PERGAMON

European Journal of Cancer 38 (2002) 1951–1952

European Journal of Cancer

www.ejconline.com

Letter

Heterogeneity of vascularisation in invasive breast carcinoma

P.B. Vermeulen[a,*], S.B. Fox[b], L.Y. Dirix[c], C. Colpaert[a], E.A. Van Marck[a]

[a]*Department of Pathology, University Hospital Antwerp, Edegem, Antwerp, Belgium*
[b]*Tumour Pathology Unit, Nuffield Dept. of Clinical Laboratory Sciences, John Radcliffe Hospital, Headington, Oxford, UK*
[c]*Angiogenesis Group, Oncology Centre, General Hospital St. Augustinus, Wilrijk, Antwerp, Belgium*

Received 4 February 2002; accepted 5 March 2002

With great interest we read the publication of Ahlgren and colleagues [1] in the first issue of the *European Journal of Cancer* of 2002. The authors have analysed the variation of microvessel density (MVD), highlighting vessels according to the methodology described in an international consensus publication on angiogenesis quantification [2], in sections derived from different paraffin blocks from the same tumour in a small group of 21 invasive breast carcinomas. They concluded that the value of MVD assessment in hot spots as a measure of angiogenesis is questionable due to the observation that the variation among sections contributed more to the total variance than the variation between different tumours. We would like to argue against this conclusion.

Firstly, we would like to emphasise that MVD is not a measure of angiogenesis, but reflects only the number of functional and afunctional blood vessels in a tumour. This is the end result of integration in time of blood vessel growth, subsequent remodelling and angioregression. A more accurate measure of angiogenesis, the methodology of which we have described in the second international consensus of angiogenesis quantification [3], is the fraction of proliferating endothelial cells. This difference is important to make since it is likely that MVD will have limited value in predicting the response of anti-angiogenic or vascular targeting drugs. Nevertheless, all tumours are indeed dependent on the presence of blood vessels, newly formed or co-opted and despite its limited predictive value, MVD has repeatedly been proven to have a prognostic value, especially in breast cancer [4].

Ahlgren and colleagues have correctly stated that the most observer-dependent step of determining vascularity in tumour sections, as first described by Weidner and colleagues [5], is the selection of the most vascularised areas, or the so-called 'hot spots', at low magnification. Although automation techniques might be of help to increase the reproducibility of this step [6], as described in detail in our second consensus report [3], the authors have neglected the second most important variability of MVD assessment, the decision the investigator frequently has to make of whether two adjacent immunostained structures are one blood vessel or two separate vessels. In contrast to the recommendations suggested in the first consensus report on angiogenesis quantification in human tumours, Ahlgren and colleagues did not use the Chalkley point-overlap technique, which abolishes this observer-dependent variability and used a sub-optimal technique to measure the vascularity of the breast cancer specimens.

For angiogenesis quantification to be implemented in the daily practice of surgical pathologists, only one representative section through the tumour is likely to be analysed, as is the case for the oestrogen receptor, progesterone receptor and c-erbB-2 status in breast cancer. There are no data supporting the idea that intratumour heterogeneity of these parameters is smaller than the intratumour heterogeneity of MVD, as reported by Ahlgren and colleagues. Furthermore, the usual representative section of a tumour allows estimation of the microscopic size, grade, contains the central area of fibrosis or necrosis surrounded by vascular hot spots [7], and provides the maximal interface of tumour and normal tissue. Moreover, this method of histological analysis gives the most extensive surface area to measure these different parameters, thereby reducing the danger of neglecting tumour areas that determine the biology of an individual tumour.

We have re-analysed the data of Ahlgren and colleagues and have calculated the mean of the counts in columns 'C' and 'D' for each tumour (their Table 2). This mean is the best estimate of the MVD assessment that would

* Corresponding author. Tel.: +32-3-821-3730; fax: +32-3-821-4753.
E-mail address: peter.vermeulen@uza.be (P.B. Vermeulen).

