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Reticulin fibre content of bone marrow infiltrates of malignant non-Hodgkin's lymphomas (B-cell type, low malignancy) – a morphometric evaluation before and after therapy

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Summary. A morphometric study was performed on bone marrow infiltrates of non-Hodgkin's lymphomas (B-cell type, low malignancy) to evaluate the content of argyrophilic (reticulin) fibres in the various subtypes before and after therapy. In congruence with the corresponding lymph node lesions, subtypes consisted of lymphocytic lymphoma – chronic lymphocytic leukaemia (CLL, $n=39$), centroblastic-centrocytic lymphoma (CB-CC, $n=35$), lymphoplasmacytoid immunocytoma (LPI, $n=22$) and finally hairy cell leukaemia (HCL, $n=21$). In comparison with control specimens, morphometric measurements on trephine biopsies (initial staging procedure) disclosed a borderline or minimal increase in reticulin in CLL and moderate fibrosis in CB-CC and LPI, whereas HCL had the greatest increase in fibres. The marrow surrounding focal or patchy lymphoma infiltrates of CLL and CB-CC displayed no relevant changes in fibre density with respect to the control samples. Following chemotherapy, repeated trephine biopsies (restaging procedure) were obtainable from 38 patients. There was no significant decrease in the fibre content of CLL, CB-CC and LPI infiltrates. In HCL an incomplete reduction was recorded after interferon treatment. So-called benign lymphoid lesions may be distinguished from focal-patchy infiltrates of CB-CC and LPI not only by showing a central localization, but also by the absence of significant amounts of reticulin. However, considering the density of the reticulin fibres, a clear-cut discrimination of these lymphoid aggregates from an early nodal-central growth pattern of CLL is not feasible in many cases.

Key words: Argyrophilic (reticulin) fibres – Malignant non-Hodgkin's lymphomas – Bone marrow infiltrates – Benign lymphoid lesions – Morphometry

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Introduction

In malignant non-Hodgkin's lymphomas (NHLs) bone marrow infiltrates have been studied extensively with respect to their cytological and immunohistological features. Classification into different subtypes associated with various growth patterns and with prognostic implications have been made (Georgii 1979; Bartl et al. 1982, 1984, 1988; Vykoupil and Georgii 1984; Han et al. 1984; Rozman et al. 1984; Frisch et al. 1985; Pangalis et al. 1987; Montserrat and Rozman 1987; Paoletti et al. 1988; Orfao et al. 1988, 1989; Lerma-Puertas et al. 1988; Frisch and Bartl 1988). Although some degree of marrow fibrosis has been described as accompanying these lymphoma infiltrates, no exact data are available (Bartl et al. 1982; Burkhardt et al. 1982; Navone and Colombano 1984; Frisch et al. 1985; Frisch and Bartl 1985, 1988). In the small cell NHLs of low or intermediate malignancy in particular, it might be assumed that evolution of reticulin fibres could be expressed differently in each subgroup. In this context it is also questionable whether argyrophilic fibres are induced in the surrounding non-involved marrow area. Furthermore, effects of cytotoxic therapy on reticulin fibrosis have been determined only in hairy cell leukaemia by several groups who have used gross quantifications or employment of a scoring system (Naeim and Jacobs 1985; Bardawil et al. 1986; Hasselbalch et al. 1988; Laughlin et al. 1988). It is also tempting to speculate that discrimination between small focal lymphoma infiltrates and so-called benign lymphoid nodules of the bone marrow (Frisch et al. 1982; Burkhardt et al. 1982; Navone et al. 1985; Faulkner-Jones et al. 1988) may be facilitated by considering the fibre content of these lesions in addition to immunohistochemistry (Frisch et al. 1985; Hall et al. 1988). For this reason, the aim of this study was to provide a comprehensive survey on argyrophilic (reticu-

