

## Letters

### Methods for analysing AgNORs

In their interesting review on proliferation markers in tumours, van Diest and coworkers<sup>1</sup> emphasise the importance of the assessment of AgNORs (nucleolar organiser regions), since AgNOR scores correlate well with other proliferation markers and can be used to estimate cell cycle time. The authors finalise the section with the statement "but at present these methods are difficult to apply in daily practice." At the moment, indeed, most of the working groups determine the silver stained area in the nucleus, which requires morphometry of course, and is time consuming. Yet we would like to remind readers that there are alternative methods for AgNOR analysis that do not depend on image analysis systems and can easily be applied in daily routine work. In our own investigations we have always emphasised the different morphological appearances of silver precipitations. AgNOR staining of cytological preparations from acute leukaemias allows the differentiation of clusters (aggregations of precipitations within a common matrix in the nucleolus) and dots (small singular precipitations without a matrix).<sup>2,3</sup> Our staining and counting procedures are standardised and the inter- and intraobserver variability is low. This alternative approach is justified because for acute leukaemias there is a good correlation between the BrdU index and the mean number of clusters ( $r = 0.60$ ) or the percentage of cells with one cluster ( $r = -0.63$ ), bearing in mind that the correlation between the mean AgNOR size and BrdU shows a very similar value ( $r = -0.63$ ).<sup>3</sup> For chronic lymphocytic leukaemia (CLL) we also recommend making a differential count, separating cells with one or two compact nucleoli and cells with clusters.<sup>4,5</sup> The percentage of cells with clusters correlates well with the tumour mass score ( $r = 0.72$ ) and lymphocyte doubling time ( $r = -0.74$ ) and permits one to differentiate well between stable and progressive CLL. Furthermore the AgNOR pattern in CLL helps in the follow up monitoring of the patients and their response to chemotherapy.<sup>4,5</sup>

In summary, although we agree with others that measuring the AgNOR area provides very important information, especially with regard to the cell cycle time,<sup>1</sup> we believe that there are alternative ways of analysing AgNORs which can be more easily applied in daily practice, but nevertheless are of equal pathophysiological and clinical relevance.

KONRADIN METZE

IRENE LORAND-METZE

Faculty of Medicine, State University of Campinas,  
BR 13081-970 Campinas-SP, Brazil

- 1 Van Diest PJ, Brugal G, Baak JPA. Proliferation markers in tumours: interpretation and clinical value. *J Clin Pathol* 1998;51:716-24.
- 2 Grotto HZM, Metzke K, Lorand-Metze I. Pattern of nucleolar organizer regions in human leukemic cells. *Anal Cell Pathol* 1993;5:203-12.
- 3 Lorand-Metze I, Carvalho MA, Metzke K. Relationship between morphometric analysis of nucleolar organizer regions and cell proliferation in acute leukemias. *Cytometry* 1998;32:51-6.

- 4 Lorand-Metze I, Metzke K. AgNOR clusters as a parameter of cell kinetics in chronic lymphocytic leukaemia. *J Clin Pathol Mol Pathol* 1996;49:M357-60.
- 5 Lorand-Metze I, Chiari AC, Andrade FL, et al. Nucleolar organizer regions as a proliferation marker in chronic lymphoid leukemia. *Eur J Histochem* 1998;42(suppl 1):19.

### Cervical intraepithelial glandular neoplasia

Kurian and Al-Nafussi<sup>1</sup> deserve our gratitude for shedding further light on the difficult subject of cervical intraepithelial glandular neoplasia. In particular, by establishing a ratio of 1.12:1 between the incidence of invasive and non-invasive disease and of 0.96:1 for low grade and high grade in situ lesions, they provide a means by which pathologists may monitor their diagnostic performance using the principles outlined by Wakely *et al* in 1998.<sup>2</sup> This will be particularly informative in the case of low grade lesions which could be easily overlooked or passed off as reactive changes. The classification of in situ glandular lesions of the cervix is the subject of much controversy—for example, two methods are described in a standard British textbook of gynaecological pathology,<sup>3</sup> each of which differs from the method used in the current study, which has also dispensed the term *adenocarcinoma in situ*. Variations in diagnostic criteria and terminology between papers may go some way to explaining why the conclusions in this paper, where progression from low grade to high grade disease is assumed to occur, differ from those of Goldstein *et al*,<sup>4</sup> who suggested that there was no morphological evidence to support the existence of a spectrum of endocervical glandular changes culminating in what they recognised as adenocarcinoma in situ. Finally, Kurian and Al-Nafussi's study highlights the importance of the cervical smear test in detecting these lesions when it is used as part of a screening programme.

