Intrasinusoidal bone marrow infiltration and splenic marginal zone lymphoma: a quantitative study

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Intrasinusoidal bone marrow infiltration and splenic marginal zone lymphoma: a quantitative study

Pich A, Fraire F, Fornari A, Davico Bonino L, Godio L, Bortolin P, Chiusa L, Palestro G. Intrasinusoidal bone marrow infiltration and splenic marginal zone lymphoma: a quantitative study.

Abstract: Intrasinusoidal infiltration (ISI) is a pattern of invasion that is rarely found on bone marrow (BM) biopsies, and is considered as a hallmark of splenic marginal zone cell lymphoma (SMZL). We analysed BM biopsies showing intrasinusoidal infiltration from 54 consecutive patients with different types of lymphoma to verify if ISI quantity was a diagnostic criterion for SMZL. There were 35 primary splenic lymphoma (PSL) and 19 non-PSL; 28 SMZL, three non-splenic MZL, six mantle cell, six small lymphocytic, four follicular, four diffuse large B cell, one peripheral T cell, one lymphoplasmacytic and one anaplastic large-cell lymphoma. The quantity of BM infiltrate was assessed on CD45, CD20 and CD3 stained sections. The mean percentage of total (TI) and intrasinusoidal (ISI) lymphocytes was calculated in 10 areas for each case. TI quantity was 21.57 in PSL and 35.05 in non-PSL ($P = 0.04$). ISI quantity was 5.23 in PSL and 7.62 in non-PSL ($P = 0.08$), 5.83 in SMZL and 2.83 in other types of PSL ($P = 0.12$), 4.46 in non-splenic MZL and 8.21 in other types of non-PSL ($P = 0.28$). No difference in ISI quantity was found among the lymphoma subtypes, either in PSL ($P = 0.74$) or non-PSL ($P = 0.3$). The data demonstrate that ISI quantity in BM biopsies is not a reliable diagnostic parameter for SMZL.

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Key words: bone marrow pathology; intrasinusoidal infiltration; splenic marginal zone lymphoma; diagnosis

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Intrasinusoidal infiltration (ISI) is a recently recognised pattern of bone marrow (BM) invasion rarely found in BM biopsies performed for diagnostic or staging purpose (1). This peculiar feature was described to be associated with rare entities, that share a distinctive intravascular growth pattern, such as the intravascular large B-cell lymphoma (malignant angioendotheliomatosis) (2) and the hepatosplenic γδT-cell lymphoma (3). Subsequently, ISI was reported to be a very frequent type of marrow invasion in patients with splenic marginal zone cell lymphoma (SMZL) with or without villous lymphocytes (4–8), and was regarded as a possible hallmark of the disease (4, 6).

Splenic marginal zone cell lymphoma (with or without villous lymphocytes) is a chronic B-cell lymphoproliferative disorder (9), that usually has an indolent course. The diagnosis of SMZL, as well as of other types of primary splenic lymphoma (PSL), should be established after splenectomy, although a clinical diagnosis is also possible from the analysis of peripheral blood in cases with circulating elements. However, splenectomy may induce a worsening of BM infiltration in patients with SMZL (10). Therefore, it would be very helpful if the pattern of invasion could allow a reliable diagnosis of SMZL on BM biopsy.

Unfortunately, intrasinusoidal BM infiltrates have also been described in other small B-cell lymphomas, such as follicular lymphoma (FL) and chronic lymphocytic leukaemia (CLL) (11), mantle cell lymphoma (MCL) (11, 12) and hairy cell leukaemia (11, 13), as well as in anaplastic large B-cell lymphoma, intravascular large B-cell lymphoma and T large granular lymphocyte leukaemia (7).

As it has been claimed that a prominent intrasinusoidal infiltrate is a distinctive pattern of BM infiltration in patients with SMZL (10, 11), whereas an association of ISI with other patterns of BM infiltration has also been described in different NHL types, it appears of great importance to exactly quantify the extent of the intrasinusoidal infiltrate.

In the present study, we analysed 54 BM biopsies showing intrasinusoidal infiltration by means of a quantitative approach. The purpose was to verify if ISI quantity on BM biopsy could represent a reliable diagnostic criterion for SMZL.