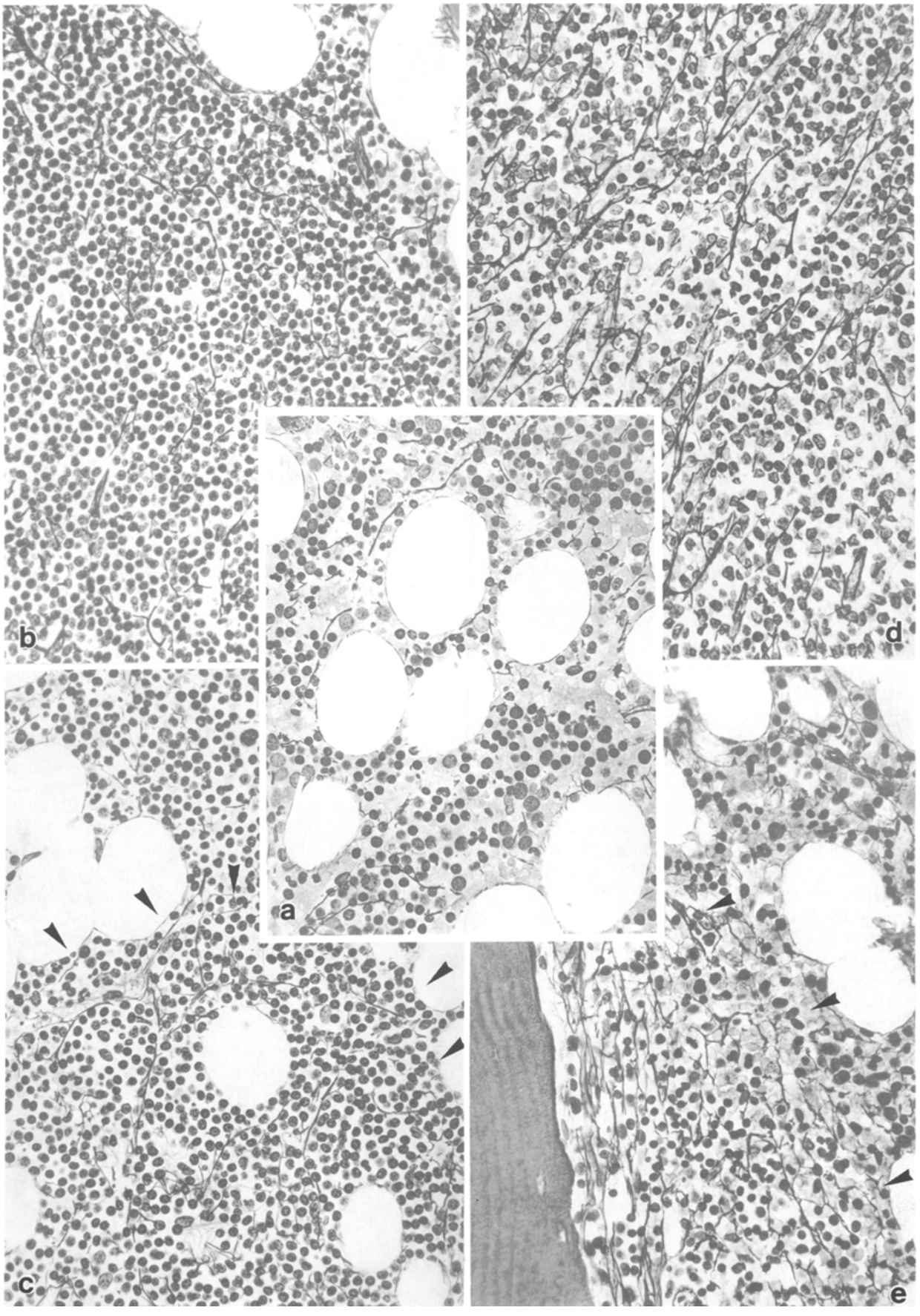

Fig. 1 a–e. Argyrophilic (reticulin) fibres in bone marrow infiltrates of non-Hodgkin's lymphomas (NHLs) in comparison with a control specimen. **a** Normal bone marrow with scanty and thin reticulin fibres. **b** Chronic lymphocytic leukaemia (CLL) showing diffuse involvement and an only borderline increase in thin argyrophilic fibres. **c** CLL displaying a focal-patchy infiltrate (*arrowheads*) with a minimal amount of fibres. **d** Centroblastic/centrocytic lymphoma (CB-CC) with diffuse growth pattern and a conspicuous argyrophilic fibrosis consisting of irregularly arranged thick fibres. **e** CB-CC exhibiting a focal paratrabecular (endosteal) infiltrate (*arrowheads*) with a striking increase in thick reticulin fibres. **a–e** Silver impregnation after Gomori, × 370

lin) fibres in NHL infiltrates of the bone marrow, based on a retrospective analysis and a morphometric evaluation of 155 specimens including 38 sequential biopsies.

