KP, Schiller AL, Wang CC, Suit HD. Primary lymphoma of bone. The relationship of morphological diversity to clinical behavior. *Cancer* 1982;**50**:1009–14.

[65] Mann RB, Jaffe ES, Berard CW. Malignant lymphomas—a conceptual understanding of morphologic diversity. *Am Ass Pathol* 1979;**94**:105–57.

[66] Rappaport H. Tumours of the hematopoietic system. In: *Atlas of tumor pathology.* Section 3, vol 8. Washington: Armed Forces Institute of Pathology, 1966.

[67] Leonard RCF, Cuziek J, MacLennan ICM, *et al.* Prognostic factors in non-Hodgkin's lymphoma: the importance of symptomatic stage as an adjunct to the Kiel histopathological

classification. *Br J Cancer* 1983;**47**:91–102.

[68] Lennert K, Burkert M. Lymphoplasmacytic/lymphoplasmacytoid lymphoma (LP Immunocytoma). In: van den Tweel JG *et al*, eds. The Hague, Boston, London: Martinus Nijhoff Publishers Co, 1980:245–7.

[69] Taylor CR, Parker JW, Pattengale PK, Lukes RJ. Malignant lymphomas: an exercise in immunopathology. In: Crowther DG, ed. *Leukemia and non-Hodgkin-lymphoma. Advances in Medical Oncology. Research and Education,* vol VII. Oxford: Pergamon Press, 1979:125–40.

[70] Bartl R, Frisch B, Hill W, Burkhardt R, Sommerfeld W, Sund M. Bone marrow histology in hairy cell leukemia: identification of subtypes and their prognostic significance. *Am J Clin Pathol* 1983;**79**:531–45.

[71] Petterson D, Mellstedt H, Holm G, Björkholm M. Monoclonal blood lymphocytes in benign monoclonal gammopathy and multiple myeloma, in relation to clinical stage. *Scand J Haematol* 1981;**27**:287–93.

[72] Warner TFCS, Krueger RG. Circulating lymphocytes and the spread of myeloma: review of the evidence. *Lancet* 1978;i:1174–6.

[73] Berrebi A, Talmor M, Vorst E, Resnitzky P, Shtalrid M. IgM Lambda globular cytoplasmic inclusions in chronic lymphocytic leukaemia resembling immunocytoma. *Scand J Haematol* 1983;**30**:43–9.

[74] Han T, Ozer H, Bloon M, Sagawa K, Minowada J. The presence of monoclonal cytoplasmic immunoglobulins in leukemic B cell from patients with chronic lymphocytic leukemia. *Blood* 1982;**69**:435–8.

[75] Spagnolo DV, Papadimitriou JM, Matz LR, Walters MNI. Nodular lymphomas with intracellular immunoglobulin inclusions: report of three cases and a review. *Pathology* 1982;**14**:415–27.

[76] Dardick I, Setterfield G, Hall R, Bladon T, Little J, Kaplan G. Nuclear alterations during lymphocyte transformation. Relationship to the heterogenous morphologic presentations of non-Hodgkin's lymphomas. *Am J Pathol* 1981;**103**:10–20.

[77] Hannam-Harris GJ, Wright DH, Smith JL. Correlation between Ig-synthesis patterns and lymphoma classification. *Br J Cancer* 1982;**46**:1467–72.

[78] Grossi CE, Zicca A, Leprini A, Cadoni A, Pilstoia V, Ferrarini M. Acid hydrolases as markers of maturation in B-cell chronic lymphocytic leukemia. *Blood* 1982;**60**:220–7.

[79] Barcos M, Herrmann R, Pickren JW, Naeher C, Han T, Stutzman L, Henderson ES. The influence of histologic type on survival in non-Hodgkin's lymphoma. *Cancer* 1981;**47**:2894–900.

[80] Economopoulos T, Fotopoulos S, Hatzioannou J, Gardikas C. "Prolymphocytoid" cells in chronic lymphocytic leukaemia and their prognostic significance. *Scand J Haematol* 1982;**28**:238–42.

[81] Warnke RA, Kim H, Fuks Z, Dorfman RF. The coexistence of nodular and diffuse patterns in nodular non-Hodgkin's lymphomas. Significance and clinicopathologic correlation. *Cancer* 1977;**40**:1229–33.

[82] Baccarani M, Cavo M, Gobbi M, Lauria F, Tura S. Staging of chronic lymphocytic leukemia. *Blood* 1982;**6**:1191–6.

[83] Geisler C, Hansen MM. Chronic lymphocytic leukaemia: a test of proposed new clinical staging system. *Scand J Haematol* 1981;**27**:279–86.

[84] Jansen J, Hermans J. Clinical staging system for Hairy-Cell leukemia. *Blood* 1982;**6**:571–6.

