Microvascular Density as a Prognostic Factor in Women with Breast Cancer: A Systematic Review of the Literature and Meta-Analysis

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Abstract

We performed a meta-analysis of all 87 published studies linking intratumoral microvessel density (MVD), reflecting angiogenesis, to relapse-free survival (RFS) and overall survival (OS). With median MVD as cutoff, MVD impact was measured by risk ratio (RR) between the two survival distributions. Seventeen studies did not mention survival data or fit inclusion criteria. Twenty-two were multiple publications of the same series, leaving 43 independent studies (8936 patients). MVD was assessed by immunohistochemistry, using antibodies against factor VIII (27 studies; \( n = 5262 \)), CD31 (10 studies; \( n = 2296 \)), or CD34 (8 studies; \( n = 1726 \)). MVD might be a better prognostic factor when assessed by CD31 or CD34 versus factor VIII (\( P = 0.11 \)). For RFS, statistical calculations were performed in 25 studies (6501 patients). High MVD significantly predicted poor survival [RR = 1.54 for RFS and OS with the same 95% confidence interval (CI), 1.29–1.84]. Twenty-two studies analyzed separately lymph node-negative patients (\( n = 3580 \)), for whom predictors of poor survival are requested. This latter meta-analysis included 15 studies for RFS (2727 patients) and 11 for OS (1926 patients). High MVD significantly predicted poor survival [RR = 1.99 for RFS (95% CI, 1.33–2.98) and RR = 1.54 for OS (95% CI, 1.01–2.33)]. Between-study variations could result from patient selection criteria, techniques to stain and count microvessels, and cutoff selection. MVD was a significant although weak prognostic factor in women with breast cancer. Standardization of MVD assessment is needed.

Introduction

Breast cancer is the most frequent neoplasm in women and the most frequent cause of death in women between 35 and 55 years of age. Five-year overall survival (OS) is estimated to be 65%, with large disparities between stages. Several prognostic indicators have been demonstrated, including tumor size, axillary lymph node status, histological grade, tumor type, vascular invasion, and estrogen receptor status (1, 2). These prognostic factors allow a better comprehension of the natural history of the disease and the identification of homogeneous populations of patients with a similar outcome profile. Numerous putative markers have been reported, yet very few have actually gained widespread clinical use. Recent developments in cytogenetics and molecular biology have provided new ways to assess prognosis (3). Among all of these prognostic factors, lymph node status remains the most important. In the United States Surveillance, Epidemiology, and End Results database, the 5-year OS was 92% in node-negative patients, 81% in patients with one to three positive axillary lymph nodes, and 57% in those with more than four involved nodes (4). However, 20–30% of all lymph node-negative patients would still develop a recurrence within 10 years of initial treatment of the primary tumor (5). It would be crucial to identify such patients, who should benefit from adjuvant chemotherapy, which has sometimes been shown to improve survival (6), although this issue is still controversial, especially in older women with estrogen receptor-positive breast cancer (7). This strategy would avoid such adjuvant treatment to be generalized to all of the node-negative patients, considering its heavy burden of side effects and its uselessness in many patients.

Angiogenesis (or neovascularization) consists in the formation of new blood vessels from the endothelium of the existing vasculature. When a new tumor reaches the size of 1–2 mm, its ulterior growth requires the induction of new blood vessels, which may consequently lead to the development of metastases, via the penetration of malignant cells into the circulation. Vascular endothelial growth factor (VEGF), platelet-derived endothelial cell growth factor, and basic fibroblast growth factor, produced by tumor and stromal cells, are potent inducers of the angiogenic switch. However, angiogenesis is necessary but not sufficient to the development of metastases. Angiogenic activity is heterogeneous within a given tumor type. Concerning the relationship between angiogenesis and clinical outcome, breast cancer has been the most studied tumor. For >10 years, microvessel density (MVD), a surrogate marker of tumoral angiogenesis, has been proposed to identify patients at high risk of recurrence more precisely than classical indicators, particularly in node-negative patients. The identification of such patients at an early stage of their disease would be of great interest, allowing for a more appropriate and effective treatment (by adjuvant chemotherapy or in the near future by specific antiangiogenic drugs) of patients at higher risk and possibly predicting the activity of these latter drugs.

