A panel of antibodies for the immunostaining of Bouin’s fixed bone marrow trephine biopsies

Jean-Luc Gala, Frédérique Chenut, Khang Bui Thi Hong, Jean Rodhain, Philippe Camby, Marianne Philippe, Jean-Marie Scheiff

Abstract

Aims—To assess a panel of antibodies on Bouin’s fixed bone marrow trephine (BMT) biopsies. These biopsies are widely used in routine diagnosis of various haematological malignancies and may be the sole material available in many centres; however, information regarding the immunostaining of this material is lacking.

Methods—Biopsies were taken from 72 patients presenting with various haematological malignancies (leukaemia, 38; lymphoma, 14; multiple myeloma, 20). A panel of antibodies was assessed on Bouin’s fixed BMT biopsies by the alkaline phosphatase-antialkaline phosphatase method.

Results—Three B (MB2, LN-2, Ki-B5) and two T cell lineage antibodies (UCHL-1, CD3-r) reliably identified lymphoid cells, while MPO-r, Leu-M1/CD15, and KP-1/CD68 recognised cells from the myeloid or histiocytic/macrophage series. Reed-Sternberg cells were stained by LN-2, Leu-M1, and CD30. Antibodies specific for plasma cells (VS38) and hairy cells (DBA-44) gave a variable pattern of staining. Among the proliferation markers, proliferative cell nuclear antigen but not Ki-67 related antibodies were effective.

Conclusion—This study presents a panel of antibodies with reactivity not restricted to common fixatives that are also suitable for Bouin’s fixed BMT biopsies. (J Clin Pathol 1997;50:521–524)

Keywords: Bouin’s fixative; bone marrow trephine biopsy; immunostaining

Table 1 Haematological neoplasms

<table>
<thead>
<tr>
<th>Neoplasm</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukaemia</td>
<td>38</td>
</tr>
<tr>
<td>Acute lymphoblastic B type</td>
<td>4</td>
</tr>
<tr>
<td>Acute lymphoblastic T type</td>
<td>2</td>
</tr>
<tr>
<td>Chronic lymphocytic B type</td>
<td>19</td>
</tr>
<tr>
<td>Chronic lymphocytic T type</td>
<td>1</td>
</tr>
<tr>
<td>Hairy cell</td>
<td>3</td>
</tr>
<tr>
<td>Acute myeloid</td>
<td>9</td>
</tr>
<tr>
<td>Lymphomas</td>
<td>14</td>
</tr>
<tr>
<td>Follicular with t(14:18)</td>
<td>2</td>
</tr>
<tr>
<td>Mantle zone with t(11:14)</td>
<td>3</td>
</tr>
<tr>
<td>Burkitt’s</td>
<td>5</td>
</tr>
<tr>
<td>Hodkin’s</td>
<td>4</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>20</td>
</tr>
</tbody>
</table>

Diagnoses were based on typical features found by cytology or histology, cytogenetics, immunophenotype, biochemistry, and molecular biology using conventional methods.

Material and methods

Seventy two samples were obtained from patients presenting with various haematological neoplasms (table 1): leukaemia (n = 38), lymphoma (n = 14), and multiple myeloma (n = 20). Bouin’s fixed BMT biopsies from patients with non-malignant haematological disorders (n = 8) were also included. All diagnoses were based on typical features found by cytology or histology, cytogenetics, immunophenotype, biochemistry, and molecular biology using conventional methods.

Bone marrow biopsies were all placed in Bouin’s fixative (copper acetate monohydrate 25 g, picric acid 40 g, distilled water 1000 ml, formalin 100 ml, acetic acid 15 ml) for no longer than 24 hours, decalcified for six hours in 7.5% nitric acid, and embedded in paraffin. Before immunostaining, each Bouin’s fixed BMT biopsy was dewaxed and rehydrated. The
alkaline phosphatase-anti-alkaline phosphatase staining, used to avoid the problem of endogenous peroxidase, was performed as described previously.6

Antibodies tested and optimal dilutions are listed in table 2. They were incubated for one hour, except for CD3-r (r = rabbit), Ki-67-r, lambda, and kappa that were incubated overnight. Isotype matched monoclonal antibodies were included as a control for non-specific binding. Slides were examined under a light microscope and evaluated for staining intensity, graded from 0 to 5+. Each antibody unreactive after conventional procedure was reassessed after microwave heating (five cycles of three minutes each in citrate buffer pH 5.7) or enzymatic digestion with pronase, according to the manufacturer’s instructions and literature.7

Results

The results are summarised in table 3.

B CELL AND PLASMA CELLS ASSOCIATED ANTIBODIES

Kappa and lambda—Both polyclonal antibodies positively stained (3+ or 4+) plasma cells in multiple myelomas, allowing the detection of monotypy.

L26, 4KB5, Ki-B3, LN-1—We confirmed a lack of staining with L26 1 to B cell malignancies (chronic lymphocytic, hairy cell) regardless of microwave heating procedure. Unlike previous data,3,4 neither Reed-Sternberg nor hairy cell leukaemia biopsies reacted with LN-1, and B cell malignancies were either inconsistently positive or very weakly stained (0+ to 1+) with 4KB5 and Ki-B3. LN-2, MB2, Ki-B5—LN-2, MB2, and the more recently described MoAb Ki-B5 strongly labelled all chronic lymphocytic B cell and hairy cell leukaemia biopsies (fig 1A), as well as follicular lymphomas, mantle zone lymphomas, and normal B lymphocytes (3+ to 5+). To the best of our knowledge, the reactivity of Ki-B5 recognising normal and neoplastic B cells has not been previously reported in BMT biopsies. LN-2 also identified Reed-Sternberg and Hodgkin’s cells in all Hodgkin’s disease biopsies assessed (3+ or 4+). MB2 reacted as expected with endothelial cells but stained only 50% of multiple myelomas.

