J Clin Pathol 1998;51:638–640

Letter

Antibody panel in diagnosis of lymphoid neoplasms

The recent article by Gala and colleagues[1] raises once again the problem of the applicability of an antibody panel in the diagnosis of lymphoid neoplasms in routinely processed bone marrow trephine biopsies. In Bouin fixed bone marrow biopsies, these investigators found that two important antibodies used routinely (CD20/L26 and DBA-44) gave unsatisfactory results. Other B cell markers (LN-2, MB-2, and Ki-B-5) showed strong reactivity and still others (4KB5 and Ki-B-3) had inconsistent reactivity.

In most surgical pathology laboratories there is not such an ample panel of B cell markers. In these cases, L26 is the marker of choice since its value in the differential diagnosis of reactive and neoplastic small cell lymphoid aggregates in bone marrow biopsies has been demonstrated.[2] Unreliable results obtained using L26 in bone marrow biopsies poses an important diagnostic problem. For this reason, we would like to stress the advantage of fixing bone marrow in Zenker/glacial acetic acid solution (20:1). Addition of 1 ml glacial acetic acid to 20 ml of Zenker's solution must be undertaken immediately before use. Fixation time lasts 16 to 24 hours, and there is no need for additional decalcification after fixation. The specimen must be washed in running water for three to six hours and routinely processed for embedment in paraffin.[3] If undesided mercury pigment persists, sections can be treated with a 5% iodine solution in alcohol 70%, for three minutes, before staining. The level of morphological detail preserved is excellent.[4] Although L26 has been reported not to work on Zenker fixed bone marrow biopsies,[5] our experience with this method has been very satisfactory, using either a panel of antibodies indicated for haematological neoplasms[6] or for metastasis of solid tumour. In particular, we have had consistently satisfactory results using L26 (Dakopatts) and DBA-44 (G. Delsol, Toulouse, France), as shown in fig 1. The retrieval procedure consists of boiling slides in 0,01M citrate buffer, pH 6.0, in a household microwave (900 W, two cycles, 7 minutes each). Under our conditions, all the antibodies indicated[6] work well; only CD61/gpIIIa (Dakopatts) is consistently unreactive.

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The authors comment:

In a previous issue of this journal,[1] we presented a list of antibodies suitable for immunostaining of Bouin's fixed paraffin embedded bone marrow trephine biopsies. Very few data indeed report the reactivity of currently available antibodies on Bouin's fixed bone marrow. Accordingly, we assessed a panel of antibodies including many of the most currently used. Some of them were inconsistently reactive (4KB5/CD45RA ; Ki-B3/CD45RA, DBA-44, VS38) or unreactive (CD 20/L26 ; LN-1/CDw75 ; Bcl-1/PRAD1 ; DO-7 ; rabbit-Ki-67).

We read with interest the comments of Vassallo and Pinot relating to this work. They argue that "in most surgical pathology laboratories, there is not such an ample panel of B cell markers." While we understand their concern, the goal of our study was precisely to define those giving consistent and reproducible results, knowing that only a few would be effective with this fixative. This study shows that a limited number of appropriate antibodies can be used by laboratories working with Bouin's fixative. Immunotyping of lymphoid cells on Bouin's fixed bone marrow may be performed with a combination of three antibodies for B cells (Ki-B5 ; LN-2/CD74 and MB2) and two antibodies for T cells (UCHL-1/CD45RO and CD3-Rabbit). In our opinion, this limited panel appears neither excessive nor particularly uncommon. Moreover, some of these pan-B and pan-T antibodies have been assessed many years ago on Zenker fixed decalcified bone marrow, the fixative chosen by Vassallo and Pinot.[2]

They also state that "in these cases, L26 is the B cell marker of choice since its usefulness in the differential diagnosis of reactive and neoplastic small cell lymphoid aggregates in bone marrow biopsies has been demonstrated," citing a reference by Bluth *et al.*[3] We would like to modify this comment. First of all, the material used in the study cited was paraffin embedded marrow aspirates, not fixed and decalcified bone marrow. It is well known that both the bone marrow trephine fixation and decalcification are critical factors for lymphocyte antigen detection, and that problems of staining or antibody specificity depend on the methodology.