Microvessel density assessment is the most commonly used technique to quantify intratumoral angiogenesis in breast cancer. It was first developed by Weidner et al. (8) in 1991 and uses panendothelial immunohistochemical staining of blood microvessels [mainly with Factor VIII antigen (F. VIII Ag or von Willebrand’s factor), CD31, PECAM-1, or CD34; rarely with integrin \( \alpha_\beta_3 \), CD105, or type IV collagen]. The first step in Weidner’s approach is the identification by light microscopy of the area of highest neovessel density (the so called “hot spot”), by scanning the whole tumoral section at low power. Then, individual microvessels are counted at a higher power (×200 field) in an adequate area (e.g., \( 0.74 \text{ mm}^2 \) per field using ×20 objective lens and ×10 ocular). Any stained endothelial cell or clusters separate from adjacent vessels are counted as a single microvessel, even in the absence of vessel lumen. Each single count is expressed as the highest number of microvessels identified at the hot spot. Some authors use Chalkley count or computerized image analysis systems, both aimed to minimize the subjectivity in the quantification of MVD. The Chalkley count consists of applying a 25-point eyepiece graticule on several hot spots (usually 3). The graticule is oriented to allow the maximum number of points to hit on or within the areas of stained microvessel profiles (Chalkley grid area: 0.196 mm²; Ref. 9).

Recent evidence suggests that tumor angiogenesis is associated with patient outcome in a number of cancers, especially breast and...
lung (10, 11). Many observational studies (either prospective or retrospective studies) have concluded that MVD is a prognostic factor in invasive breast cancer, but others reached the opposite conclusion (10). The data are so confounded that, in his review of prognostic factors in breast cancer, Hayes et al. (2) did not recommend the use of MVD as a basis for making clinical decisions. To determine whether angiogenesis, assessed by its surrogate end point MVD, is a prognostic factor in breast cancer, we undertook a systematic review of the literature with a meta-analysis. Although meta-analyses of observational studies offer much more challenges than meta-analyses of randomized controlled trials, due to inherent biases and differences in study design, they may provide a useful tool for helping to understand and quantify sources of variability in results across studies (12). The aim of our meta-analysis was to test the hypothesis that initially assessed MVD would be able to predict OS (breast cancer-related death) and/or relapse-free survival (RFS, recurrence at any site), either in the global population of operated breast cancer women without metastases or exclusively in lymph node-negative patients. By doing so, we tried to contribute to convert MVD from candidate to accepted prognostic factor in breast cancer (2). Actually, we performed four different meta-analyses, the first two including studies involving either lymph node-negative or node-positive patients or both (one meta-analysis for OS the other one for RFS), the two others restricted to studies with a majority (≥75%) of or only node-negative patients.

This article aims to comply with the recommendations for reporting meta-analyses of observational studies (12).

Materials and Methods

Publication Selection. We did this meta-analysis according to a predetermined written protocol. To be eligible for our meta-analysis, studies had to deal with breast cancer only, with no distant metastases at inclusion, to evaluate the correlation between microvessel count and density and survival, to measure MVD in the primary tumor, and to be published in English, French, or German languages. We also excluded studies when their recruitment came from two distinct retrospective cohorts with a different prognosis (13–15), because we deemed their results could be biased.

Studies (full articles and abstracts) published between January 1991 and December 31, 2002, were identified by an electronic search using online Pubmed, with the following key words used simultaneously, breast cancer, neovascularization, prognosis, and no special limits except time. Last query was updated on September 4, 2003. We also screened references from the relevant literature, including all of the identified studies, but also reviews (10, 16) and editorials (17–19). We tried carefully to avoid duplication of data, by examining for each publication the names of all authors and the different medical centers involved. When additional data were needed for statistical analysis, the authors were kindly requested by letter or e-mail to send them. Abstracts (n = 6) were only included in a descriptive analysis.