[85] Rozman C, Hernandez-Nieto L, Montserrat E, Bougues P. Prognostic significance of bone marrow patterns in chronic lymphocytic leukaemia. *Br J Haematol* 1981;**47**:529–37.

[86] Skinnider LF, Tan L, Schmidt J, Armitage G. Chronic lymphocytic leukemia. A review of 745 cases and assessment of clinical staging. *Cancer* 1982;**50**:2951–5.

[87] Vercelli D, Cozzolino R, Di Guglielmo R. A comparison of two staging systems for myeloma. *Nouv Rev Franc Hématol* 1982;**23**:107–10.

[88] Cullen MH, Lister TA, Brearley RL, *et al.* Histologic transformation of non-Hodgkin's lymphoma. A prospective study. *Cancer* 1979;**44**:645–51.

[89] Erickson DJ, Cousar JB, Flenner JM, *et al.* Transformation of follicular center cell (FFC) lymphomas (Lukes-Collins classification): progression of small cleaved cell (SCC) type to transformed cell type. *Lab Invest* 1981;**44**:16A.

[90] Fisher RI, Jones RB, DeVita VT jr, *et al.* Natural history of malignant lymphomas with divergent histologies at staging evaluation. *Cancer* 1981;**47**:2022–5.

[91] Goffinet DR, Warnke R, Dunniok NR. Clinical and surgical (laparotomy) evaluation of patients with non-Hodgkin's lymphomas. *Cancer Treat Rep* 1977;**61**:981–92.

[92] Greenberg BR, Miller C, Cardiff RD, MacKenzie MR, Walling P. Concurrent development of preleukaemic, lymphoproliferative and plasma cell disorders. *Br J Haematol* 1983;**53**:125–33.

[93] Hoppe RT. Histologic variation in non-Hodgkin's lymphomas: commentary. *Cancer Treat Rep* 1981;**65**:11–12.

[94] Hubbard SM, Chabner BA, DeVita VT, *et al.* Histologic progression in non-Hodgkin lymphoma. *Blood* 1982;**59**:258–64.

[95] Jennete JC, Reddick RL, Saunders AW, Wilkman AS. Diffuse T cell lymphoma preceded by nodular lymphoma. *Am J Pathol* 1982;**78**:242–8.

[96] Jones RB. Garvin AJ, Canellos GP, Osborne CK, Young RC. Histologic progression in Hodgkin's lymphoma. *Blood* 1982;**59**:258–64.

[97] Kim H, Henrickson MR, Dorfman RF. Composite lymphoma. *Cancer* 1977;**40**:959–76.

[98] Leyser S, Variakojis D, Mintz U, Vardiman JW, Ultmann JE. Multiple histologic subtypes of non-Hodgkin's lymphoma: clinical and pathologic features. *Cancer* 1981;**48**:2063–9.

[99] Ostrow SS, Diggs CH, Sutherland JC, *et al.* Nodular poorly differentiated lymphocytic lymphoma: changes in histology and survival. *Cancer Treat Rep* 1981;**65**:929–33.

[100] Harousseau JL, Flandrin G, Tricot G, Brouet JC, Seligmann M, Bernard J. Malignant lymphoma supervening in chronic lymphocytic leukemia and related disorders. Richter's syndrome: a study of 25 cases. *Cancer* 1981;**48**:302–8.

[101] Emmerich B, Pemsl M, Wüst I, *et al.* Conversion of an IGM Secreting immunocytoma in a high grade malignant lymphoma of immunoblastic type. *Blut* 1983;**46**:81–4.

[102] Falini B, deSolas I, Levine AM, Parker JW, Lukes RJ, Taylor CR. Emergence of B-immunoblastic sarcoma in patients with multiple myeloma: a clinicopathologic study of 10 cases. *Blood* 1982;**59**:923–33.

[103] Suchman AL, Coleman M, Mouradian JA, Wolf DJ, Saletan S. Aggressive plasma cell myeloma. A terminal phase. *Arch Intern Med* 1981;**141**:1315–20.

[104] Woda, BA, Knowles DM. Nodular lymphocytic lymphoma eventuating into diffuse histocytic lymphoma. Immunoperoxidase demonstration of monoclonality. *Cancer* 1979;**43**:303–7.

[105] Stern N, Shemesh J, Ramot B. Chronic lymphatic leukemia terminating in acute myeloid leukemia: review of the literature. *Cancer* 1981;**47**:1849–51.

[106] Bergsagel DE. Plasma cell neoplasms and acute leukaemia. *Clin Haematol* 1982;**11**:221–34.

[107] Zalcberg JR, Cornell FN, Ireton HJC, McGrath KM, McLachlan R, Woodruff RK, Wiley JS. Chronic lymphatic leukemia developing in a patient with multiple myeloma. Immunologic demonstration of a clonally distinct second malignancy. *Cancer* 1982;**50**:594–7.