Materials and methods

Patients who presented with the clinical signs and symptoms of NHLs were enrolled in this study. They underwent lymph node biopsy (with the exception of 17 cases with hairy cell leukaemia) as well as a representative trephine biopsy of the bone marrow performed within 1 week after admission and prior to any therapy (initial staging procedure). Further criteria for recruitment included:

1. Classification of lymph node lesions by following the Kiel classification (Stansfeld et al. 1988) into well-defined subtypes in consideration of their cytological and immunohistological characteristics.
2. Bone marrow infiltrates exceeding at least 1 mm^2 in discrete focal-nodular involvement and in accordance with point 1 above.
3. Haematological and laboratory data, but particularly cytological findings (smears of peripheral blood and bone marrow) in patients with chronic lymphocytic and hairy cell leukaemia.
4. Repeated trephine biopsies of the bone marrow (re-staging procedure) taken at an interval of at least 6 months (range 0.5–4 years) following first course of chemo- or interferon therapy (the latter was used in 38 cases).

Using these strict criteria sufficient NHLs of the B-cell type and of low-grade malignancy according to the updated Kiel classification (Stansfeld et al. 1988) were entered into the study. Subtypes consisted of lymphocytic lymphoma – chronic lymphocytic leukaemia (CLL), centroblastic-centrocytic lymphoma (CB-CC), lymphoplasmacytoid immunocytoma (LPI) and hairy cell leukaemia (HCL). The characteristics of the patients, the number of NHLs selected for each subgroup and therapeutic modalities are listed in Table 1.

Bone marrow specimens from 20 individuals (9 males, 11 females; median age 59 years) without any haematological disorder and a peripheral lymphocyte count within the normal range served as controls.

Large trephine biopsies (18 ± 5 mm^2) with a mean artifact – free marrow area evaluable for morphometry of 13 mm^2 (range 7–21 mm^2) were performed from the posterior iliac crest (Jamshidi and Swaim 1971). Further processing included fixation in an aldehyde solution (2 ml of 25% glutaraldehyde, 3 ml of 37% formaldehyde and 1.58 g calcium acetate with 100 ml distilled water), decalcification in neutral buffered EDTA for 3 days and finally paraffin embedding (Schaefer 1984). The following methods were employed on re-cut paraffin blocks taken from our files for the identification of certain histological features: Giemsa-survey (haematopoietic, lymphoid and adipose tissue), periodic acid-Schiff (PAS) reagent – megakaryocytes and adipose tissue, naphthol-AS-D-chloroacetate esterase – neutrophilic granulopoiesis and easy recognition of small focal lymphoma infiltrates, Gomori's silver impregnation – argyrophilic (reticulin) fibres.

Morphometric evaluation was done using a manual optic planimeter (MOP-A-MO1-Kontron) with a standard program set (Kontron software). Total marrow area, amount of adipose tissue and extent of lymphoma infiltrates were determined in Giemsa-stained slides at 500× magnification. The reticulin fibre content was measured in specimens following silver impregnation (Gomori's stain) by counting the number of intersections (i) with the lines of a grid ocular at a magnification of 500× in 20 fields free from trabecular bone (equalling 1.14 mm^2). These fields were selected in a strictly random fashion. The area covered by fat cells was substracted and the reticulin fibre density distribution expressed as number of intersections per square millimetre fat cell-free haematopoietic tissue (i/mm^2). Because of the significant inverse correlation between amount of fat cells and density of argyrophilic fibres, this method of calculation has been applied to avoid an interference with alterations of cellularity particularly in higher age groups and as the sequel of therapy. Unfortunately the areas occupied by the so-called benign lymphoid lesions (lymphoid aggregates) were far too small to allow an appropriate morphometric and comparative statistical evaluation with the NHLs and therefore had to be discarded.

A multivariate computer-based statistical analysis was performed by utilizing selected BMDP programs (Dixon 1983). To calculate differences between the variables for the density of argyrophilic fibres in each subtype of NHLs and the non-involved as well as normal marrow, the Newman-Keuls test was applied (Winer 1971). All correlations were statistically checked using Student's t-test (Fisher 1972) ($P \leq 0.05$).