Methodological Assessment. Information was carefully extracted from all of the publications in duplicate by two of us (B. U. and P. N.), using a standardized data collection form, with the following items, complete reference of the publication, original publication or update of a former publication, mode of making up of the series of cases, median duration of follow-up, number of women included in the study, mean or median age, menopausal status, antinecancer treatment(s) during follow-up, histological type (ductal invasive or lobular), tumor size, nodal status, optical reading with or without Chalkley count or image analysis system, number of readers, blinded reading, type(s) of immunohistochemical staining, number of hot spots examined, magnification used, area of the field read, cutoff value for MVD (median MVD, unless otherwise stated), number of events in each category of MVD, RFS or OS or both, survival curves for the global population or separately for node-negative patients, and results of uni- and multivariate analyses. Disagreements were resolved by consensus between the two readers. In case of persistent disagreement, the final decision was made by our experts (G. P. for clinical evaluation, and M. C. for methodological and statistical evaluation of data). There was only one disagreement between the two readers on clinical data, but several on the statistical working of the data, resolved by M. C.

We did not set a predefined minimal number of patients for a study to be included in our meta-analysis, nor a minimal duration of median follow-up. We did not weigh each study by a quality score, because no such score has received general agreement for use in a meta-analysis, especially of observational studies, making more difficult the evaluation of its quality (20). Studies were not blinded to the readers, but decisions of exclusion were always taken without knowledge of the global result of each study.

When duplicate studies were retrieved, we included in our systematic review the study involving the highest number of patients from which data could be extracted (usually the latest). This was done to avoid overlapping between cohorts, with two exceptions, the study by Toi et al. (21) was included, as was the study by Gasparini et al. (22) published in 1998, a large multicenter study which only included 140 Japanese patients, possibly members of the Japanese cohort of Toi, and the two studies of Guidi et al. (23, 24), because the authors confirmed that they corresponded to distinct protocols and patients.

Studies were usually retrospective, but sometimes consisted of a cohort of consecutive patients. Although their methodological quality and the reliability of their conclusions were variable, their design was almost similar, a favorable condition for our meta-analysis. One study included the complete population of breast cancer patients from the area of Odense University Hospital (25, 26). Usually, observers (i.e., readers of stained sections of tumor blocks) were unaware of the clinical outcome of patients.

Statistical Methods. A study was considered significant when the P for the statistical test comparing survival distributions between the groups with high and low MVD (usually with median MVD as cutoff) was inferior to 0.05 in univariate analysis. The same threshold was adopted for multivariate analysis. A study was termed “positive” or conclusive when a high MVD predicted poorer survival and “negative” or inconclusive when a high MVD did not predict a poor survival, or even once predicted a better survival (27).

For the quantitative aggregation of survival results, we measured the impact of MVD on survival by estimating the risk ratio (RR) between the high or low MVD groups. For each trial, this RR was estimated by a method depending on the data provided in the publication. The simplest method consisted in the direct collection of RR, hazard ratio, or odds ratio, and their 95% confidence interval (CI) from the original article (5 studies only). If not available, we looked at the total numbers of events and the numbers of patients at risk in each group to determine the RR estimate. When data were only available as graphical survival plots, the calculations were done only if the number of steps on the curves equaled the number of events given in the publication, assuming that the rate of censored patients was constant during the study follow-up (28). In 5 studies, the data were obtained directly from the authors (see Table 1). Whenever possible, the parameter MVD was dichotomized by using its observed median.

Considering the many sources of heterogeneity between studies and consequently between their individual RR estimates, we calculated the overall RR by using a random-effect model (Der Simonian and Laird’s method). By convention, an observed RR > 1 implied a worse prognosis in the high MVD group. The detrimental impact of angiogenesis on survival was deemed statistically significant when the lower boundary of the 95% CI of the overall RR was > 1.

Comparisons of proportions of studies with or without various characteristics were made by χ² tests.

The statistical calculations for our meta-analyses were performed with EasyMA.net Internet distributed application (Department of Clinical Pharmacology, Cardiology Hospital, Lyons, France;1 Ref. 29).