Materials and methods

All BM biopsies performed in patients with non-Hodgkin lymphoma (NHL) from 1998 to 2002 were retrieved from the files of the Section of Pathology of the Department of Biomedical Sciences and Human Oncology of Turin University. Of a total of 1325 cases showing marrow infiltration, 54 (4.07%) consecutive and initial BM biopsies with intrasinusoidal infiltration were selected.

Twenty-nine patients were females (53.7%) and 25 (46.3%) males. The mean age of patients was 60.2 yr (median, 63; range, 30–79). According to WHO classification (14), there were 31 (57.4%) marginal zone lymphoma (MZL), six (11.1%) MCL, six (11.1%) small lymphocytic lymphoma/chronic lymphocytic leukaemia (SLL/CLL), four (7.4%) FL, four (7.4%) diffuse large B-cell lymphoma (DLBCL), one (1.9%) peripheral T-cell lymphoma (PTL), one (1.9%) lymphoplasmacytic lymphoma (LDL) and one (1.9%) anaplastic large-cell lymphoma (ALCL). Twenty-five patients underwent splenectomy: the mean weight of the spleen was 1722 g (median, 1500; range, 340–4700).

According to Warnke *et al*. (15), 35 (64.8%) were primary lymphomas of the spleen (PSL). Fifteen patients (42.9%) were males and 20 (57.1%) females. The mean age of patients was 58.5 yr (median, 61.5; range, 30–79). There were 28 (80%) SMZL, two (5.7%) MCL, two (5.7%) SLL/CLL, one (2.9%) DLBCL, one (2.9%) PTL and one (2.9%) LDL. Twenty-four patients underwent splenectomy.

Nineteen cases were non-PSL. Ten patients (52.6%) were males and nine (47.4%) females. The mean age of patients was 63.3 yr (median, 65; range, 34–77). There were three (15.8%) MZL, four (21.1%) FL, four (21.1%) MCL, four (21.1%) SLL/CLL, three (15.8%) DLBCL and one (5.3%) ALCL. One patient underwent splenectomy.

The diagnostic material available for subtyping the lymphomas was represented by surgically removed lymph nodes, spleen, peripheral blood and BM aspirates and/or biopsies. Diagnosis was made based on histologic examination of lymph nodes, spleen and/or BM integrated with immunophenotypic and cytologic data.

Immunohistochemistry was performed on deparaffinised and rehydrated sections, following an immuno-peroxidase technique with an automatic stainer device (Dakoautostainer, DakoCytomation, Glostrup, Denmark), according to manufacturer's instructions. Samples were analysed with a monoclonal antibody panel comprising: CD3 (Medac, Wedel, Germany), CD5 (Neomarkers, Fremont, CA, USA), CD10 (Medac), CD20 (Dako), CD21 (Dako), CD23 (The Binding Site, Birmingham, UK), CD43 (Becton Dickinson, San José, CA, USA), Bcl-2 (Dako), Bcl-6 (Dako), Ki-67 (Dako), p53 (24/28, Medac), cyclin D1 (Neomarkers).

Surface antigens have been studied with a three-colour operating FACS (FACScalibur, Becton Dickinson), on mononuclear cells obtained from lymph nodes or biopsy specimens. The following monoclonal antibody combinations were used for analysis of B cells: CD43/CD23/CD19, FMC7/CD79b/CD19, CD5/CD20/CD45, CD20/CD10/CD19, kappa/lambda/CD19, CD103/CD22/CD19 and CD25/CD11c/CD19. Antibodies to CD11c and FMC7 were obtained from Caltag (Burlingame, CA, USA). Antibodies to CD103 and CD22 were obtained from IQP (Immunoquality Product, Groningen, the Netherlands). Antibodies to CD43 and CD5 were obtained from Immunotech (Marseille, France). Antibody to CD25 was obtained from Dako. Antibody to CD79b was obtained from Southern Biotechnology (Birmingham, AL, USA). Antibodies to CD19, CD23, CD20, CD10, CD45, kappa and lambda were obtained from Becton Dickinson. T cells as a control population also were evaluated with an appropriate immunophenotyping panel.

In selected cases, polymerase chain reaction (PCR) analysis of IgH rearrangements, heteroduplex analysis, DNA sequencing and mutational status analysis, and PCR analysis of Bcl-2/IgH rearrangement were also performed as previously described (16).