Results

There is a striking variety in the appearance of NHL infiltrates in the bone marrow, not only regarding the different growth pattern and localization (microfocal, extended or diffuse/endosteal or central) but also the amount of reticulin fibres. In comparison with normal samples (Fig. 1a), patients with CLL show either a diffuse-extended or focal-patchy involvement with only a marginal or no increase in argyrophilic fibres (Fig. 1b, c). In contrast to these findings, in germinal centre cell types of NHLs (CB-CC) and in immunocytomas (LPI), there is a conspicuous coarse and irregularly arranged reticular fibrosis enmeshing small groups of atypical lymphoid elements (Figs. 1d, e, 2a). As was expected, the greatest amount of fibres was encountered in HCL with a diffuse network-like aspect (Fig. 2c). Following cytotoxic therapy (Table 1) for at least 6 months in CLL, CB-CC and LPI no obvious changes were observable in the fibre content, whereas the adipose tissue revealed a tendency to expand simultaneously with reduction of the lymphoma infiltrates (Fig. 2b). In HCL after interferon treatment (interval 6–25 months) there was a remarkable, but partial decrease not only in the atypical lymphoid population but also in reticulin fibres, together with a focal regeneration of haematopoiesis (Fig. 2d). Bone marrow specimens with one or two so-called benign nodular lymphoid lesions were derived from about 20 patients with underlying inflammatory disorders (chronic rheumatoid arthritis, hepatitis, Crohn's disease, autoimmune haemolytic anaemia). These focal lymphoid aggregates revealed mostly a central perisinusoidal, rarely an intermediate localization, but no relevant fibrosis in comparison with the control group (Fig. 2e).

Results of morphometric measurements of the density of reticulin (argyrophilic) fibres in each subtype of NHLs before and after therapy and the amount of adipose tissue are listed in Table 2 with comparisons of the control group. A discriminate analysis of these variables disclosed significant differences between single lymphoma subgroups with exception of CB-CC and LPI (Table 3). In case of nodular-focal infiltrates of CLL and CB-CC not only the fibre content of the involved areas but also that of the surrounding apparently normal marrow was determined (Table 2). While the non-involved bone marrow was equivalent to control specimens, the focal lymphoma infiltrates revealed a significant increase in reticulin fibres. Quantitatively this fibrosis was not different from the extended and diffuse patterns of lymphoma growth in these subtypes (Table 4). Following