DBA.44—Proposed as a hairy cell marker,4 this MoAb labelled only one of the three hairy cell biopsies (2+). Noteworthy, staining was markedly intensified (4+) by pronase and by microwave heating, but only in the single positive case.

VS38—The use of microwave heating was mandatory with this recently discovered plasma cell marker.4 Variable immunostaining of normal and malignant plasma was observed (0 to 4+). On most slides, strongly positive malignant plasma cells, observed on the border of heavily infiltrated slides (fig 1B), coexisted with totally negative cells in the centre of the biopsy, demonstrating the variability of labeling due to the quality of the fixation (poorer in the middle of the tissue).

T CELL ASSOCIATED ANTIBODIES

UCHL-1, CD3—Both T lineage antibodies, which are recommended for the diagnosis of T cell lymphomas in paraffin wax embedded sections,10 stained clearly normal and malig-
Antibodies for the immunostaining of Bouin’s fixed bone marrow trephine biopsies

nant T cells. Staining with UCHL-1 was usually stronger (4+ to 5+) than with CD3-r (2+ to 3+). In concert with others, we found staining of myeloid cells with UCHL-1.

MYELOID/MONOCYTE/MACROPHAGE ASSOCIATED ANTIBODIES

KP1/CD68, MPO, Leu-M1/CD15—KP1 reacted with marrow monocytes and macrophages in normal and pathological Bouin’s fixed BMT biopsies (4+ or 5+) (fig 1C), and strongly labelled myeloid cells in eight of nine acute myeloid leukaemias. Rabbit PoAb (MPO) against myeloperoxidase labelled blast cells in nine of nine myeloid malignancies (4+ or 5+) (fig 1D). Leu-M1 stained mature granulocytic cells and monocytes (5+), three of seven acute myeloid leukaemias (4+ or 5+), and Reed-Sternberg cells in four of four Hodgkin’s disease biopsies (3+ or 4+) (fig 1E).

NON-LINEAGE ASSOCIATED ANTIBODIES

Bcl-2, Bcl-1, DO-7—Among the antibodies to oncoproteins, bcl-2 but not DO-7 or bcl-1 (PRAD D1/cyclin D1) reacted on Bouin’s fixed BMT biopsies. Anti-bcl-2 MoAb consistently stained follicular lymphoma (5+) (fig 1F), chronic lymphocytic B cell leukaemia (3+ to 5+), acute lymphoblastic leukaemia (4+ or 5+), and multiple myeloma (2+ to 5+), but, as expected, none of the Burkitt’s lymphomas. CD30—Like LN-2, CD30 labelled the Reed-Sternberg cells in four of four Hodgkin’s disease biopsies.

PCNA, MiB-1, Ki-67—Despite microwave heating, no staining was observed with either

Figure 1 (A) Group of hairy cells reacting with Ki-B5; (B) VS38 positive multiple myeloma cells; (C) Cellular contact between KP1 positive macrophage and plasma cells; (D) Staining of myeloblasts by MPO in a case of acute myeloid leukaemia; (E) Typical Reed-Sternberg cell reacting with Leu-M1; (F) bcl-2 labelling of infiltrating follicular lymphoma cells.
MoAb or PoAb recognising the Ki-67 antigen. On the contrary, nuclear localisation of proliferative cell nuclear antigen (PCNA) was demonstrated in more than 80% of the malignant cells in highly proliferative Burkitt’s lymphoma (4+ or 5+) without antigen retrieval.

Discussion
Among the antibodies tested, several demonstrated excellent immunoreactivity on tissues from Bouin’s fixed BMT biopsies. The antigenic specificity on pathological marrows was in accordance with previously reported data. However, the weak and inconsistent immunolabelling with some MoAb that recognise formalin resistant antigen (4KB5, Ki-B3) renders them inappropriate for immunostaining of Bouin’s fixed BMT biopsies. Lack of staining was observed with DBA.44 from two florid hairy cell leukaemia biopsies, whereas they were strongly positive with MB2, suggesting that DBA.44 labelling may depend on the exposure to Bouin’s fixative. This seems also to apply to VS38 where strong positive or totally negative labelling of normal and malignant plasma cells was striking on the same slides. Interestingly, this staining variability was not observed with other antibodies (MB2, LN-2, Ki-B5, UCHL-1, CD3-ր, PCNA, CD30, Leu-M1). Noteworthy, preliminary microwave heating, recommended by the manufacturer’s instruction, was not mandatory for immunostaining of Bouin’s fixed BMT with Pab CD3-ր or CD30.

In conclusion, reliable immunophenotyping of archival Bouin’s fixed BMT biopsies is feasible. While demonstrating the variable degree of reactivity obtained on Bouin’s fixed and decalified tissues with some widely used or more recent antibodies, this study presents a panel of antibodies suitable for phenotyping the most common haematological neoplasms on this material.

This study was fully supported by the association Salus Sanguinis. We thank Professor Barrett, Danish Cancer Society, Copenhagen, Denmark and Professor Parwaresch, Institut für Hämatopathologie, Kiel, Germany for the generous gift of antibody, Dr M Zandecki, Hôpital Calmette, Lille, France for critical reading of the manuscript and useful discussions, and Prosan, Dako, Belgium for its contribution.

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*J Clin Pathol* 1997 50: 521-524
doi: 10.1136/jcp.50.6.521

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