If a patient did not undergo splenectomy, the diagnosis of PSL was based on splenomegaly without lymphadenopathy, and the presence of clonal (light chain-restricted) circulating CD19/CD20 positive B lymphocytes that were negative for CD5, CD10, CD23 and CD43.

All BM biopsies were taken at presentation as a part of the staging procedure, from both right and left posterior–superior iliac crests, using a Jamshidi needle. Biopsies were immediately fixed in buffered acid formol for 24 h, decalcified in Osteodec° (Bioptica, Milan, Italy) [ethylenediaminetetraacetic

acid (EDTA), HCl mixture] for 6 h, dehydrated and embedded in paraffin. Only sections measuring at least 8 × 2 mm, with a minimum marrow area (excluding periosteal tissues, cortical bone and artifacts) of 9 mm^2 were included in the study. Serial sections (3 μm thick) were stained with haematoxylin–eosin, periodic-acid Schiff (PAS), Giemsa and reticulin staining. Immunohistochemistry was performed in each case. Sections were deparaffinised in xylene and rehydrated in a series of graded alcohols. Slides were incubated in 10 mmol/L citrate buffer, pH 6, at 121°C in a steam autoclave. Immunohistochemistry was performed with an automatic stainer device (Dako-autostainer, Dako), using the Labelled Streptavidin-Biotin 2 System detection kit (Dako), diaminobenzidine as chromogen and monoclonal antibodies anti-CD45, CD20 (Dako) and CD3 (Novocastra, Newcastle, UK). In selected cases, the analysis was supplemented by monoclonal antibodies anti-CD79a, CD76 (clone DBA44), CD8, CD30 (Dako), CD5, CD4 (Novocastra), TIA-1 (Immunotech), granzyme B (Monosan, Uden, the Netherlands), and the polyclonal antibody anti-ALK (Zymed, San Francisco, CA, USA). When intrasinusoidal infiltrate was scanty, BM biopsies were stained with anti-CD34 monoclonal antibody (Clone QBEnd/10) (Neomarkers) to highlight the vascular structure, thus allowing a more precise quantification of ISI extent.

Evaluation of the extent of BM invasion

In each case, the assessment of the quantity of BM invasion was independently performed by two observers (F.F. and A.F.) on CD45, CD20 and CD3 stained sections, using a Zeiss Axioskop microscope (Carl Zeiss, Jena, Germany) equipped with a 10× ocular and a 20× objective.

The mean percentage of CD20 (or CD3 in peripheral T-cell lymphoma) stained lymphocytes over the total number of BM nucleated cells, including lymphoma cells, was calculated by examining 10 randomly selected areas from both left- and right-side biopsies for each case. The value was regarded as the quantity of total invasion (TI) of the BM. Then, the mean percentage of CD20 (or CD3 in peripheral T-cell lymphoma)-stained lymphocytes located within the sinusoidal spaces in the same areas was calculated for each case. The value was considered as the quantity of ISI (Figs 1 and 2). The interobserver variation was $< 5\%$.

Statistical analysis

Associations between the quantity of BM infiltrate and the origin of lymphoma (PSL, non-PSL), or the type of lymphoma were assessed by the two-tailed *t*-test (for TI) or the Mann–Whitney *U*-test (for ISI). The independence between categorised variables (patterns of non-ISI infiltration) and the type of lymphoma in PSL was estimated by the Yates' corrected chi-squared test. The relationship between continuous variables (TI, ISI) and the weight of resected spleen was assessed by the linear correlation (Pearson's correlation coefficient). All data were processed with BMDP selected programs (2D, 3D, 6D, 7D, 4F) (17).

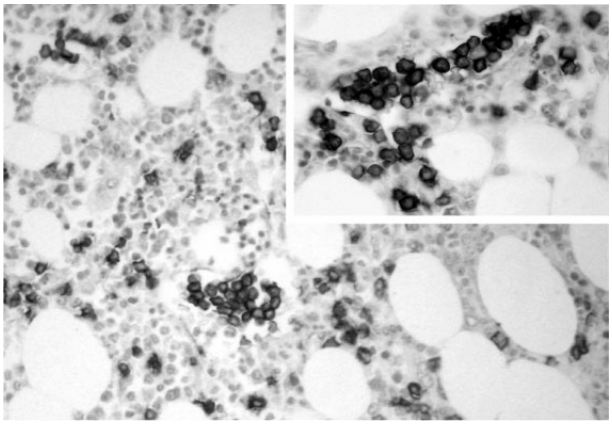

Fig. 1. Bone marrow intrasinusoidal infiltration by splenic marginal zone lymphoma: the quantity of ISI is about 15% of all nucleated marrow cells (CD20 immunostaining; original magnification ×250; inset: ×400).