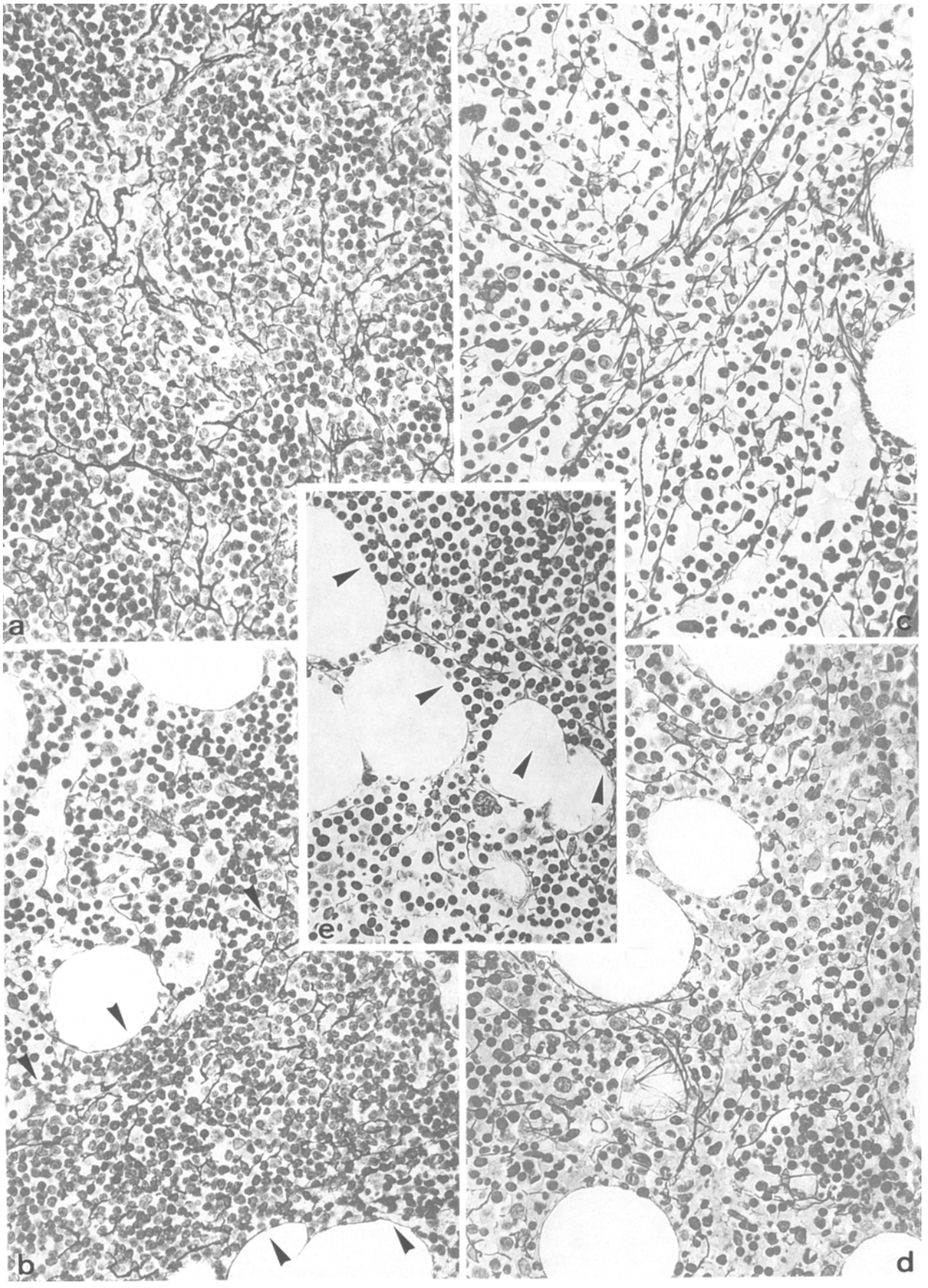
a
c
e
b
d

Table 1. Characteristics of patients with bone marrow infiltrates of non-Hodgkin-lymphomas (NHLs) involved in this study

	n	Male/ female	Age (median years)	Therapy
CLL	39	21/18	60	Cyclophosphamide, vincristine and prednisone (COP) or COP and procarbacine (COPP) or chlorambucil and prednisone (Knospe protocol)
CB-CC	35	22/13	57	COP
LPI	22	13/ 9	62	COP or Knospe protocol
HCL	21	16/ 5	56	Recombinant interferon (IFN) alpha-2b

CLL, Chronic lymphocytic leukaemia; CB-CC, centroblastic/centrocytic lymphoma; LPI, lymphoplasmacytoid immunocytoma; HCL, hairy cell leukaemia

chemotherapy (Table 1) no significant changes in the fibre content could be calculated in CLL, CB-CC and LPI. However, most cases with HCL (ratio 6/7) showed an incomplete reversal of reticulin fibrosis ($P \leq 0.001$).

Discussion

The aetiology of the increased content of argyrophilic (reticulin) fibres in bone marrow associated with various haematological malignancies is still debatable (McCarthy 1985). The same applies to the reversal of myelofibrosis during therapeutic modalities. Presumably degradation and removal of reticulin and collagen is mediated by monocytes, macrophages and granulocytes which are thought to contain collagenase (Horwitz et al. 1977) and/or by a reduced synthesis and release of plate-

Table 2. Density of argyrophilic (reticulin) fibres determined in bone marrow infiltrates of various subtypes of NHLs before and after therapy together with percentages of adipose tissue and evaluation of a control group

	n	Density of fibres ($i \times 10^2/mm^2$)	Adipose tissue %
Controls	20	16.2±4.5	48.0±9.2
CLL			
Total	39	35.0± 7.3	15.9±12.1
Growth pattern			
Diffuse	30	34.9± 5.9	15.0±13.0
Nodular	9	40.8± 8.9	19.0± 9.1
Non-involved marrow	–	17.3± 3.3	–
After therapy	10	34.6±11.3	9.3±11.4
CB-CC			
Total	35	52.6±10.7	16.3±11.1
Growth pattern			
Diffuse	15	53.4±10.9	14.3±13.7
Nodular	20	52.1±10.8	17.7± 8.9
Non-involved marrow	–	18.1± 3.2	–
After therapy	11	49.8± 9.6	20.4± 7.3
LPI			
Total	22	52.2± 7.4	6.8± 7.9
Growth pattern			
Nodular			
After therapy	10	54.8±14.4	20.0±21.9
HCL			
Total	21	66.4±12.8	11.2±10.8
Growth pattern			
Diffuse			
After therapy	7	51.6± 9.6	25.7±13.5