[108] Zucker-Franklin D, Amorosi EL, Ritz ND. Evolution of Sézary syndrome in the course of hairy cell leukemia. *Blood* 1982;**59**:1181–90.

[109] Jacobsen N, Theilade K, Videbaek A. Two additional cases of coexisting polycythaemia vera and chronic lymphocytic leukaemia. *Scand J Haematol* 1982;**29**:405–10.

[110] Longo DL, Young RC, DeVita VT. What is so good about the

"good prognosis" lymphomas? In: Williams CJ, Whitehouse JMA, eds. *Recent advances in oncology*. London: Churchill Livingstone, 1982:223–31.

[111] Durie BGM, Salmon SY, Moon TE. Pretreatment tumor mass, cell kinetics, and prognosis in multiple myeloma. *Blood* 1980;**55**:364–72.

[112] Latreille J, Barlogie B, Johnston D, Drewinko B, Alexanian R. Ploidy and proliferative characteristics in monoclonal gammopathies. *Blood* 1982;**59**;43–51.

[113] Pennec Y, Mottier D, Youinou P, *et al.* Critical study of staging in multiple myeloma. *Scand J Haematol* 1983;**30**:183–90.

[114] Rosenberg SA, Dorfman FR, Kaplan HS. The value of sequential bone marrow biopsy and laparotomy and splenectomy in a series of 127 consecutive untreated patients with non Hodgkin's lymphoma. *Br J Cancer* 1975;**31** suppl II:221–7.

[115] Vercelli D, Guglielmo R, Guidi G, Scolari L, Buricchi L, Cozzolino F. Bone marrow percentage of plasma cells in the staging of monoclonal gammopathies. *Nouv Rev Franc Hématol* 1980;**22**:139–45.

[116] Wutke K, Varbiro M, Rüdiger KD, Kelenyi G. Cytological and histological classification of multiple myeloma. *Haematologia* 1981;**14**:315–29.

[117] Child JA, Crawford SM, Norfolk DR, O'Quigley J, Scarffe JH. Evaluation of serum β 2-microglobulin as a prognostic indicator in myelomatosis. *Br J Cancer* 1983;**47**:111–4.

[118] Kyle RA. Long-term survival in multiple myeloma. *N Engl J Med* 1983;**308**:314–6.

[119] Buckman R, Cuzick J, Galton DAG. Long-term survival in myelomatosis. A report to the MRC Working Party on leukaemia in adults. *Br J Haematol* 1982;**52**:589–99.

[120] Jacquillat CI, Auclerc G, Auclerc MF, Andrieu JM, Weil M, Bernard J. Maladie de Hodgkin: caractéristiques e pronostic des formes avec atteinte médullarie initiale. *Nouv Presse Méd* 1981;**10**:95–100.

[121] Kaplan HS. Hodgkin's disease: unfolding concepts concerning its nature, management and prognosis. *Cancer* 1980; **45**:2439–74.

[122] Myers CE, Chabner RA, DeVita VT, Gralnick HR. Bone marrow involvement in Hodgkin's disease, pathology and response to MOPP chemotherapy. *Blood* 1974;**44**:197–204.

[123] Bartl R, Frisch B, Burkhardt R, Huhn D, Pappenberger R. Assessment of bone marrow histology in Hodgkin's disease: correlation with clinical factors. *Br J Haematol* 1982;**51**:345–60.

[124] Lukes RJ, Tindle BH. Immunoblastic lymphadenopathy. A hyperimmune entity resembling Hodgkin's disease. *N Engl J Med* 1975;**292**:1–8.

[125] Bluming AZ, Cohen HG, Saxon A. Angioimmunoblastic lymphadenopathy with dysproteinemia. A pathogenetic link between physiologic lymphoid proliferation and malignant lymphoma. *Am J Med* 1979;**67**:421–8.

[126] Schauer PD, Straus DJ, Bagley CM, *et al.* Angioimmunoblastic lymphadenopathy: clinical spectrum of disease. *Cancer* 1981;**48**:2493–8.

[127] Pangalis GA, Moran EM, Rappaport H. Blood and bone marrow findings in angioimmunoblastic lymphadenopathy. *Blood* 1978;**51**:71–83.

[128] Schnaidt U, Vykoupil KF, Thiele J, Georgii A. Angioimmunoblastic lymphadenopathy. Histopathology of bone marrow involvement. *Virchows Arch (Pathol Anat)* 1980;**389**:369–80.

Requests for reprints to: Professor Dr med Reiner Bartl, Abteilung für Knochenmarksdiagnostik an der Medizinischen Klinik Innenstadt der Universität München, D-8000 München 2, Ziemssenstrasse 1, Germany.