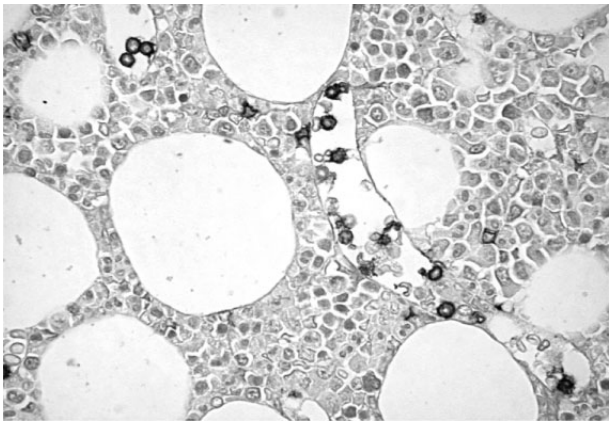

Fig. 2. Bone marrow intrasinusoidal infiltration by splenic marginal zone lymphoma: the quantity of ISI is about 5% of all nucleated marrow cells (CD20 immunostaining; original magnification ×400).

Results

Association between the quantity of BM infiltration and PSL or non-PSL

The mean TI for the whole series was 26.31 (median, 20; SD = 20.97; range, 1–80). It was

greater in non-PSL (mean, 35.05; median, 25; SD = 25.25; range, 1–80) than in PSL (mean, 21.57; median, 15; SD = 16.79; range, 5–70) ($P = 0.04$).

The mean ISI for the whole series was 6.07 (median, 4.82; SD = 5.29; range, 0.1–21.3). It was greater (mean, 7.62; median, 7.2; SD = 5.59; range, 0.2–21.3) in non-PSL than in PSL (mean, 5.23; median, 3.7; SD = 5; range, 0.1–20.1); however, the difference is only of borderline statistical significance ($P = 0.08$). The results are shown in Table 1.

Association between the quantity of ISI and the type of lymphoma in PSL and non-PSL

No difference was found between the extent of ISI and the type of lymphoma in PSL. ISI was 5.83 for SMZL, 4.1 for splenic SLL/CLL, 3.15 for splenic MCL, 0.1 for DLBCL, 5 for LDL and 0.26 for PTL ($P = 0.74$). The results are shown in Table 2.

No difference was found between the extent of ISI and the type of lymphoma in non-PSL. ISI was 4.46 for MZL, 7.4 for SLL/CLL, 13.47 for MCL, 6.63 for DLBCL, 5.27 for FL and 7 for ALCL ($P = 0.3$). ISI quantity tended to be greater in MCL than MZL ($P = 0.07$). When all non-MCL were grouped in a single category, the difference in ISI extent between MCL (13.47) and non-MCL (6.06) was significant ($P = 0.03$). The results are reported in Table 3.

Table 1. Quantity of marrow infiltration in primary splenic and non-primary splenic lymphomas

	No.	TI (mean ± SD)	ISI (mean ± SD)
Whole series	54	26.31 ± 20.97	6.07 ± 5.29
Primary splenic	35	21.57 ± 16.79	5.23 ± 5
Non-primary splenic	19	35.05 ± 25.25	7.62 ± 5.59
P-value		0.04	0.08

TI, total invasion; ISI, intrasinusoidal invasion.

Table 2. Quantity of intrasinusoidal marrow infiltration in primary splenic lymphomas according to the lymphoma categories

	n	Mean ± SD
Whole series	35	5.23 ± 5
SMZL	28	5.83 ± 5.28
SLL/CLL	2	4.1 ± 4.8
MCL	2	3.15 ± 1.2
DLBCL	1	0.1
LDL	1	5
PTL	1	0.26

$P = 0.74$.
SMZL, splenic marginal zone lymphoma; SLL/CLL, small lymphocytic lymphoma/chronic lymphocytic leukaemia; MCL, mantle cell lymphoma; DLBCL, diffuse large B-cell lymphoma; LDL, lymphoplasmacytic lymphoma; PTL, peripheral T-cell lymphoma.