Results

Studies Selection and Characteristics. Our electronic search retrieved a total of 237 references (see Appendix 1). Our global literature search collected 87 studies (8, 13–15, 21–27, 30–105) including 6 abstracts (43, 90, 91, 96, 104, 105). Only 58 came from the electronic database (8, 13, 15, 21–27, 30, 31, 33, 34, 36–42, 44–50, 69, 79, 103, 104). The statistical calculations for our meta-analyses were performed with EasyMA.net Internet distributed application (Department of Clinical Pharmacology, Cardiology Hospital, Lyons, France;1 Ref. 29).

1 Internet address: http://www.spc.univ-lyon1.fr/easyma.net.
### MVD, A PROGNOSTIC FACTOR IN BREAST CANCER

Forty-three independent studies fulfilled our inclusion criteria, and were excluded (13–15). Two studies were rejected because they included patients coming from two distinct retrospective cohorts and were excluded (26, 45, 51, 56, 68, 69, 71, 76–78, 81–83, 85, 90, 92, 94, 95, 97, 99, 101, 102, 106). One publication was written in German with an English abstract (98), but it very probably corresponded to the subsequent full article by Fregene et al. (93).

Among all of these 87 studies, 17 did not mention survival data or were out of the scope of our systematic review and were excluded (8, 31, 32, 36, 39, 47, 53, 57, 63, 65, 67, 70, 72, 86, 89, 97, 104). One of these 17 studies was rejected because it included metastatic cancers (32). These excluded studies are listed in Appendix 2, with informations on the cause(s) of rejection. Twenty-two studies corresponded to multiple publications of the same series of cases and were also excluded (26, 45, 51, 56, 68, 69, 71, 76–78, 81–83, 85, 90, 92, 94, 95, 97, 99, 101, 102, 106). Three studies included patients coming from two distinct retrospective cohorts and were excluded (13–15). Two studies were rejected because meta-analytic calculations were obviously impossible (54, 101). Forty-three independent studies fulfilled our inclusion criteria, representing 8936 patients (21–25, 27, 30, 33, 35, 37, 38, 40–44, 46, 48–50, 52, 55, 58–62, 64, 66, 73–75, 79, 80, 84, 87, 88, 91, 93, 96, 100, 102, 105). These studies included at least 3616 post-menopausal women from the 27 studies providing a description of the clinical characteristics of 5917 patients. The main features of the eligible studies are summarized in Table 1. The study design was more often a retrospective (trococ; n = 18) than a prospective cohort study (n = 12). In 13 studies, the study design could not be inferred. In several studies, a lot of patients from the initial series were excluded because of insufficient histological material, insufficient clinical data, or other reasons, leading to a potential recruit-

