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 eosin (H&E) staining and for its ease of use. (Following three to six hours of fixation in Bouin, biopsies are decal-
cified overnight in 10% formic acid. A short daytime processing cycle allows H&E sections to be reviewed late on the day following biopsy.) For immunohistochemistry we use antigen retrieval in antigen unmasking solu-
tion (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 find-
ing we have also seen with B5 fixed material. Nuclear staining, described in epithelial and ectodermal cells, 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 and so we have reviewed all the immu
nostains 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 (4K85/ CD45RA, Ki-B3/CD45RA, DBA-44, VS38) or unreactive (CD 20/L26, LN-1-CDw75, Bcl-1-PRAD, Ki-67). In a let-
ter to the editor, Vassallo and Pinto com-
mented on lack of reactivity of our L26 stain-
ing with Bouin’s fixed material. They pointed out the usefulness of L26 staining for differential diagnosis of reactive and neoplas-
tic small cell lymphoid aggregates in bone marrow specimens, and stressed the satisfac-
tory results that were obtained with their Zenker fixed marrow. 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) immunoreactivity on Bouin’s fixed and decalcified material. O’Brien and Murphy used three to six hours of fixation, decal-
cification 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 simi-
lar 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% in nitric acid). In agree-
mend 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 an adequate antigen retrieval procedure. While the reac-
tivity of our panel of antibodies was com-
pared with and without microwave heating on archival bone marrow biopsies, our evalu-
ation with microwave retrieval was unfortu-
nately 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, delivered 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 those of O’Brien and Murphy.

Regarding the statement of these investiga-
tors that L26 is one of the most useful lymphoid markers, we would like to emphasise 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-67, a very effective antigen in distinguishing normal and malignant B cells. The advan-
tage of these antibodies, compared with L26, is that they do not require prior antigen retrieval. The use of this panel of B cell anti-
body, together with marker antigen, easy identification and quantification of malignant B cells in bone marrow trephine biopsies.

The complementary list of other immuno-
reagents 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 antityrosine (AA1). CD79a also appears immunoreactive in our hands after micro-
wave 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 the haematological malignancies as well as solid tumors, as follows: CD4, CD8, CD15, prostate specific antigen (PSA, prediluted), carci-
noembryonic antigen (CEA, prediluted), mouse anti-human osteoclast (MAE, predi-
luted), and cytokeratin (clone MNF116, predi-
luted), all from Dakopatts, Proscan, Bel-
gium. Preliminary results with CD5 and CD1a also seem encouraging and 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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chemical evaluation of neoplasms in bone mar-
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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 situ1 we have encountered a further case. A 54 year old woman had microcalcifications detected in her right breast on routine breast screening. Core biopsies taken under x ray guidance showed ductal carcinoma in situ, but the only calcifications seen in the biopsies after examination of multiple tissue sections were of calcium oxalate (Weddellite) crystals in the lumen of a duct involved by high grade solid pattern ductal carcinoma in situ. A subsequent x ray guided wide excision of the microcalcifications revealed extensive high grade solid and comedo pattern ductal carcinoma, associated with (predominantly) ordinary-type microcalcifications, but also with luminal Weddellite crystals. This contrasted with our previously reported case in which the ductal carcinoma in situ was of papillary type.2

As previously discussed,3 Weddellite-type microcalcifications are usually associated with benign breast disease—particularly with apocrine microcysts.3 Interestingly, the wide excision specimen in this case also contained apocrine microcysts with Weddellite crystals, which were immediately adjacent to the Weddellite containing ductal carcinoma in situ (fig 1).

Ductal carcinoma in situ associated with Weddellite-type microcalcifications remains a rare finding. The coexistence of Weddellite crystals in apocrine microcysts (not present within previously reported cases of ductal carcinoma in situ in this case) lends further support to the "bystander" theory for this phenomenon; that is, that ductal carcinoma in situ may involve a previously benign duct containing Weddellite crystals associated with pre-existing benign changes.

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


Virginia Woolf wrote, "for the desire to read, like all the other desires which distract our unhappy souls, is capable of analysis." You may have noticed a few unhappy trainee souls around at the time of the MRCPPath examination, so will the arrival of the fourth edition of Postgraduate Haematology bring a little light into their lives? The question of whether any book has a role for future trainees in the age of electronic publishing is certainly worthy of analysis. Remember when videos were going to sweep aside the cinema audience? The internet is unrivalled for current information but only the most hardened of computer nerds could suggest that the internet will ultimately replace the textbook. The local trainees who have had a look at Postgraduate Haematology agree with my assessment that it is an excellent overview and very clearly laid out. Some of the tables will be familiar to those who have attended the Hammersmith course, but all the 41 authors are to be complimented on their presentation of tables and diagrams. The morphology slides are also particularly good.

