L26 (CD20) staining of Bouin fixed bone marrow biopsies

L26 (CD20) is one of the most useful markers in the diagnosis of lymphoid neoplasms, but when Gala et al tested a large panel of antibodies for use in Bouin fixed bone marrow, L26 was one of the few antibodies which failed to stain. Vassallo and Pinto now suggest that if Zenker solution is used instead of Bouin solution, satisfactory L26 staining may be obtained. However, Zenker has its own technical and safety disadvantages and we note that, in contrast to the findings of Gala et al, successful immunostaining of Bouin fixed material for L26 has previously been noted and we get consistent, strong immunostaining for L26. We have fixed marrow biopsies in Bouin fluid for many years because of its excellent morphology in haematoxylin and cosin (H&E) staining and for its ease of use. (Following three to six hours of fixation in Bouin, biopsies are decalcified overnight in 10% formic acid. A short daytime processing cycle allows H&E sections to be reviewed late on the day following biopsy.) For immunohistochimistry we use antigen retrieval in antigen unmasking solution (Vector Laboratories) with pressure cooking for two minutes before applying L26 (Dako) followed by the avidin-biotin complex (ABC) technique. In addition to staining B cells, we note that L26 may give weak to moderate staining of megakaryocytes, a finding we have also seen with B5 fixed material. Nucleolar staining, described in epithelial and other cells types and regarded as non-specific, may be seen in some marrow blast cells. Figure 1 shows L26 staining of an infiltrate of hairy cell leukaemia in a Bouin fixed marrow biopsy. Gala et al indicate that there is limited information on the range of antibodies which stain in Bouin fixed marrow so we have reviewed all the immunostains on our bone marrow biopsies for the past year. In addition to the antibodies described by Gala we have found successful staining for L26, leucocyte common anti-

body, CD79a, CD34, CD68 (PGM1), epithelial membrane antigen, glycophorin A and C, S-100, and tryptase.

Authors' reply: In a previous issue of this journal, 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. Some of the antibodies assessed were inconsistently reactive (4KB5/CD45RA, Ki-B3/CD45RA, DBA-44, V538) or unreactive (CD 20/L26, LN-1/CDw75, Bcl-1/PRAD1, DO-7, rabbit-Ki-67). In a letter to the editor, Vassallo and Pinto commented on lack of reactivity of our L26 staining with Bouin's fixed material. They pointed out the usefulness of L26 staining for differential diagnosis of neoplastic and non-neoplastic small cell lymphoid aggregates in bone marrow specimens, and stressed the satisfactory results that were obtained with their Zenker fixed material. As pinpointed in our reply, it is of note that the major difference between their method and several previous discrepant reports of unsuccessful staining with L26 on Zenker's fixed material was the use of a microwave retrieval procedure.

In this issue, O'Brien and Murphy also stress the value of L26 for diagnosis of lymphoid neoplasms, and point out that they, and another team, have consistent L26 (CD20) immunostaining of Bouin's fixed and decalcified material. O'Brien and Murphy used three to six hours of fixation, decalcification overnight in 10% formic acid, followed by antigen unmasking solution with pressure cooking for two minutes. The other team reported successful L26 staining on Bouin's fixed bone marrow with a very similar procedure, including Bouin's fixation for 12 hours, decalcification in EDTA for two hours, and retrieval step by pressure cooking. This is highly comparable with our procedure (less than 24 hours of fixation, decalcification for six hours in 7.5% nitric acid). In agreement with others, however, we have experienced a lack of reactivity of L26 on Bouin's fixed material. Here again, the major difference between successful and unsuccessful L26 staining appeared to be the use of an adequate antigen retrieval procedure. While the reactivity of our panel of antibodies was compared with and without microwave heating on archival bone marrow biopsies, our evaluation with microwave retrieval was unfortunately hampered by the frequent unsticking and destruction of part or whole of the bone marrow core biopsy sample. Moreover, the length of fixation in several Bouin's fixed samples, described from other institutions, was close to 24 hours and it is thought that overfixation may damage the L26 epitope. Mounting processed bone marrow (fixation less than 12 hours) on coated slides now allows an adequate retrieval procedure. In such conditions and with microwave heating as prerequisite, our current L26 results are in full accordance with these of O'Brien and Murphy.