### Table 1 Main characteristics of the 43 studies fulfilling the inclusion criteria

<table>
<thead>
<tr>
<th>First author (ref)</th>
<th>Year</th>
<th>Study from PubMed (y)</th>
<th>Blinded reading</th>
<th>Reader (n)</th>
<th>Median follow-up (y)</th>
<th>Antibody</th>
<th>Counted area (mm²)</th>
<th>RR estimation</th>
<th>Analysis</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bosant (102)⁶</td>
<td>1992</td>
<td>Yes Retro.</td>
<td>&gt; 1</td>
<td>Optical</td>
<td>FVIII</td>
<td>Data-extrapolated RFS, RFS — Positive</td>
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<tr>
<td>Van Hoef (106)⁶</td>
<td>1993</td>
<td>No Retro.</td>
<td>?</td>
<td>Optical</td>
<td>FVIII</td>
<td>Missing OS, RFS Negative</td>
<td></td>
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<tr>
<td>Commer (91)⁶</td>
<td>1994</td>
<td>No Retro.</td>
<td>?</td>
<td>Optical</td>
<td>FVIII</td>
<td>Missing OS, RFS — Negative</td>
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<tr>
<td>Fregene (93)⁶</td>
<td>1994</td>
<td>No Retro.</td>
<td>Yes &gt; 1</td>
<td>Optical</td>
<td>FVIII</td>
<td>0.190 Data-extrapolated RFS, RFS — Positive</td>
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<tr>
<td>Sightler (96)⁶</td>
<td>1994</td>
<td>No</td>
<td>?</td>
<td>Optical + CD31</td>
<td>0.747 Missing OS</td>
<td>Positive</td>
<td></td>
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<td>Axelsson (75)⁶</td>
<td>1995</td>
<td>Yes Prosp.</td>
<td>&gt; 1</td>
<td>Optical</td>
<td>FVIII</td>
<td>0.340 Data-extrapolated OS, RFS, OS –, RFS — Negative</td>
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<tr>
<td>Costello (79)⁶</td>
<td>1995</td>
<td>Yes Retro.</td>
<td></td>
<td>Optical</td>
<td>FVIII</td>
<td>0.220 Data-extrapolated OS, OS, OS —, RFS Negative</td>
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<tr>
<td>Fox (80)⁵</td>
<td>1995</td>
<td>Yes Retro.</td>
<td>?</td>
<td>Optical + CD31</td>
<td>1.55 Missing OS</td>
<td>Positive</td>
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<tr>
<td>Goulding (84)⁵</td>
<td>1995</td>
<td>Yes Retro.</td>
<td>?</td>
<td>Optical +</td>
<td>CD31</td>
<td>0.170 Missing OS</td>
<td>Negative</td>
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<tr>
<td>Obermair (87)⁶</td>
<td>1995</td>
<td>No Prosp.</td>
<td></td>
<td>Optical</td>
<td>FVIII</td>
<td>0.250 Data-extrapolated RFS, RFS — Positive</td>
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<tr>
<td>Ogawa (88)⁶</td>
<td>1995</td>
<td>No Retro.</td>
<td>&gt; 1</td>
<td>Optical</td>
<td>FVIII</td>
<td>0.785 Data-extrapolated OS, OS, OS —, RFS Positive</td>
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<tr>
<td>Toi (21)⁶</td>
<td>1997</td>
<td>Yes Prosp.</td>
<td></td>
<td>Optical</td>
<td>FVIII</td>
<td>0.785 Survival curves OS, RFS — Negative</td>
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<tr>
<td>Acenero (50, 51)⁶</td>
<td>1998</td>
<td>Yes Prosp.</td>
<td>&gt; 1</td>
<td>Optical</td>
<td>FVIII</td>
<td>0.740 Data-extrapolated RFS — Positive</td>
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<tr>
<td>Chalkley (29)⁶</td>
<td>1998</td>
<td>Yes Prosp.</td>
<td></td>
<td>Optical</td>
<td>CD31</td>
<td>0.740 Data-extrapolated OS, RFS, OS Positive</td>
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<tr>
<td>Gasparini (43)⁶</td>
<td>1999</td>
<td>No Prosp.</td>
<td>?</td>
<td>Optical</td>
<td>CD31</td>
<td>0.340 Data-extrapolated OS, RFS, OS Positive</td>
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<tr>
<td>Guidi (24)⁶</td>
<td>2000</td>
<td>Yes Prosp.</td>
<td>?</td>
<td>Optical +</td>
<td>FVIII</td>
<td>0.630 Given by author OS, RFS Negative</td>
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<tr>
<td>Hansen (25, 26)⁶</td>
<td>2000</td>
<td>Yes Prosp.</td>
<td>Yes &gt; 1</td>
<td>Optical</td>
<td>CD31</td>
<td>0.740 Data-extrapolated OS, RFS — Positive</td>
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<tr>
<td>Medici (27)⁶</td>
<td>2000</td>
<td>Yes Prosp.</td>
<td>Yes &gt; 1</td>
<td>Optical</td>
<td>CD31</td>
<td>0.720 Data-extrapolated OS, RFS, RFS — Negative</td>
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<tr>
<td>Takei (40)⁶</td>
<td>2000</td>
<td>Yes Prosp.</td>
<td>Yes &gt; 1</td>
<td>Optical</td>
<td>CD31</td>
<td>0.740 Missing OS, RFS Negative</td>
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<tr>
<td>Tai (41)⁶</td>
<td>2000</td>
<td>Yes Prosp.</td>
<td>Yes &gt; 1</td>
<td>Optical</td>
<td>CD31</td>
<td>0.720 Data-extrapolated OS, RFS Negative</td>
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<tr>
<td>Kato (35)⁶</td>
<td>2001</td>
<td>Yes Prosp.</td>
<td></td>
<td>Optical</td>
<td>CD31</td>
<td>0.740 Missing OS, RFS, OS Positive</td>
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<tr>
<td>Lee (35)⁶</td>
<td>2001</td>
<td>No Prosp.</td>
<td>?</td>
<td>Automated  CD34</td>
<td>0.785 Survival curves OS, RFS — Negative</td>
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<tr>
<td>Vinet-Salomon (37)⁶</td>
<td>2002</td>
<td>Yes Prosp.</td>
<td>?</td>
<td>Automated  CD34</td>
<td>0.785 Survival curves OS, RFS — Negative</td>
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<tr>
<td>Guidi (23)⁶</td>
<td>2002</td>
<td>Yes Prosp.</td>
<td>&gt; 1</td>
<td>Optical</td>
<td>CD31</td>
<td>0.740 Given by author OS, RFS Negative</td>
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<tr>
<td>Gunel (30)⁶</td>
<td>2002</td>
<td>Yes Prosp.</td>
<td>&gt; 1</td>
<td>Optical</td>
<td>CD31</td>
<td>0.740 Given by author OS, RFS Negative</td>
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52, 55–57, 61, 63, 64, 69–75, 77, 79–82, 84, 86, 89, 92, 94, 95, 97, 99, 101, 102, 106). One publication was written in German with an English abstract (95), but we did not include it because there were no separate results by nodal status, and it included only 52 node-negative patients, and finally the series of 54 node-negative patients was completed and later published by the same authors (87). No article used French language. One publication was excluded because it was written in Czech language, and its English abstract did not mention survival data (36). All of the other articles were written in English language. One reference was actually a chapter of a book we could not obtain (98), but it very probably corresponded to the subsequent full article by Fregene et al. (93).
ment bias. A great majority of tumors, the histological type of which was mentioned, were ductal invasive cancers (4834 of 5693; 85%). Among these 43 studies, 4 were in abstract form and were only included in the descriptive analysis (43, 91, 96, 105). The number of patients included in the studies ranged from 50 (66) to 836 (Ref. 25; mean number, 208). Thirty-two studies (74% of the total number of independent studies) were published between years 1995 and 2000, including 19 positive studies.