M K HEATLEY

Department of Pathology, The Royal Liverpool  
Hospital, Liverpool L7 8XP, UK

- 1 Kurian K, Al-Nafussi A. Relation of cervical glandular intraepithelial neoplasia to microinvasive and invasive adenocarcinoma of the uterine cervix: a study of 121 cases. *J Clin Pathol* 1999;52:112-17.
  - 2 Wakely SL, Baxendine-Jones JA, Gallagher PJ, et al. Aberrant diagnoses by individual surgical pathologists. *Am J Surg Pathol* 1998;22:77-82.
  - 3 Griffin NR, Wells M. Premalignant and malignant glandular lesions of the cervix. In: Fox H, Wells M, eds. *Obstetrical and gynaecological pathology*, 4th ed, vol 1. Edinburgh: Churchill Livingstone, 1985:323-44.
  - 4 Goldstein NS, Ahmad E, Hussain M, et al. Endocervical glandular atypia; does a preneoplastic lesion of adenocarcinoma in situ exist? *Am J Clin Pathol* 1998;110:200-9.
- Authors' response

### Author's response

It is a pleasure to read Dr Heatley's response to our paper. The reason for abandoning the term *adenocarcinoma in situ* in our report is to conform with the new terminology for glandular lesions of the cervix. This is due to be released shortly in the *Guidelines of Royal College of Pathology* for reporting cervical biopsies.

A AL-NAFUSSI

Department of Pathology,  
University of Edinburgh Medical School

## Book review

**Manual of Diagnostic Antibodies for Immunohistochemistry.** By A S-Y Leong, K Cooper, and F J W-M Leong. (£45.00.) Greenwich Medical Media, 1999. ISBN 1 900151 31 6.

Immunohistological staining has rapidly become accepted as an important, and in some cases indispensable, adjunct to histopathological examination and diagnosis. Histopathology laboratories have to be proficient in immunostaining procedures and fully conversant with the sensitivities and specificities of the primary antibodies used, the nature of the epitope demonstrated by each antibody, and its sensitivity to common fixatives. A thorough knowledge of tissue processing as well as methods of antigen retrieval is essential for routine practice.

This excellent manual provides a comprehensive list of diagnostic antibodies (151) presented in alphabetical order, and covers the background and applications of each reagent together with relevant references. Most of the antibodies described are immunoreactive in fixed paraffin embedded tissue sections as these remain the mainstay of routine diagnostic histopathology. There is much useful practical technical information in each of these brief descriptions—evidence of the authors' extensive expertise—and as such this manual is much more than another antibody catalogue.

The authors rightly emphasise that immunohistological diagnosis is critically linked to an assessment of the morphological appearances and stress the need to employ a panel of antibodies to establish the immunoprofile of a tumour and thus reduce the risk of misinterpreting false positive and false negative staining. The text is cross referenced to an appendix that contains an extensive assembly of selected antibody panels (35) to a wide variety of specific diagnostic situations, providing an easy to use practical guide. A further appendix addresses the value of heat induced epitope retrieval and provides a succinct and helpful protocol for antigen retrieval using microwaves. Surprisingly there is no section on proteolytic digestion techniques, though experience has shown that these techniques have advantages over heat induced unmasking methods for some antibodies—for example, S100, CD21, CD68, 34 E12, CMV, cytokeratins, and so on.

This manual provides a wealth of essential technical information for biomedical scientists and pathologists who regularly employ diagnostic antibodies in their work. There will, however, be a need for early revision as new antibodies are becoming available with increasing frequency and immunodiagnostic practices will be continually evolving with their use. This book will become an important practical reference source for all immunohistopathological laboratories and will be regularly consulted by both trainee and experienced biomedical scientists and pathologists as part of their routine work and research.

E L JONES

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  - 2 Washington JA. Conventional approaches to blood culture. In: Washington JA, ed. *The detection of septicemia*. West Palm Beach, Florida: CRP Press, 1978:41-87.
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