Regarding the statement of these investigators that L26 is one of the most useful lymphoid markers, we would like to emphasize that immunotyping of lymphoid cells on Bouin's fixed bone marrow may also be performed with surrogate B cell markers, such as LN-2/CD74 and MB2, but also by Ki-56, a very effective anti-cytokeratin antibody, either normal and malignant B cells. The advantage of these antibodies, compared with L26, is that they do not require prior antigen retrieval. The use of this panel of B cell antibodies, together with some non-specific markers and additional immunohistochemical feature, are easy identification and quantification of malignant B cells in bone marrow trephine biopsies.

The complementary list of other immunoreactive antibodies given by O'Brien and Murphy (LCA, CD79a, CD34, CD68, glycophorin A and C, S-100, and tryptase) is very useful for laboratories testing Bouin's fixed material, and we thank these workers for providing this valuable information. Like them, we previously reported and illustrated the strong reactivity of mast cells with antibody antitryptase (AA1). CD79a also appears immunoreactive in our hands after microwave heating. In the light of these new data, we would like to upgrade the current list of antibodies suitable for the immunostaining of Bouin's fixed bone marrow trephine biopsies; this includes antibodies for diagnostic and differential diagnosis of lymphoid neoplasms and haematological malignancies as well as solid tumors, as follows: CD4, CD8, CD15, prostate specific antigen (PSA, prediluted), carci-noembryonic antigen (CEA, prediluted), mouse, and rabbit antibodies (MAE, prediluted), and cytokeratin (clone MNF116, prediluted), all from Dakopatts, Prosan, Belgium. Preliminary results with CD5 and CD1a also seem encouraging but require confirmation on a larger series of clinical specimens. It should be borne in mind that prior microwave heating appears necessary for all these newly tested antibodies.

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Calcium oxalate (Weddellite) crystals within ductal carcinoma in situ

Following our short report of a rare example of calcium oxalate (Weddellite) crystals within papillary pattern ductal carcinoma in situ we have encountered a further case. A 54 year old woman had microrcalifications detected in her right breast on routine breast screening. Core biopsies taken under mammographic guidance showed ductal carcinoma in situ, but only the calcifications seen in the biopsies after examination of multiple tissue sections were of calcium oxalate (Weddellite) crystals in apocrine microcysts (not present in previously reported cases).

The case was immediately adjacent to the excision specimen in this case also contained calcium oxalate (Weddellite) within the secretions of ductal carcinoma in situ, photographed under partially polarized light. Haematoxylin and eosin stain.

Figure 1 Weddellite crystals within high grade ductal carcinoma in situ, photographed under partially polarized light. Haematoxylin and eosin stain.

Book reviews


In this book the authors aim to provide “a single source for describing and illustrating normal cytology that may be seen in day to day practice.” Following introductory chapters covering the structure of the cell and cytological techniques, there are sections devoted to specific cell types and individual organisms. There is a section on gynaecological cytology, although the majority of the book is devoted to fine needle aspiration and exfoliative cytology from other organs.

Each section is arranged in a similar fashion, covering anatomy, histology, normal cytology, and potential pitfalls. This results in a pleasing uniformity that is often missing in multiauthor texts. The text, while brief, is clearly written and the sections in each chapter on potential pitfalls are particularly useful. The illustrations provide a good mix of Papanicolaou and Diff-quick stained material, together with relevant haematoxylin and eosin stained histological sections. They are of generally good quality, although some of the photomicrographs of Diff-quick stained material are a little dark, and in some sections there is quite marked variation in the photomicrograph background. These minor problems do not, however, detract from what is a high quality text.