Twenty-two independent studies (n = 3580) included only lymph node-negative patients or gave separate results for such patients (21, 22, 25, 27, 34, 42, 48, 52, 55, 58–60, 62, 74, 75, 79, 87, 88, 92, 93, 100, 102).

**Studies Results Reports.** Twenty-two studies (4779 patients) found an inverse relationship between survival and MVD (so-called positive studies), whereas 21 studies (4157 patients) found no relation (negative studies). All of the studies were dispatched into three classes according to the length of median follow-up: ≤5 years (n = 11), 5–10 years (n = 18), and >10 years (n = 11). The proportion of positive studies was not higher among those with short follow-up. Among the 9 largest studies (>300 patients each), representing 4547 patients (half of the global population), 4 were positive. There were significantly more negative studies among those including ≤100 patients (10 negative versus 2 positive studies), than among those including >100 patients (11 versus 20; P = 0.005 by χ²-test). This finding argues against a major publication bias. The proportion of positive studies was exactly the same whether the study mentioned explicitly more than one reader of the immunostained slides or not (5 of 10 versus 16 of 31). Nine studies (2367 patients) used a graticule (Chalkley count; Refs. 26, 46, 52, 58, 64, 68, 79, 80, 100). The proportion of positive studies was similar whether or not a graticule was used (6 of 9 versus 17 of 34). Too few studies (3 positive and 5 negative; Refs. 35, 38, 51, 60, 64, 74, 84, 96) used computerized image analysis to draw any firm conclusion. The area of the field examined optically did not influence the results (7 of 14 positive studies with a field area ≤0.384 mm² versus 4 of 15 with a field area >0.384 mm²; P = not significant). Immunostaining was obtained by use of antibodies directed against F. VIII Ag in 27 studies (5262 patients; Refs. 21, 23, 27, 34, 42, 48, 50, 59, 61, 62, 64, 66, 73, 75, 79, 87, 88, 91, 93, 100, 102, 105), CD31 in 10 studies (2296 patients; Refs. 22, 44, 49, 52, 58, 60, 64, 80, 84, 96), and CD34 in 8 studies (1726 patients; Refs. 25, 35, 41, 43, 46, 55, 64, 74). When pooling the results from CD31 and CD34, the proportion of positive studies was somewhat higher compared with that of F. VIII Ag (11 of 18 versus 10 of 27; P = 0.11 by χ²-test).