Table 3. Quantity of intrasinusoidal marrow infiltration in non-primary splenic lymphomas according to the lymphoma categories

	n	Mean ± SD
Whole series	19	7.62 ± 5.59
MZL	3	4.46 ± 3.43
SLL/CLL	4	7.4 ± 2.01
MCL	4	13.47 ± 7.06
DLBCL	3	6.63 ± 8.9
FL	4	5.27 ± 2.98
ALCL	1	7

$P = 0.3$; MZL vs. MCL = 0.07.
Considering all non-MCL as a single group, the difference in ISI extent between MCL (13.47) and non-MCL (6.06) is significant ($P = 0.03$).
MZL, marginal zone lymphoma; SLL/CLL, small lymphocytic lymphoma/chronic lymphocytic leukaemia; MCL, mantle cell lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; ALCL, anaplastic large-cell lymphoma.

Table 4. Association between the quantity of intrasinusoidal marrow infiltration and the type of lymphoma in primary splenic and non-primary splenic lymphomas

	n	Mean ± SD
Primary splenic		
Whole series	35	5.23 ± 5
SMZL	28	5.83 ± 5.28
Non-SMZL	7	2.83 ± 2.79
P-value		0.12
Non-primary splenic		
Whole series	19	7.62 ± 5.59
MZL	3	4.46 ± 3.43
Non-MZL	16	8.21 ± 5.79
P-value		0.28

SMZL, splenic marginal zone lymphoma; MZL, marginal zone lymphoma.

Because of the small number of cases of some categories, all non-SMZL (in PSL) and non-MZL (in non-PSL) were categorised as a single group. When all non-SMZL were considered as a single group, the quantity of ISI was greater in SMZL than in non-SMZL, although not statistically different ($P = 0.12$). No difference in ISI quantity was also seen between MZL and non-MZL in non-PSL ($P = 0.28$). The results are summarised in Table 4.

Association between the pattern of non-ISI marrow infiltrate in PSL

None of the different types of small B-cell lymphoma showed intrasinusoidal infiltration as the only expression of BM infiltration. Interestingly, not even MZL had an exclusive ISI pattern. A number of patterns of BM infiltration could be seen in association with ISI in all cases (non-ISI pattern) (Fig. 3). According to the most currently applied classification (18–20), the patterns of BM infiltration associated with ISI were nodular, four cases (11.2%); interstitial, nine (25.7%); nodular-inter-

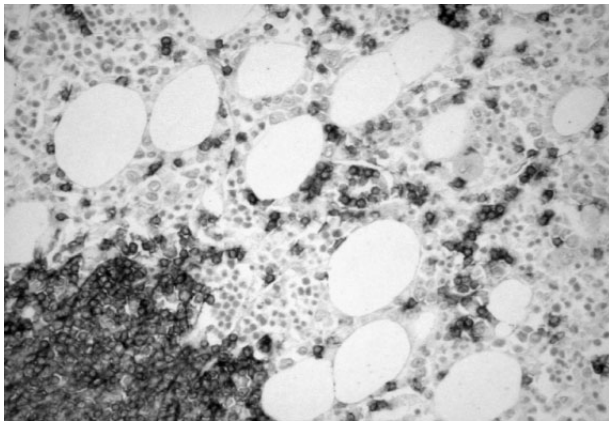

Fig. 3. Splenic marginal zone lymphoma showing a nodular, interstitial and intrasinusoidal pattern of invasion (CD20 immunostaining; original magnification ×250).