This book would be of use to any practising cytopathologist and would also be of benefit to trainees. As such, it would be an important addition to any cytopathologist’s library.

This is a new edition of the standard UK text on killing microbes or preventing their growth. The first half of the book is devoted to chemical disinfection and covers all the relevant agents, how they work, what can interfere with successful disinfection, assessing activity, specific target organisms, and some applications in the wider field of health care. The rest of the book is split between preservation and sterilisation. It is the preservation section that contains the most diversity in application, from familiar ground such as medicines and food to the preservation of wood, leather, and museum specimens. The sterilisation section covers sterilisation by heat, radiation, gases and filtration, the application of heat processes to both medical items and foods (the latter including substerilisation). This book is aimed at investigators and scientists looking for a review of data on the role of inflammatory mediators derived from fatty acids and their biological products in pathological physiology. Our increased understanding of this intricate molecular interplay between several classes of receptors and their ligands inevitably brings with it a buzz of excitement about potential therapeutic interventions targeted at specific interactions. With this perspective in mind, I think that scientists will welcome this excellent new book of protocols designed to characterise adhesion molecules experimentally at both structural and functional levels. The book draws together a wide spectrum of analytical strategies written by experts and is highly accessible to both the beginner and the experienced scientist. Each chapter concisely defines a single key aspect of study which scientists from many disciplines could usefully adapt to their own specific needs. Key references included with each chapter provide additional interesting and useful information. I was particularly delighted with the “notes” at the end of every chapter which highlight potential pitfalls and provide the kind of empirical wisdom which only experience brings.

LYNN MORGAN


The past years have seen an explosion of knowledge and interest in the central position of adhesion molecules and their ligands in the processes of cellular recognition, activation, and migration. More recently studies of leucocyte migration have also highlighted the critical roles played by chemokines and cytokines in recruiting specific cells to defined microenvironments in both normal and pathological physiology. Our increased understanding of this intricate molecular interplay between several classes of receptors and their ligands inevitably brings with it a buzz of excitement about potential therapeutic interventions targeted at specific interactions. With this perspective in mind, I think that scientists will welcome this excellent new book of protocols designed to characterise adhesion molecules experimentally at both structural and functional levels. The book draws together a wide spectrum of analytical strategies written by experts and is highly accessible to both the beginner and the experienced scientist. Each chapter concisely defines a single key aspect of study which scientists from many disciplines could usefully adapt to their own specific needs. Key references included with each chapter provide additional interesting and useful information. I was particularly delighted with the “notes” at the end of every chapter which highlight potential pitfalls and provide the kind of empirical wisdom which only experience brings.

LYNN MORGAN


This is an exploration of the role of fatty acids in the skin, a much underexplored area. The authors concentrate on describing biosynthetic pathways for ceramides in the skin, but principally on the role of arachidonic acid, eicosanoids, and leukotrienes in diseases of the skin. Topics reviewed include the role of eicosanoids in inflammation, their modulation as produced in the dermis and by keratinocytes, and also the role of these molecules in eczema and psoriasis. Brief sections describe basic analytical techniques and the effects of fatty acid compositions and their retinoid modulators in keratinocytes. The book is aimed at investigators and scientists looking for a review of data on the role of inflammatory mediators derived from fatty acids in dermatological disease. It is a good summary of the field, clearly written to a high standard throughout, with good illustrations and diagrams. However, it offers little for clinicians, as most of the information on the pathogenesis of dermatological inflammation but in its treatment in clinical practice.

ANTHONY WIERZBICKI

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Correction

Lam KY. Oesophageal mesenchymal tumours: clinicopathological features and absence of Epstein-Barr virus, October issue (52:758–760).

There is an error in the legend to figure 1, which should read: “Coexisting carcinoma (arrows) noted in oesophageal leiomyoma.”

The reference to the figure in the text (Results, para 6, 3 lines from the end) should be moved to the end of the next sentence: “Coexisting squamous cell carcinoma of the oesophagus was found in 12 patients (fig 1).”
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