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Contra:

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The Editors of the *European Journal of Cancer* gave Professor Walker the task of summarising the contra position, focusing on microvessel density as an expression of angiogenic activity and its relationship to patient outcome, since this is a feature which could be determined in a routine setting.

THE MAJOR biological and clinical problem of solid epithelial tumours is their capacity to metastasise; in the majority of cases, this is what causes the death of patients. Metastasis is a complex process and several different components can be identified: alterations in cell–cell and cell–stromal interactions; the degradation of stroma and basement membrane; the penetration of tumour cells into vessels; the ability of tumour cells to survive in the circulation; extravasation; and the ability of tumour cells to grow at their final resting state [1]. One factor which is important to several of the components of the metastatic cascade is angiogenesis. The growth of solid tumours is dependent on this, since if new vessels do not form, the tumour will not grow beyond 2–3 mm [2]. This is important for both primary and secondary growth. New vessels will provide a route for tumour cells into the circulation. There has been a considerable body of important research into angiogenesis in non-neoplastic and neoplastic conditions and the development of agents which act against angiogenic factors involved in new vessel formation is clearly of major therapeutic importance [3]. Another way in which the biologically important area of angiogenesis has been translated to the clinical situation has been in the determination of the microvessel density of tumours and its evaluation of the relationship to patient outcome. If such approaches are to be employed in histopathology laboratories to provide information that will be used for the selection of therapy (e.g. adjuvant chemotherapy versus no therapy), then the tests have to be reproducible between laboratories, with good quality control.

Various tumour types have been studied for microvessel density. One group reported that the quantitation of microvessels was of value in predicting the behaviour of malignant melanoma [4, 5]. Associations between microvessel counts and a higher incidence of metastasis have been found for prostatic carcinoma [6], gastric carcinoma [7] and head and neck tumours [8]. Non-small cell carcinoma of the lung has been analysed by several different research groups. Macchiarini and associates [9] showed that in node-negative, operable cases, microvessel count was the only independent prognostic parameter. A study of adenocarcinomas of the lung of different stages failed to show a correlation between microvessel count and overall survival [10]. A recent study [11] of different non-small cell types, both node-positive and node-negative, concluded that microvessel count was an independent prognostic indicator. However, this was only after excluding node status from the statistical analysis, because of the highly significant correlation between it and microvessel count.

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The main body of work on microvessel density and prognosis has related to breast cancer and here the findings are conflicting. Interest in this area was stimulated by the report from Weidner and colleagues [12] in 1991, of the correlation between microvessel density in the areas of most intense neovascularisation and metastasis. In view of the continual search for new and better markers of prognosis of breast cancer, it was predictable that microvessel density would result in numerous publications. One of the first, by Horak and associates [13], reported an independent association with survival. This was shortly followed by a further paper from Weidner and colleagues [14], who found an independent association with relapse-free and overall survival. There have been subsequent reports of an association between microvessel density and survival, either from the same groups [15, 16] or from independent researchers [17–19]. However, there have been several studies which have failed to find any association [20–25]. Why this discrepancy?

The problems relate to two factors: the application and evaluation of immunohistochemistry and the heterogeneity of breast cancer. These problems are common to studies of other markers. The first point to consider is which is the best antibody to use to identify endothelium. Antibodies against factor VIII-related antigen have been favoured by Weidner and colleagues [12, 14, 16] and have been found to be better than CD31 antibodies [25], or give similar results [18]. Harris' group [13, 15] consider CD31 to be a more sensitive endothelial marker. Both CD31 and CD34 antibodies were used by Goulding and associates [24], who found no significance with survival; CD34 antibodies were employed in a recent paper demonstrating an association with survival [19]. It would seem that there is no agreement about the best endothelial antigen to detect. Fixation and antigen retrieval can certainly modify reactivity. The main problem, though, seems to be in the quantification of microvessels, particularly with regard to the intrinsic variability of the microvessel count and the subjectivity of selection of areas for counting. Counting of three separate, non-overlapping fields from areas with the most intense vascularisation, from one tumour block, has been proposed [12, 14], although the Oxford group favour Chalkley counting. This relies on the identification of the region within a tumour with the highest microvessel density and counting the number of points in a Chalkley eyepiece graticule that fall on stained vessels in three areas [26]. Even when guidelines are followed, considerable variability can occur between the results for the same tumours for different analysts and among regions for the same tumours [22]. Other groups following the guidelines suggested by Weidner and colleagues have [19] or have not

[23–25] found an association between microvessel density and survival. Goulding and associates [24] also evaluated random field selection and image analysis to provide an objective and unbiased estimate, but found no relationship with survival.

The other problem relates to the heterogeneity of breast cancer and the need to take into consideration stage, age and type of carcinoma. This is well illustrated by the carefully documented study of infiltrating lobular carcinomas [25] which failed to show any associations between microvessel density and prognosis in this specific group.

The evidence to date about the value of microvessel counting is still inconclusive. The main problems appear to relate to evaluation. Until these are overcome the assessment of microvessel density is not an appropriate test to use outside of a very limited number of laboratories. Also, a static count of microvessel density does not necessarily reflect the dynamic process of metastasis. Seeking information about the factors stimulating angiogenesis, which may be targets for therapy, would appear to be a more profitable approach.

In conclusion, determining microvessel density in human tumours is not clinically useful.

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ELLIS IN his elegant article (pp. 609–613) highlights the theoretical issues which are the basis of the potential relevance of the determination of angiogenic activity in human neoplasia. Bi-directional research from the laboratory to the clinic provides rapid information on the pivotal role of angiogenesis in tumour growth, progression and metastasis, as well as on anti-angiogenic therapy [1].

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