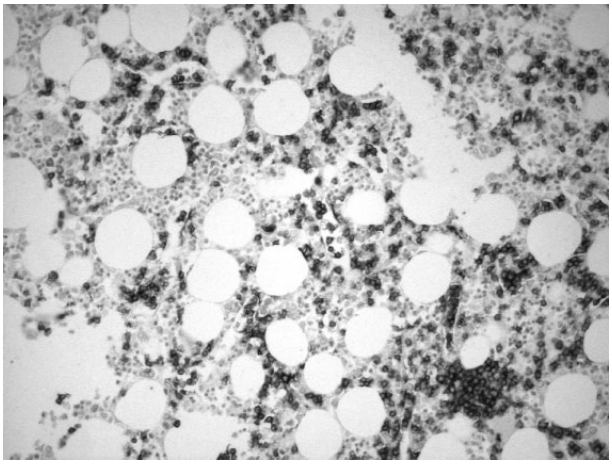

Fig. 4. Bone marrow involvement by B-chronic lymphocytic leukaemia showing intrasinusoidal, interstitial and nodular pattern of infiltration (CD20 immunostaining; original magnification ×250).

stitial, 19 (54.3%); diffuse, two (5.7%) and interstitial-paratrabecular, one (2.9%).

The distribution of the patterns of marrow invasion different from ISI was not different between SMZL and non-SMZL ($P = 0.16$). The nodular pattern was present in SMZL only. The results are shown in Table 5.

Association between the quantity of BM infiltration and the weight of the spleen in PSL

A direct correlation was found between the weight of the spleen and TI quantity in patients who underwent splenectomy ($r = 0.41$, $P = 0.04$), while no correlation was seen between ISI quantity and the weight of the spleen ($r = 0.04$, $P = 0.84$).

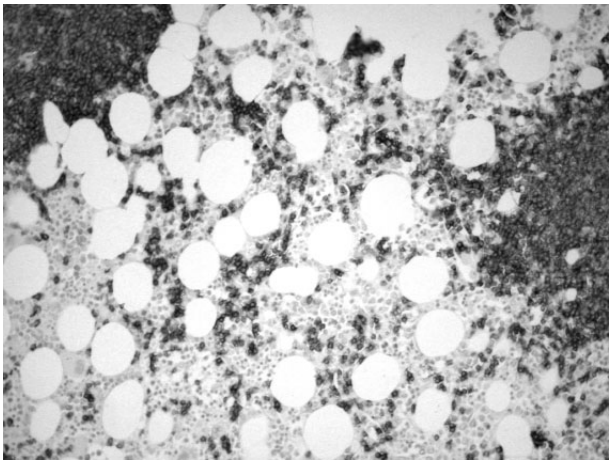

Fig. 5. Bone marrow involvement by mantle cell lymphoma. A predominant nodular pattern of infiltration is associated with intrasinusoidal infiltration (CD20 immunostaining; original magnification ×250).

Discussion

Intrasinusoidal infiltration is a pattern of invasion that is rarely found on BM biopsies performed for diagnostic or staging purpose (1) and is considered as a hallmark of SMZL with or without villous lymphocytes (4, 6). In our series, ISI was detected in only 54 of 1325 consecutive NHL showing marrow invasion (4.07%). However, although most of the cases (57%) with ISI were SMZL or non-splenic MZL, other lymphoma categories, in particular, SLL/CLL (Fig. 4), MCL (Fig. 5), FL (Fig. 6), DLBCL, LDL, PTL and ALC also showed intrasinusoidal infiltration. This is in accordance with a recent study reporting that ISI is a common growth pattern for different lymphoma subtypes (7).

Moreover, intrasinusoidal infiltration was not the exclusive pattern of BM infiltration in the different types of small B-cell lymphoma, not even

Table 5. Association between non-intrasinusoidal patterns of marrow invasion and lymphoma categories in primary splenic lymphomas

N (%)	Nodular	Interstitial	Nodular interstitial	Diffuse	Interstitial paratrabecular	Whole series
SMZL	4 (14.3)	8 (28.6)	15 (53.6)	1 (3.6)	0	28 (100)
Non-SMZL	0	1 (14.3)	4 (57.1)	1 (14.3)	1 (14.3)	7 (100)
Whole series	4 (11.2)	9 (25.7)	19 (54.3)	2 (5.7)	1 (2.9)	35 (100)

$\chi^2 = 6.58$; $P = 0.16$.
SMZL, splenic marginal zone lymphoma.

