Effect on lymph node status of triple levelling and immunohistochemistry with CAM 5.2 on node negative colorectal carcinomas

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Abstract

Several papers have recently assessed whether immunohistochemistry increases the accuracy of staging in node negative colorectal carcinomas, by showing micrometastases. The results have been contradictory. In this paper 542 nodes which were negative for tumour on routine sectioning, were stained with CAM 5.2. In addition, the tissue was levelled three times, this technique being more economical, to see if either or both techniques increased the pick up of micrometastases. Six nodes showed positive staining of occasional cells in the subcapsular and paracortical sinuses, but these were cytologically benign on review and were not thought to represent metastatic tumour. Triple levelling did not show any additional micrometastases. (Gut 1994; 35: 1447-1448)

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The prognosis of colorectal carcinomas is known to be directly related to lymph node status with Dukes's staging^{1 2} still being used today. Accuracy in assessment of lymph nodes is known to be increased by xylene clearance of mesenteric fat³⁻⁵ with potential improvements in correlation between pathological staging and prognosis. Papers advocating the use of multiple levelling of lymph nodes in staging of breast carcinomas have recently been published, groups finding a higher incidence of metastasis to the axillary lymph nodes.⁶

The technique has not been evaluated with regard to colorectal tumours. Recent published reports have argued over the benefit of immunohistochemistry on node negative carcinomas but the significance remains unclear.⁷⁻⁹

In this paper, we have taken 33 cases of Dukes's A and B carcinomas, all of which had undergone xylene clearance of submitted fat. In addition to staining the retrieved lymph nodes with CAM 5.2, we levelled each node containing block three times to find out if this more economical technique would prove as, or more effective. Cases were taken from between 1984 and 1986 to assess five year survival should any change in staging result.

Methods

Thirty three cases of Dukes's A and B carcinomas were selected at random from between 1984 and 1986. All cases had undergone clearance of pericolic fat by first fixing the submitted tissue in 10% formal saline, followed by dehydration in 99% industrial methylated spirits, and finally clearance of the fat with xylene. This technique did not change the staining characteristics with CAM 5.2. Slides containing lymph nodes were reviewed by two of the authors (AGN/MGC) and absence of tumour was confirmed. One section was then taken from each block containing nodes and, together with one section of the primary tumour for control purposes, were stained with CAM 5.2 using an avidin-biotin immunoperoxidase method. These same blocks, containing nodes, were subsequently levelled three times at 75 micrometre intervals and stained routinely with haematoxylin and eosin.

Results

A total of 542 nodes were examined, average per case 16.4 (range 5-37) and none showed metastatic tumour on triple levelling. In six nodes, there was positive staining of cells in the subcapsular and paracortical sinuses with CAM 5.2 (Fig 1). These cells seemed cytologically benign, however, lacking the malignant characteristics of those comprising the primary tumour - that is, cellular pleomorphism, nuclear hyperchromatism, and nuclear atypia (Fig 2).

Discussion

Carcinoma of the breast has been evaluated

Figure 1: Subcapsular sinus in a lymph node containing cytologically benign cells staining positively with CAM 5.2, (original magnification $\times 200$).

Figure 2: Primary colorectal carcinoma from the same case as Fig 1. Note the difference in cell morphology between the adenocarcinoma cells and the positively staining cells in the node (haematoxylin and eosin, original magnification $\times 200$).

using multiple levelling and opinion is divided as to its value. In a paper on the prognostic importance of occult lymph node metastases, the Ludwig group advocate the technique to increase the accuracy of lymph node status as a prognostic index⁶ and Saphir and Amromin sequentially levelled axillary nodes, finding a 30% increase in positive nodes.¹⁰ In that particular paper, however, the average number of sections per case was 332 and we and others feel that such time consuming techniques are not worthwhile.¹¹ The purpose of this work was to see if triple levelling of lymph node blocks, a technique that could be practically administered within the financial and temporal frameworks of a general pathology laboratory, would provide an increase in the accuracy of pathological staging. With none of 542 nodes changing from negative to positive status, our results suggest that it does not.

Published reports on using immunohistochemistry to uncover micrometastases is widespread but results differ as to its value with regard to colorectal carcinomas. Davidson *et al*⁷ found only one additional metastatic deposit in 249 nodes with epithelial membrane antigen and carcinoembryonic antigen while a recent study by Haboubi *et al*,⁹ combining xylene clearance with CAM 5.2 staining, changed the staging of 12 of 41 cases of colorectal carcinoma. Cutait *et al*⁸ also found positive staining of cells within the

subcapsular sinuses in 22 of 603 nodes, but it is interesting that these cases showed no change in their five year survival within their groups.

Our own results suggest that this technique is not of value in increasing the accuracy of pathological staging above that of xylene clearance alone. We feel that the positively staining cells are benign because they lack the distinct malignant cytological characteristics seen in the primary tumour. Although the possibility that they are degenerate malignant cells cannot be excluded, we believe that they are more likely to represent drainage of benign epithelial cells, reported in the cases of naevus cells¹² and postulated in the spread of endometriosis.¹³

In summary, we feel that immunohistochemistry and levelling of nodes, performed to an extent that could realistically be managed on a routine basis, do not increase the accuracy of the Dukes's staging system above that of xylene clearance.

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