PII: S0959-8049(02)00156-9

have been performed by pathologists analysing only the usual section of the 21 breast carcinomas. If we categorise these values according to the median and compare this with the categorisation of maximal counts found in other regions according to their median value, a concordance ratio of 76% is obtained. Moreover, a strong correlation is found between the maximal MVD in the central part (blocks 'C' and 'D') of the breast cancers and the highest MVD elsewhere ($r = 0.64$; $P = 0.0012$; 95% Confidence Interval (CI) of the correlation coefficient r: 0.3–0.8). Moreover, since Ahlgren and colleagues have provided the tumour size in their Table 2, we have also stratified the breast tumours by the TNM-system into T1 and T2 tumours. Mean MVD of the central blocks 'C' and 'D' was 82.8 (Standard Deviation (S.D.) of 19.3; median of 78.5) for the T1 tumours and 104.9 (S.D. of 25.6; median of 107) for the T2 tumours (Mann–Whitney U-test P value of 0.048). Since it is well established that T2 tumours carry a significantly worse prognosis compared with T1 tumours, the difference in MVD between both groups implies that MVD measured in the central portion of breast cancer, even in this small group of 21 tumours, may have a prognostic value.

Taken together, we believe that the quantification of vascularity in the central representative section of breast carcinoma specimens using the Chalkley technique in vascular hot spots immunostained with antibodies directed at CD34, which is the standard methodology detailed in the second international consensus report of angiogenesis quantification in human tumours [3], will be useful as an aid in the assessment of the prognosis of individual breast cancer patients. This has been unequivocally demonstrated by Hansen and colleagues [4] using Chalkley counting in 836 patients with invasive breast carcinomas with a median follow-up time of more than 11 years. The mean value of the counts in the three most vascular areas showed that independent of other prognosticators, there was a 1.6-fold higher risk of dying when the tumour had a Chalkley count between 5 and 7 and a 2.3-fold higher risk of dying associated with a Chalkley count $\geqslant 7$. Indeed, the Chalkley count predicted overall and disease-free survival, both in the node-negative and node-positive patient groups. We believe that it is time to use tumour vascularity in clinical trial protocols and seriously consider implementing the assessment of vascularity in the routine pathology of breast cancer.

References

1. Ahlgren J, Risberg B, Villman K, Bergh J. Angiogenesis in invasive breast carcinoma—a prospective study of tumour heterogeneity. *Eur J Cancer* 2002, **38**, 64–69.
2. Vermeulen PB, Gasparini G, Fox SB, *et al*. Quantification of angiogenesis in solid human tumours: an international consensus on the methodology and criteria of evaluation. *Eur J Cancer* 1996, **32A**, 2474–2484.
3. Vermeulen PB, Gasparini G, Fox SB, *et al*. Second international consensus on the methodology and criteria of evaluation of angiogenesis quantification in solid human tumours. *Eur J Cancer* 2002, **38**(12), 1564–1579.
4. Hansen S, Grabau DA, Sorensen FB, Bak M, Vach W, Rose C. The prognostic value of angiogenesis by Chalkley counting in a confirmatory study design on 836 breast cancer patients. *Clin Cancer Res* 2000, **6**, 139–146.
5. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis: correlation in invasive breast carcinoma. *N Engl J Med* 1991, **324**, 1–8.
6. Belien JAM, Somi S, de Jong JS, van Diest PJ, Baak JPA. Fully automated microvessel counting and hot spot selection by image processing of whole tumour sections in invasive breast cancer. *J Clin Pathol* 1999, **52**, 184–192.
7. Colpaert C, Vermeulen P, Jeuris W, *et al*. Early distant relapse in "node negative" breast cancer patients is not predicted by occult axillary lymph node metastases, but by the features of the primary tumour. *J Pathol* 2001, **193**, 442–449.