Regarding the relation between the choice of fixative and the quality of CD20 (L26) immunolabelling of bone marrow, B5 fixative was indeed reported to give stronger staining than formalin,[4] and has been recommended for phenotyping of leukaemias and lymphomas.[5] With regard to Vassallo and Pinto's illustration of hairy cells positively stained by L26 in Zenker's fixed bone marrow, previous immunomorphological analysis of these cells showed that positive staining is more common with DBA.44 than with L26 on formalin, Zenker, or Bouin's fixed bone marrow.[6] Other investigators willing to identify residual hairy or lymphoid neoplastic cells have reported the lack of staining with L26 in Zenker's fixed,[7 8] as well as formalin or Bouin's fixed, bone marrow.[9] In one of the above studies, it is worth noting that the overnight Zenker/acetic acid (19:1, vol/vol) protocol was very similar to that described by Vassallo and Pinot, and that the lack of staining was not confined to L26 but also extended to CD45RA (4KB5).[7] This is another discrepancy with Vassallo and Pinot's data, since they reported that "all the antibodies indicated [by Perkins and Kjeldsberg[5]] work well,"—including CD45RA. On the contrary, results with Bouin's fixed bone marrow[9] were in perfect accord with our own data.[1] Interestingly, very high background was reported with the widely used antibody UCHL-1 in the study cited by Vassallo and Pinot,[3] as well as in other studies.[7 10]

Besides the fixative, decalcification is another significant factor contributing to the alteration of L26 immunoreactivity.[4] Depending of the strength of the acids, bone decalcification may indeed influence antigenic reactivity.[4 11 12] Decalcification, which is mandatory before sectioning paraffin embedded bone marrow, is part of the traditional methods of preparation. Vassallo and Pinot stated that no additional decalcification was performed after fixation/decalcification.

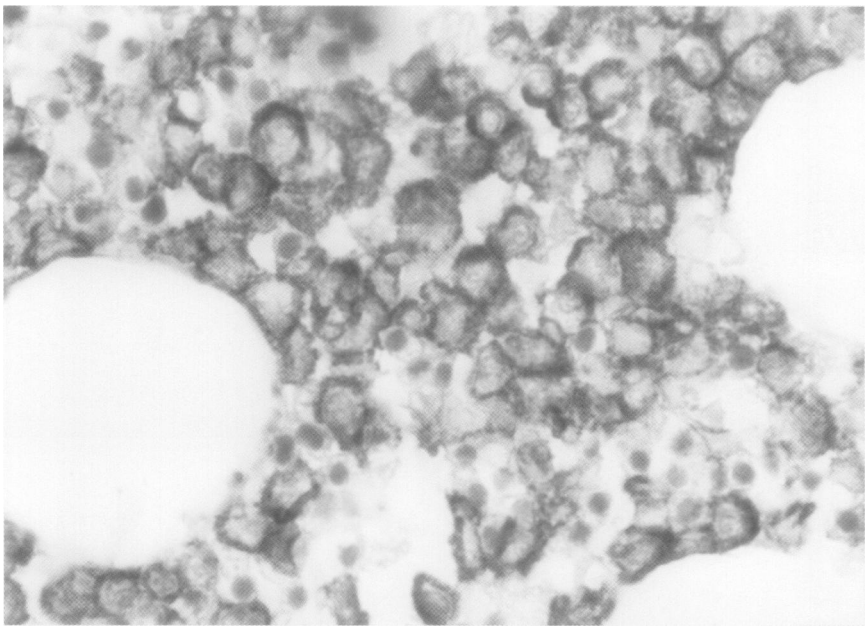

Figure 1 Hairy cell leukaemia. Strong positivity for CD20/L26 (bone marrow biopsies; streptavidin-biotin-peroxidase; ×1045; haematoxylin counterstain).

Among the other two reports using Zenker's fixative,[7 8] only one used additional decalcification in RDO,[8] a compound known to alter reactivity with antibody CD61/GIIIa and elsastase.[11] However, both reports pinpointed the lack of L26 staining regardless of these methodological differences. In our opinion, satisfactory results obtained by Vasello and Pinot with L26 may therefore not be related to the fixation/decalcification procedure but rather to the retrieval procedure, which was not used in the other two studies.

We would like to make a further modification to Vassallo and Pinot's comment. In our opinion, no cell marker shows such restricted reactivity that it allows a clear distinction to be drawn between malignant and reactive neoplastic lymphoid aggregates, except for the light chain restriction markers and Bcl-2.[13 14] It is, however, true that the pattern of staining (homogeneous or mixed), together with the size and number of aggregates, may help to discriminate between reactive or malignant aggregates. Accordingly, any B cell marker—including MB-2 or Ki-B5—may be as satisfactory as L26.[14]

Finally, we would like to update the list of antibodies effective on Bouin's fixed bone marrow, taking advantage of recent additional investigations. Bone marrow samples were collected from patients presenting with systemic malignant mastocytosis (n = 4), and stained using an antibody antitryptase (AA1; Dakopatts, Prosan, Belgium). Bone marrow fixation/decalcification, alkaline phosphatase-antialkaline phosphatase staining, and evaluation of staining intensity were described in our previous report.[1] Intestinal, splenic, and cutaneous infiltration were present in the four patients, all of whom complained of diarrhea and one presented with peripheral blood cytopenia. Immunostaining of the bone marrow clearly showed a diffuse infiltration with positively stained (4+ or 5+) mast cells in three cases, and scattered positive (4+ or 5+) mast cells in one (fig 1). Microwave heating or enzymatic digestion were not necessary. These complementary data are in agreement with previous data,[15] and confirm that AA1 antibody is suitable for detecting marrow mastocytosis in Bouin's fixed bone marrow.

