Correspondence

1 PCNA immunohistochemical expression (evaluated with the PC10 mono-
clonal antibody) seems to be related to cellular proliferation in many normal tissues and in some cases in tumors, such as gastro-
intestinal lymphomas, central nervous system tumours, lung neuroendocrine neoplasms, and prostatic carcinomas.

However, in other tumours, like breast and gynaecological, PCNA (PC10) expression seems aberrant and not strictly related to proliferative activity. 

Various factors unrelated to cell proliferation may influence the immunohistochemical expression of PCNA, including tran-
scriptional regulation (and deregulation) of the PCNA gene, long half-life of the PCNA protein, involvement of PCNA pro-	ein in DNA repair synthesis, and tissue section processing—type and degree of the fixatives, fixation time, section heating, immunohistochemical techni-
ques.

Further problems in PCNA immunohisto-
chemical staining, as in other kinetic quan-
titative immunohistochemical studies, concern evaluation and scoring methods. Should we use quantitative or semiquan-
titative methods? How can cell counts be obtained? Which tumour areas should be evaluated (the most positive or random selec-
ted areas) to which immunoreactive cells should be evaluated (all positive cells or only the most intensely stained)? Particular attention should be also drawn to the kind of antibody used to localise PCNA. Different staining patterns may be seen with different antibodies, and this may add to confusing and confusing results.

In our opinion PCNA immunostaining should be evaluated with great caution and in some fields even with scepticism. More work is needed to assess the extent and range of PCNA staining in different tissues and lesions (neoplastic and non-neoplastic). PCNA counts should be evaluated concurrently with the different anti-PCNA avail-
able antibodies and the results should be compared to confirm the "proliferation index" and especially with clinical data. The possi-

bility that PCNA immunostaining may have diagnostic or prognostic value is intriguing and carefully performed clinicopathological studies are needed to assess this possibility further. This will be the only way to know if we are faced with an interesting but clinically worthless tool or with an important test to be added to the routine evaluation of neo-
plasms.

6 Harper ME, Glynne-Jones E, Goddard L, et al. Relationship of proliferating cell nuclear anti-
11 Sivji MKK, Kenny MK, Wood RD. Proliferat-
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4 Cheville JC, Clamon GH, Robinson RA. Silver stained nucleolar organiser region in the differentia-

6 Derezini M, Farabegoli F, Trerè D. Relationship between interphase AgNOR distribution and nuclear size in cancer cells. Histochem J (in press).

Method for grading breast cancer

Parham and colleagues have proposed a new and "simplified" method for grading breast cancer that claims to be superior to the Bloom and Richardson method, 5 which they rightly criticise for its lack of precision. We agree entirely with this criticism, but are rather surprised that we do not refer to our recent publication in which, for precisely this reason, we have devised modifications which provide objective criteria for the evaluation of the three morphological components of his-
torial grade. 6 We have shown in a study of over 1500 patients that histological grade, using this method, provides powerful prog-
nostic information, and in combination with tumour size and lymph node status the Nottingham Prognostic Index which can be used by clinicians to stratify patients for the temperature and temporal length of the staining reaction. These two variables are inversely related to each other: the higher the temperature, the shorter the time required for the staining reaction to be complete. The staining reaction is prolonged beyond the time for selective visualisation of NORs, but the other nuclear structures are progressively stained, until the whole nucleus appears homogeneously stained. For this reason, it is evident that different nuclear structures have been stained and counted in various laboratories, and this has caused disagreement about AgNOR numbers report-
ed in individual studies on the same neo-
plastic lesions.

In a recent investigation it was shown that the total interphase AgNOR area was closely related to the whole nucleolar area stained by silver when staining was prolonged for the optimal time for selective interphase NOR visualisation. 6

To obtain comparable data between differ-
ent laboratories the whole nucleolar ought to be silver stained and the area occupied by the silver stained nucleoli per cell measured using image analysis instead of AgNOR counting. Because AgNOR area and nucleo-
lar size (proliferative) are so strictly related, the morphometric analysis of silver stained nucleoli will certainly have the same clinical and biological relevance demonstrated for interphase AgNORs.

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10 Leonard E, Girlando S, Mauri FA, Dalla Palma P, Barbarechi M. PCNA and Ki67 expres-
13 Woods AL, Hall PA, Shepherd NA, et al. The assessment of proliferating cell nuclear anti-
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17 Woods AL, Hall PA, Shepherd NA, et al. The assessment of proliferating cell nuclear anti-
18 Woods AL, Hall PA, Shepherd NA, et al. The assessment of proliferating cell nuclear anti-
19 Woods AL, Hall PA, Shepherd NA, et al. The assessment of proliferating cell nuclear anti-
appropriate treatment. This method for histological grade has been adopted by the Royal College of Pathologists’ Working Group for use in the NHS Breast Screening Programme.  

