offer similar sensitivity and specificity to that established by immunofluorescence techniques.

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MATTERS ARISING

Diagnostic importance of cytokeratin expression in linings of odontogenic cysts

Recent studies on keratin expression by odontogenic cyst epithelium suggest that while expression of keratin 19, and possibly keratin 5, is a characteristic feature of human odontogenic epithelium, there are no clear differences in keratin expression between cyst types that might be diagnostically useful.[1-3] Our own studies[2] and the report of Morgan *et al*,[4] however, suggest that the odontogenic keratocyst may be unusual in coexpressing keratins 19 and 10. Unfortunately this observation cannot form the basis of a routine diagnostic test as the epitopes recognised by the monoclonal antibodies specific to keratins 19 and 10 are destroyed by formalin fixation and paraffin wax processing. A further complication to the potential use of the "odontogenic keratocyst specific" keratin phenotype (keratin 19^+10^+) is that it may also be detected in other types of odontogenic cyst that have undergone metaplastic keratinisation.[2]

The recent paper by MacDonald and Fletcher, however, suggests that dentigerous cysts and odontogenic keratocysts may be differentiated by the pattern of staining obtained using a commercially available monoclonal antibody to intermediate molecular weight keratins (LP34; CK1, Dako) and an indirect immunoperoxidase technique on trypsin treated, formalin fixed, paraffin wax sections.[5] While dentigerous cyst linings were darkly stained throughout their whole thickness, those of odontogenic keratocysts showed no or weak staining of basal and suprabasal cell layers with stronger surface staining. This report prompted us to review the paraffin wax sections in our files which had been stained with the two monoclonal antibodies (LP34, Dako; CAM5.2, Becton Dickinson) used by MacDonald and Fletcher.[5]

Stained sections from 18 dental (periapical) cysts, 12 dentigerous cysts, and 15 odontogenic keratocysts were examined. All the specimens had been formalin fixed and decalcified in 10% formic acid for between four and 24 hours before being embedded in paraffin wax. Trypsin treated (20–30 minutes, room temperature), 5 μm sections were stained using a conventional indirect immunoperoxidase technique. Sections were sequentially treated with 0·3% hydrogen peroxide in methanol (20 minutes), 50 mg/ml ovalbumin solution (10 minutes), monoclonal antibody (60 minutes; LP34, 1/40–1/100; CAM5·2, 1/20), peroxidase conjugated rabbit anti-mouse Ig (Dako; 1/100 dilution in buffer containing 10% normal human serum) and DAB reagent. Sections were washed between all steps after the primary antibody had been applied. All washes and reagent dilutions were performed using 0·05M TRIS-HCl (pH 7·6) containing 0·1M sodium chloride).

All cysts showed moderate to strong reactivity to strong reactivity throughout their epithelial linings with LP34 (figure). Dental and dentigerous cyst linings exhibited even, strong reactivity; those of odontogenic keratocysts occasionally exhibited more intense staining of the basal cells (figure). This latter staining pattern did not seem to correlate with any local differences within the connective tissue capsule (such as extent of inflammatory cell infiltrate). Reactivity with CAM 5·2 was restricted to some surface cells of the epithelial linings of three dental cysts and eight dentigerous cysts (figure). Staining of dental cysts was clearly associated with areas of mucous metaplasia with mucus secreting cells being CAM $5{\cdot}2^+$, $LP34^-$. The reactivity of dentigerous cysts for CAM 5·2 often seemed to be within isolated single or groups of surface cells.

Our present observations, together with those obtained using frozen sections,[2] suggest that it is not possible to differentiate between odontogenic cyst types on the basis of LP34 immunoreactivity. Although epithelial reactivity of odontogenic keratocyst linings with LP34 was subjectively less intense than that of dental and dentigerous cyst linings, the considerable differences between our results and those of MacDonald and Fletcher[5] presumably indicate that LP34 reactivity in paraffin wax sections of odontogenic keratocysts is particularly laboratory or technique dependent. Indeed, the Dako data sheet concerning LP34 states that this antibody may not give "satisfactory" results on formalin fixed, paraffin wax sections unless large amounts of keratin proteins are present.

Finally, it is generally agreed that the greatest diagnostic difficulty in distinguishing between odontogenic cyst types occurs when the typical histological appearance of the epithelial lining is masked by inflammatory changes or metaplastic keratinisation. As both events are known to affect keratin expression[3 4] it is unlikely that keratin profiles will aid the histological diagnosis of odontogenic cysts.