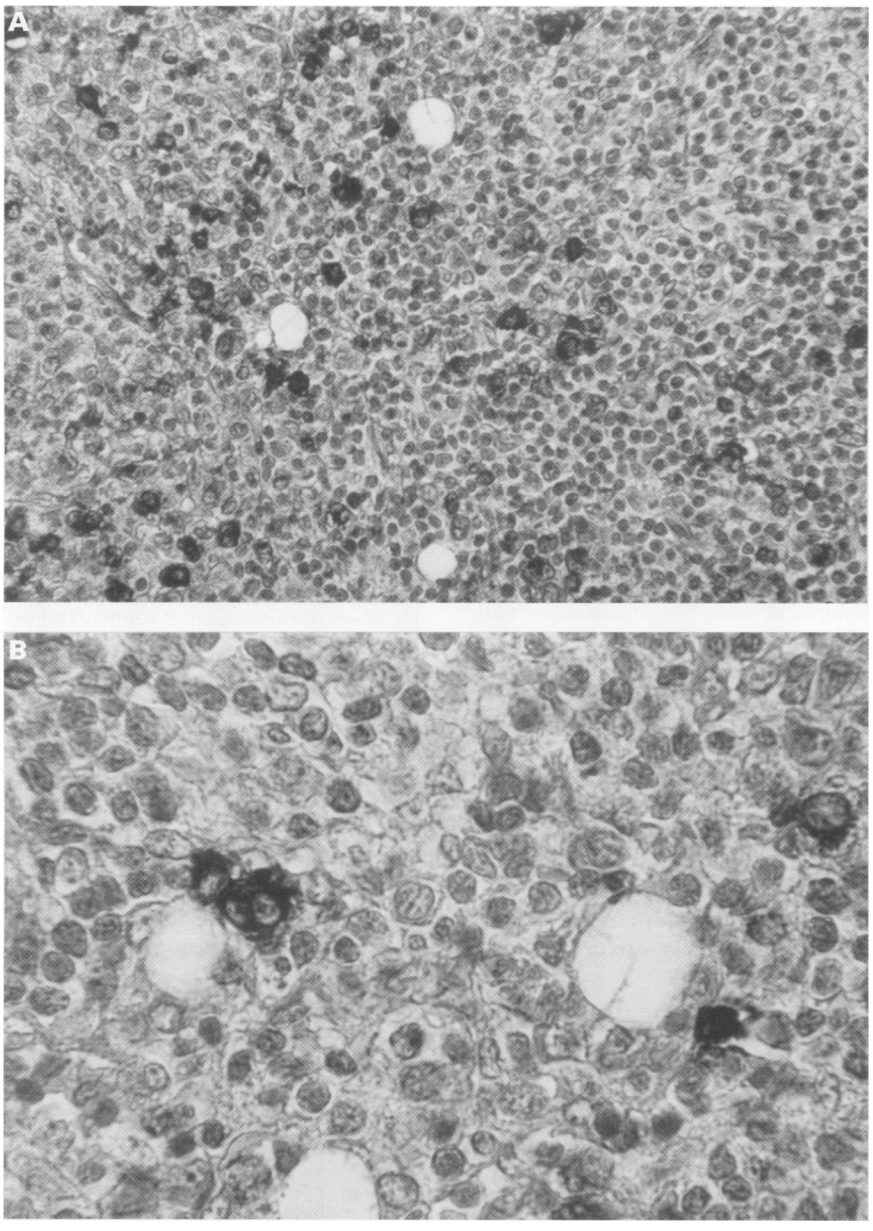

Figure 1 Bone marrow trephine infiltration by mast cells strongly reacting with the antitryptase antibody AA1. (A) Involvement of a reactive lymphoid nodule. (B) Scattered positive mast cells (details).

The study with anti-tryptase antibody was supported by the association Salus Sanguinis.

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Book reviews

Pathology of the Prostate. By C S Foster, D G Bostwick. (Pp 460; £60.) Harcourt Brace, 1997. ISBN 0 721 66951 4.

At last the relative neglect of pathology of the prostate is at an end! A new multiauthor book, which is comprehensive and extensively illustrated, is to be warmly welcomed. The