Parham and colleagues have concluded from a small series of cases (105) that mitotic counts and semiquantitative assessment of tumour necrosis are the most significant factors. Unfortunately, despite their criticism of the Bloom and Richardson method, the authors appear to have fallen foul of the same imprecision which they espoused. Although they have followed us in defining the field area for mitotic counting, they do not state in their paper how many mitoses per field were counted for each point scored. Their evaluation of tumour necrosis also lacks clarity. It is admirable to define the dimensions of an area of necrosis but there is surely a flaw in the assessment of multiple foci if only the largest focus is counted. This basis a tumour could have several foci of necrosis each of which might score 1 or 2 points, but this only qualifies it for an overall score of 2; less than a tumour with a single focus. The relative lack of numerical data in this paper is also surprising and we are not told the number of cases in each necrosis group. For these reasons we must conclude that not only are there doubts about the value of this new method, but fear that for lack of an adequate description no one else will actually be able to use it. A number of other points are pertinent. The study is confined to tumours of no special type which seriously reduces its utility, since, as we have shown recently, only 50% of cases of invasive breast carcinoma fall into this category. It is remarkable that no reference to the Breast Cancer Prognostic Index, among the many indices scores five groups of patients are identified with an annual mortality ranging from 1.5 to 32%. In practice, however, prognosis must be related to the available treatment. The information we have on the use of more than three groups serves no useful purpose.


Immunophenotype of multicellular tumours from giant cell lesions

I read the interesting paper by Dr Dousis and colleagues and I wish to focus in here in the light of our own results.

In our investigation enzyme histochemistry was applied to cryostat sections of unfixed and undecalcified specimens of 101 different tumours or tumour-like lesions of bone. In all cases the osteoclast-like giant cells showed the same pattern of reactions, which was identical with that of osteoclasts but different from that of the multinucleated neoplastic cells: a lack of demonstrable alkaline phosphatase, but clearly detectable activity of tetraritate-resistant acid phosphatase (TRACPase) activity; non-specific acid esterase, leucinaminopeptidase, and NADH-tetrazolium oxidoreductase activity. Microdensitometry of the enzyme reaction product 4 in giant cells of varying sizes in six different bone tumours exhibited the same trend in all cases: a continuous decline of the relative activities of non-specific esterase and NADH-tetrazolium oxidoreductase but an increase in the TRACPase activity with increasing cell size. Among the very large giant cells, however, there were cells with both high and very low TRACPase activities. Additional electron microscopy examination of these cases showed swollen mitochondria with cristolysis, fragmentation, and swelling of cisternae of endoplasmic reticulum and the nuclear envelope, more and larger digestive vacuoles with myelin-like materials and many vacuoles of variable size scattered throughout an electron dense cytoplasm. This pattern differed from that seen in the smaller giant cells. Thus we hypothesised that an increase in cell size osteoclast-like giant cells changed their physiological activities and that at least some of the very large cells degenerated.

It is interesting to note that in the study by Dousis et al the pattern of reactivity for anti-CD 68 was quite similar to that of non-specific esterase and NADH-tetrazolium oxidoreductase, but the giant cells with larger diameters clearly showed less density of the immunoperoxidase reaction product than the smaller ones (figs 2A and 3A of the paper by Dousis et al). We think that these photographs confirm our theory. A microdensitometric examination of these sections would certainly demonstrate a size dependent pattern of the anti-CD68 reaction product comparable with that obtained in the study of the above mentioned two enzymes.

Dousis et al show that giant cells of giant cell tumours can be distinguished from other giant cell containing bone tumours by the absence or paucity of the HLA-DR reactive. The authors mention that the above explanations, that this phenomenon might be due to differences in the nature of the giant cells. But our study of enzyme physiology and ultrastructure of osteoclast-like giant cells in various bone lesions does not support this hypothesis. Furthermore, despite some differences, osteoclast-like giant cells of both giant cell tumours and other giant cell containing tumours or bone lesions share many antigens in common. * Bearing in mind the observation that lymphokines modulate the expression of HLA-DR in human monocytes and macrophages, we suggest that this is also the case for the osteoclast-like giant cells. Therefore, we favour the alternative explanation given by Dousis et al, that the differing HLA-DR expression may reflect variations in the tissue matrix or in the immunological response, to a lower extent among the various bone tumours or tumour-like lesions.

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Method for grading breast cancer.

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