The layout is clear with the diagrams and photomicrographs organised in the appropriate section of the text so that they can be easily referred to. I recall reading a review of the second edition which criticised a didactic style which was necessary to keep the size of the book down. The fourth edition is a much more expansive text to take in the wealth of recent developments (such as new chapters on haemopoiesis and stem cell transplantation), but it does present most sides of the major issues and the bibliography is commendably concise. I particularly enjoyed the chapters on aplasia, genetic disorders of haemoglobin, and the myeloproliferative disorders.

Inevitably there are criticisms but they really are minor issues. A textbook will find that its description of current trials (for example, CML IV) will be out of date by the time it is published. You could quibble about the balance in some areas but there is a wellcome overall balance between malignant and non-malignant haematology. Do you find it a challenge to remember CD numbers? I'm almost up to double figures but true enthusiasts can test themselves on seven pages of numbers, taking them up to CD166. The female trainees were not impressed that pregnancy is considered as a systemic disease, nor by the fact that the common haematological problems in pregnancy merit only one page, while the spleen has its own chapter. One could carp over other aspects but the one positive point is that the fourth edition of this standard text book succeeds extremely well in providing a readable and visually attractive general overview of haematology. Clinical haematologists like myself attending the BSH or ASH meeting show a high incidence of narcolepsy during some of the presentations. This book will give us a good cutting edge introduction so that at least we can understand the titles of the presentations next time.

It can be highly recommended to everyone who works in a haematology department and particularly its target audience of postgraduate students.

NEIL ANDERSON
J A MURRAY

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. Most recent 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 bio-synthetic 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 and its 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 interested not in the pathogenesis of dermatological inflammation but in its treatment in clinical practice.

ANTHONY WIERZBICKI


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 approaches), novel and experimental sterilisation technologies, reuse of disposables, and quality assurance. These are not fields where there have been major advances since the previous edition in 1992, but there have been shifts in perception. There is an important new chapter on the thorny topic of inactivating the agents of transmissible encephalopathies by physical and chemical methods. New space is also given to protozoa, listeria, and biofilms, and more detail on virucidal activity and rapid methods in microbial assessments. In all, this edition has expanded by some 200 pages. The book is not aimed at those primarily interested in infection control in health care. Its prime use lies in its multidisciplinary approach. There is always a danger that, as disciplines evolve, they will separate from each other and follow increasingly disparate standards. Sometimes this may be a rational decision, but sometimes it may occur through isolation from wider contexts. The prime advantage of this book is that, in having such a wide range of approaches to microbial applications, it enables a reader to make decisions and choose approaches within a broadly informed context.

P N HOFFMAN


This book is a capacious text on the vulva and very useful from the clinical viewpoint in diagnosing and treating vulval disease. For histopathologists the clinical aspect is helpful, but for diagnostic pathology cross reference to other texts in colour may be necessary. Overall this book is a useful addition to the pathology library with well written and clear explanations of vulval conditions.

M M WALKER

Notices

The International Academy of Pathology Forthcoming meetings

Symposium on Infectious Diseases, Antwerp, Belgium, 19–20 May 2000


Further details from: British Division of the International Academy of Pathology (administrative secretary, Mrs C Harris), PO Box 73, Westbury on Trym, Bristol BS9 1RY; tel +44 (0)117 907 7940; fax +44 (0)117 907 7941; email: bdiap@cableinet.co.uk

Course on Pulmonary Pathology

London, 20–23 June 2000

This course is designed to provide histopathologists and cytopathologists with an opportunity to study diagnostic lung pathology in a comprehensive manner. It comprises lectures and practical microscopy sessions, the latter making up roughly half the time and consisting of individual study of a unique collection of cases.

Further details and application forms from: Professor B Corrin, Brompton Hospital, London SW3 6NP; fax +44 20 7351 8293; email: b.corrin@ic.ac.uk

Correction

Lam KY. Oesophageal mesenchymal tumours: clinicopathological features and absence of Epstein-Barr virus, October issue ($2.758–760).

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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