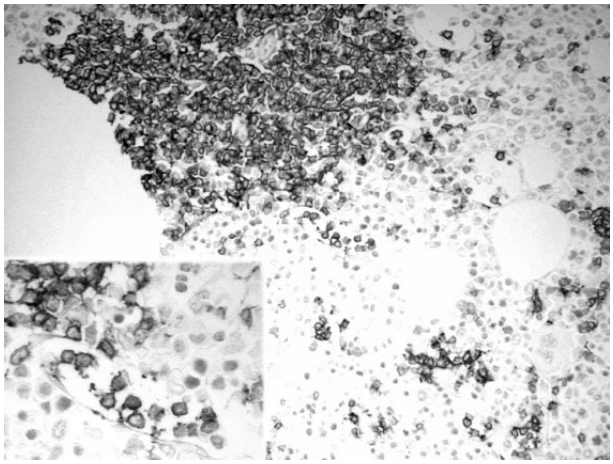

Fig. 6. Bone marrow involvement by follicular lymphoma showing a nodular–paratrabecular infiltrate associated with intrasinusoidal infiltration (CD20 immunostaining; original magnification ×250; inset: ×400).

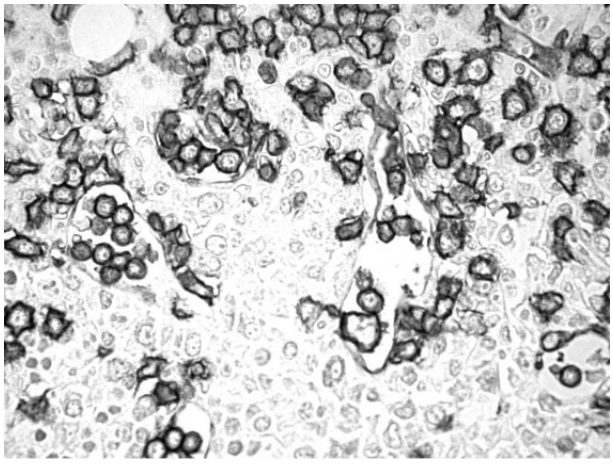

Fig. 7. Bone marrow involvement by marginal zone lymphoma showing association of a few large B cells with small cells in intrasinusoidal infiltration (CD20 immunostaining; original magnification ×400).

in MZL, but it was always associated with other patterns of infiltration. Moreover, in BM involvement by MZL, an association of a few large B cells with small cells in the intrasinusoidal infiltration was occasionally observed (Fig. 7).

A 'prominent' ISI was reported to be characteristic of SMZL (10, 11). Using strict morphologic and immunohistochemical criteria, we did not detect any difference in ISI quantity between PSL and non-PSL, but rather found that ISI quantity tended to be greater in non-PSL (7.68) than in PSL (5.23, $P = 0.08$). Therefore ISI quantity in BM biopsy does not allow the distinction between PSL and non-PSL.

When only PSL were analysed, ISI extent was greater in MZL type (5.83) than in other types (2.83); however, the difference is not statistically significant ($P = 0.12$) (Table 4). No differences were also seen among the various lymphoma categories ($P = 0.74$) (Table 2). Therefore, ISI quantity alone does not allow the diagnosis of SMZL on BM biopsy, and an 'extensive' or 'prominent' ISI is not a diagnostic criterion. This is also supported by the lack of correlation between ISI quantity and the weight of the spleen ($r = 0.04$, $P = 0.84$), whereas a direct correlation was found between TI quantity and the weight of the spleen ($r = 0.41$, $P = 0.04$).

When non-PSL were considered, ISI quantity was greater in non-MZL (8.21) than in MZL (4.46), although not significantly different ($P = 0.28$) (Table 4). Interestingly, the four non-splenic MCL of our series had an ISI quantity (13.47) greater than the other categories of non-PSL (6.06, $P = 0.03$). The finding is in accordance with a recent study reporting an extensive intrasinusoidal infiltration of BM in two cases of MCL (12).

As far as the patterns of BM infiltration different from ISI are concerned, Table 5 indicates that in PSL the nodular pattern was present in SMZL only, in agreement with reports showing that the most common finding in BM biopsy of patients with SMZL is the nodular pattern of infiltration (8, 21).

We are aware that the number of cases of small B-cell lymphoma is too small to allow a definite conclusion. However, our results suggest that although intrasinusoidal infiltration is very frequent in SMZL, ISI quantity alone, as assessed by strict morphologic and immunohistochemical criteria, is not diagnostic of SMZL, regardless of the extension. The pathologist should be cautious in referring the intrasinusoidal BM infiltration to a SMZL, unless a complete immunophenotype of the marrow infiltrate is performed.

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