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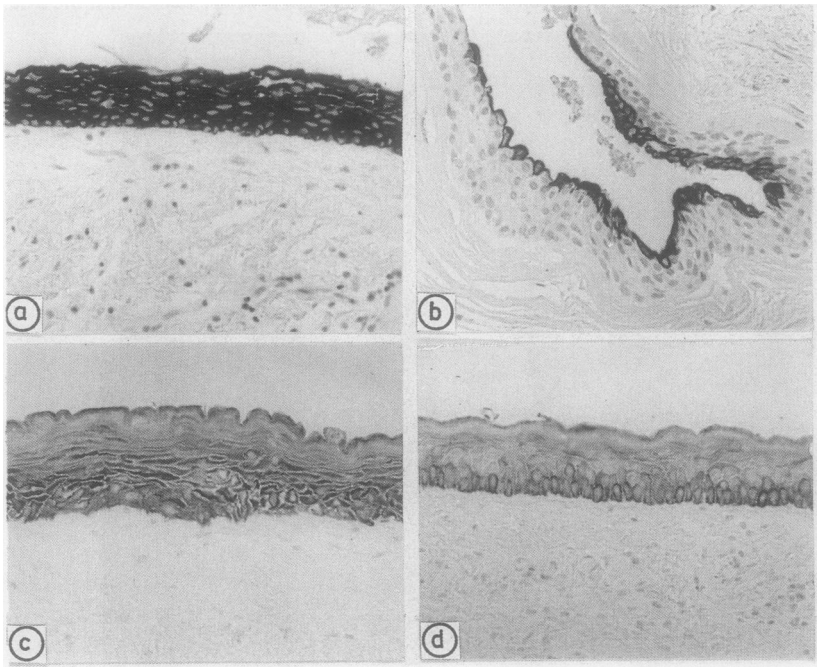

Staining patterns of epithelial linings of dentigerous cysts (a and b) and keratocysts (c and d) with LP34 (a, c, and d) and CAM 5·2(b).

4 Morgan PR, Shirlaw PJ, Johnson NW, Leigh IM, Lane EB. Potential applicaions of antikeratin antibodies in oral diagnosis. *J Oral Pathol* 1987;16:212–2.
5 MacDonald AW, Fletcher A. Expression of cytokeratin in the epithelium of dentigerous cysts and odontogenic keratocysts: an aid to diagnosis. *J Clin Pathol* 1989;42:736–9.

Drs MacDonald and Fletcher comment:

We are grateful to Matthews and Browne for their comments and we note the differences in staining pattern in odontogenic keratocyst linings using LP34 between their method and the method we described. The methods are different in several important respects. The tissue on which we based our observations had not been subjected to any form of decalcification. We used a normal rabbit serum pretreatment and incubated the section overnight with primary antibody (LP34; 1/200 dilution). This, we have found, virtually eliminates background staining while giving strong reliable reactivity. The method described by Matthews and Browne uses tissue which has been decalcified in 10% formic acid and pretreated with ovalbumin, and the incubation period and dilution of the primary antibody were different (LP34; 1/40–1/100 for 60 minutes).

Experienced immunohistochemists agree that to expect reproducible results using monoclonal antibodies, such as LP34, the processing of the tissue and methods of staining used should be similar wherever possible.

We should like to take this opportunity to reactify a typographical error and a detail from the material and methods section of our recent article. The antibody CAM 5·2 was supplied by Becton Dickinson and the secondary antibody we used was a peroxidase conjugated rabbit antimouse serum (Dako; 1/25 dilution).

AW MACDONALD
A FLETCHER

Statistics on microcomputers

The article on correlation and regression shows the correlation coefficient of r = 0·021 for the data in figure 1c, depicting a recurvilinear relation between measurements A and B (page 5).[1]

With all due respect, I believe that the correlation coefficient is inappropriate for a recurvilinear relation of this sort; instead, an appropriate quadratic equation could be derived for the relation and the correlation calculated from that; alternatively, and more simply, the correlation ratio or eta coefficient.[2]

The eta coefficient calculated for the measurements read (admittedly with some small inaccuracy) from the graph shown in fig 1c provides a value of 0·9154. In other words there is a very good recurvilinear correlation shown, and this figure certainly fits better with the "eyeball" estimate of linear quality to the points on their graph.

The eta coefficient does not seem to be widely used. It is indeed a little crude compared with deriving the proper quadratic equation, but the approximations are very close and rarely give rise to differences larger than the second decimal place.

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1 Brown RA, Swanson Beck J. Statistics on microcomputers: A non-algebraic guide to their appropriate use in biomedial research and pathology laboratory practice 4. Correlation and regression. *J Clin Pathol* 1989;42: 4–12.
2 Downie NM, Heath RW. Other correlation techniques. In: *Basic statistical methods*. New York: Harper and Row, 1974:110–14.

Drs Brown and Swanson Beck comment:

Dr Muckle seems to have misunderstood our reason for including fig 1c in article 4 of our recent series on the use of microcomputers for statistical analysis.