There are no statistically significant differences for the fibre density between diffuse or nodular-focal infiltrates of CLL and CB-CC. For abbreviations, see Table 1

Table 3. Significant correlations among bone marrow infiltrates of various subtypes of NHLs tested for density of argyrophilic (reticulin) fibres (Newman-Keuls test)

	Controls	CLL		CB-CC		LPI	HCL
		Diffuse	Nodular	Diffuse	Nodular		
Controls	–						
CLL	7.64*	–					
Diffuse growth pattern							
Nodular	10.86**	3.22*	–				
CB-CC	16.42**	8.77**	5.56**	–			
Diffuse growth pattern							
Nodular	15.84**	8.19**	4.98**	0.58*	–		
LPI	15.59**	7.94**	4.73**	0.83*	0.25*	–	
HCL	22.29**	14.65**	11.43**	5.87**	6.45**	6.70**	–

Levels of significance: not significant*, $0.05 > P \leq 0.01$**

Fig. 2a–e. Argyrophilic (reticulin) fibres in bone marrow infiltrates of NHLs before and after therapy and in comparison with a so-called benign lymphoid lesion. **a** Lymphoplasmacytoid immunocytoma (LPI) reveals a diffuse involvement and a coarse irregularly appearing fibrosis before cytotoxic therapy. **b** LPI following chemotherapy with reduction of lymphoma infiltrates (*arrowheads*), but a still preserved argyrophilic fibrosis in comparison with the surrounding regeneratory marrow and Fig. **a**. **c** Hairy cell leukaemia (HCL) showing a dense meshwork of reticulin fibres between the atypical lymphoid elements before treatment. **d** HCL after interferon therapy discloses a significant, but incomplete reversal of the atypical lymphoid population as well as argyrophilic fibres together with a focal regeneration of haematopoiesis in comparison with Fig. **c**. **e** Part of a nodular and centrally localized lymphoid aggregate in chronic rheumatoid arthritis (*arrowheads*) containing only a few thin reticulin fibres similar to the surrounding normal marrow. **a–e** Silver impregnation after Gomori, ×370

Table 4. Comparison of focal nodular and diffuse bone marrow infiltrates with the non-involved marrow area in NHLs regarding density of argyrophilic (reticulin) fibres (*t*-test)

	Controls	NHL-pattern of bone marrow involvement	
		Non-involved marrow around nodular infiltrates	Diffuse infiltrates
CLL – nodular Marrow infiltrates	0.0001		
Focal infiltrates	0.0001	0.0001	NS
Non-involved marrow	NS –	–	0.0001
CB-CC-nodular Marrow infiltrates			
Focal infiltrates	0.0001	0.0001	NS
Non-involved marrow	NS	–	0.0001 0.0001

Levels of significance (*P* values): not significant (NS)

let-derived growth factor (Groopman 1980; Oblon et al. 1983; Mehta et al. 1983; Manoharan and Pitney 1984).

In small cell NHLs of low to intermediate malignancy other than HCL, the occurrence of fine and sparse and rarely coarse argyrophilic fibres has been described infrequently. However, there has been no morphometric quantification and sometimes a detailed reference to the various subtypes was missing (Bartl et al. 1982; Burkhardt et al. 1982; Navone and Colombano 1984; Frisch and Bartl 1985; Frisch et al. 1985). In their extensive study on bone marrow histology in the malignant lymphomas, Bartl et al. (1982) were obviously not able to recognize significant differences in the reticulin content between CLL, CB-CC and LPI. This finding is not in agreement with our results, which were obtained by morphometry: in lymphocytic lymphoma (CLL) a borderline to minimal argyrophilic fibrosis was evident which is significantly different to the fibre content of the CB-CC and LPI subtypes (Table 3). Only in HCL was an approximately 50% increase in coarse fibres noticed by Bartl et al. (1982) and was calculated to present an unfavourable influence on survival, similar to the presence of an unspecified fibrosis in CLL (Frisch and Bartl 1988).