In the second paragraph we emphasised that preliminary scatter plots should be used to assess the data visually and determine whether the points were obviously distributed along a straight or curved line or whether they were scattered randomly. In the third paragraph we defined the product-moment correlation coefficient and explained that this technique is invalid when there is a strong non-linear relation between the measurements: fig 1c was presented as a serious warning on such inappropriate use of correlation coefficient.

When faced with data such as those given in fig 1c, it would be usual to attempt to fit a curve as suggested by Dr Muckle, but this was beyond the scope of the introductory series of articles. Both Minitab and Statgraphics can be used to fit a curve to data and to calculate r^2 as a measure of the amount of the variability explained by the curve. The correlation ratio to which Dr Muckle refers is synonymous with r^2: when calculated for fitting a quadratic function to the data and it was greater than 0·9. It is, however, important to remember that this expression uses the term "correlation" loosely because it properly refers to "the strength of the linear relationship between two or more variables" (Kotz S & Johnson NL, 1982 *Encyclopedia of Statistics* Vol 2, p 193).

C-myc oncogene product p62 in ovarian mucinous neoplasms

In the article by Polacarz *et al* the oncogene protein product of *c-myc* was noted to differ in localisation in ovarian mucinous cystadenoma and cystadenocarcinoma. Such a difference can be explained by either compartmentalisation or artefactual localisation. The latter was considered to be unlikely because such a difference could be seen between two different epithelial types on the same tissue block in studies on colonic polyps.

Such a conclusion is based on the assumption that the *c-myc* protein in different epithelia reacts similarly with fixatives and that the nuclear structure (especially the nuclear matrix), with respect to its ability to retain the *c-myc* protein, is in a similar state in both epithelia.

The first assumption is not always true. Abnormal *c-myc* protein is not unknown in malignancies (in Raji cell line) and these proteins in different structures may behave differently when exposed to fixatives. This has been noted in mutated *c-myc* in vitro. Lee *et al* defined the regions in *c-myc* protein which are important but not necessary for nuclear localisation. In transfected cells expressing different mutants of *c-myc*, however, there is a discrepancy in the localisation of the protein when deletions of these regions occur in the mutant cells, depending on the method of processing.

If the protein is similarly mutated in malignant epithelium we might be seeing an artefact of protein displacement during tissue processing, but structural changes in protein are probably uncommon in malignancies. In fact, there is evidence to suggest that preservation of specific regions of the *c-myc* protein is important for its transforming ability and that these regions overlap with those which are most important for nuclear localisation.[1] Whether there are contributory factors which may cause protein displacement during processing, such as structural changes in the nuclear matrix and the chromatin state, need to be investigated further.[2]

Such an analysis does not exclude a change in compartmentalisation nor does it negate the importance of consistently finding cytoplasmic localisation of *c-myc* in certain neoplastic states. After all, what histology demands is only a reproducible artefact. On the other hand, there would be important implications for the function of the *c-myc* protein if we assume that its real in vivo localisation is affected by targetting in malignant epithelium. We need to assess critically tissue processing.[3,4]

We have shown previously that fixation and embedding do affect the quantitation and localisation of *c-myc* protein in cell lines and normal tissues.[3] The optimal conditions for more reliable and consistent localisation of *c-myc* protein in frozen tissue have been defined.

Purely cytoplasmic staining is very common, even in many normal tissues in paraffin wax blocks, and a complete nuclear localisation is seen in the corresponding frozen blocks if processed using the conditions we have defined. In a case of mucinous ovarian tumour of borderline malignancy, we found the same discrepancy between the paraffin wax and frozen blocks. Although it is hard for us to prove that it is the nuclear localisation that is real, it will probably be more difficult to argue that the cytoplasmic localisation is not artefactual.

For optimal immunostaining, the frozen sections are freshly cut, incubated at 45°C for five to 30 minutes, air dried for one hour and fixed in 1% paraformaldehyde for 30 minutes. Periodate-lycine-paraformaldehyde gives similar results, while acetone or formalin give weaker staining with a tendency of more cytoplasmic than nuclear staining as the duration of fixation increases. The sections are then put into 0·1%. Triton-X-100 for 10 minutes. Standard peroxidase or APAAP techniques can then be applied.

In trying out these methods we have seen different artefacts, leading us to conclude that cytoplasmic localisation is easily simulated by manipulation of the tissue processing.

Lastly, we might also need to look at the difference in nuclear matrix between benign and malignant epithelia. It is probably not too far-fetched to imagine that cytoplasmic localisation of *c-myc* protein in paraffin wax sections is related more to the chromatin pattern. If this were the case, maybe a more reasonable speculation on the function of *c-myc* is that it helps to maintain the chromatin in a state ready for cell division.

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