In HCL a loose to dense argyrophilic network enmeshing the lymphoid cell population in the bone marrow is a characteristic feature of the histopathology (Vykoupil et al. 1976; Burke 1978; Bartl et al. 1982, 1984; Burke and Rappaport 1984; Naeim and Jacobs 1985; Frisch et al. 1985; Bardawil et al. 1986; Paoletti et al. 1988). The prevalence and response of reticulin fibres to interferon therapy (Quesada et al. 1984; Jansen et al. 1984; Worman et al. 1985; Groopman 1985; Berneman et al. 1986; Golomb et al. 1986; Ratain et al. 1988; Lauria et al. 1988; Hasselbalch et al. 1988) has been studied repeatedly, with slightly different findings (Naeim and Jacobs 1985; Bardawil et al. 1986; Hasselbalch et al. 1988; Laughlin et al. 1988). Generally in these investigations assessment of the fibre content has been done by a grading system using different scores. A complete reversal of reticulin fibres to normal levels together with a significant reduction of the amount of hairy cells was encountered in 6 of 16 patients following recombinant alpha-interferon treatment by Laughlin et al. (1988). The failure to achieve a relevant decrease in reticulin in a considerable number of patients with HCL has been reported in other studies (Naeim and Jacobs 1985; Bardawil et al. 1986; Hasselbalch et al. 1988). Our results confirm and extend these findings by showing that, although some reduction in the fibre count is apparent, the density is still greater than in the normal marrow or in CLL patients (Table 2). Experimental studies have pointed out that interferon exerted an antiproliferative activity on myeloid progenitor cells (Neumann and Fauser 1982; Carlo-Stella et al. 1987; Ganser et al. 1987). In this context it was found that in myeloid metaplasia with myelofibrosis no recognizable effect of alpha-interferon treatment on the degree of marrow fibrosis was accomplished (Parmeggiani et al. 1987; Barosi et al. 1989).

Discrimination between solitary benign lymphoid aggregates in the bone marrow and discrete microfocal infiltrates of small cell NHLs has to be considered a major diagnostic challenge (Burkhardt et al. 1982;Frisch et al. 1982, 1985; Navone et al. 1985; Faulkner-Jones et al. 1988). Nodular lymphoid hyperplasia is frequently displayed in inflammatory disorders, such as rheumatoid arthritis and autoimmune diseases; further, there is a significant association with age (Hashimoto et al. 1957; Bartl et al. 1984; Frisch et al. 1982, 1985; Navone et al. 1985). So-called benign lymphoid nodules may be classified according to Hashimoto et al. (1957) into different groups, one of which is consistent with a hyperplastic lymph follicle with a well-defined germinal centre. This lesion should not cause difficulties in differentiation from NHLs. When regarding the various growth patterns of small cells NHLs in bone marrow tissue (Georgii 1979; Bartl et al. 1982, 1984; Vykoupil and Georgii 1984; Frisch et al. 1985; Frisch and Bartl 1988), a paratrabecular (endosteal) localization or a mixture of a nodular-extended (Georgii 1979), or nodular-interstitial (Bartl et al. 1984; Frisch et al. 1985; Frisch and Bartl 1988) lymphoid proliferation is highly suspicious of malignancy. A further indication for malignancy may be a relevant reticulin fibrosis as shown in this study, particularly in patchy infiltrates of CB-CC and LPI lymphomas. However, CLL with early focal involvement of the marrow and a borderline or minimal increase in fibres is known to occupy a central localization (Georgii 1979; Bartl et al. 1982, 1984; Frisch et al. 1985). For the insignificant amount of fibres these lesions may closely resemble a benign lymphoid nodule. In a study on the eventual outcome of so-called benign lymphoid aggregates in the bone marrow, Faulkner-Jones et al. (1988) reported that in 18 out of 82 patients such lymphoid nodules later evolved into NHLs of low grade malignan-

cy; however they gave no data on the fibre content or localization of these lesions.

In summary, our study indicates a distinctive amount of reticulin fibres occurring in several subtypes of NHLs. These may be a valuable aid in their discrimination from benign lymphoid nodules. The change is not, or is only incompletely, reversible by chemotherapy and interferon therapy.

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