Among the 22 lymph node-negative studies (3580 patients), 13 were positive studies (2185 patients; 61% of node-negative patients; Refs. 21, 22, 25, 34, 42, 52, 55, 59, 74, 75, 87, 88, 91, 93, 100, 102, 105), CD31 in 10 studies (2296 patients; Refs. 22, 44, 49, 52, 58, 60, 64, 80, 84, 96), and CD34 in 8 studies (1726 patients; Refs. 25, 35, 41, 43, 46, 55, 64, 74). When pooling the results from CD31 and CD34, the proportion of positive studies was somewhat higher compared with that of F. VIII Ag (11 of 18 versus 10 of 27; P = 0.11 by χ²-test).

**Meta-Analysis.** Data extraction for meta-analytic calculations was impossible in 14 studies (22, 30, 40, 43, 50, 61, 64, 66, 80, 84, 91, 96, 100, 105), even after writing to the authors for complementary information, leaving 33 studies (7738 patients) available for the final meta-analyses (21, 23–25, 27, 33–35, 37, 38, 41, 42, 44, 46, 48, 49, 52, 55, 58–60, 62, 68, 73–75, 79, 82, 87, 88, 92, 93, 102). These 33 studies include duplicate studies by Kato et al. (34), Gasparini et al. (68, 82), and Fox et al. (92), which were not included in the corpus of 43 independent eligible studies. This is the reason why the sum of the 14 studies not suitable for meta-analytic calculations and of the 33 studies available for the final meta-analyses makes 47, i.e., 4 more than 43. Of the 14 studies not suitable for calculations, 6 were positive (22, 50, 61, 64, 80, 105) and 8 were negative studies, so that their exclusion did not bias the sample of studies. We could not include in our calculations a large study (22), because MVD was used as a continuous variable, and we could not obtain reformulated data with MVD expressed as a binary variable. Fortunately, we could analyze from the same author a smaller study of node-positive patients (68), and another one of node-negative patients (82). For the study by Fox et al. (80), we could not convert the table of events provided by tertiles into two classes of MVD and instead used another study from the same author (92). We added a second study by Kato et al. (34), specifically devoted to node-negative patients, including a greater number of these patients than his global study (33). Only 15 among 22 eligible studies could be included in our meta-analysis of lymph node-negative patients, representing 2727 patients (21, 25, 27, 34, 55, 58, 60, 62, 75, 79, 87, 88, 92, 93, 102).

The four main meta-analyses (global population/node-negative patients, RFS/OS) are shown on Figs. 1-4. The largest (global population, RFS; Fig. 1) included 25 studies (6501 patients) [RR = 1.54 (95% CI, 1.29–1.85; P < 0.001)]; the smallest (lymph node-negative, OS; Fig. 4) included only 11 studies (1926 patients) [RR = 1.54 (95% CI, 1.01–2.33; P < 0.04)]. In the global population, RR for OS (Fig. 2) was 1.54 (95% CI, 1.29–1.84). The highest RR (1.99; 95% CI, 1.33–2.98; P < 0.001) was found for RFS in node-negative patients (Fig. 3). All four of the meta-analyses gave statistically significant results, favoring a link between high MVD and poor survival.

Fourteen studies (21, 23, 24, 27, 33, 37, 38, 62, 75, 79, 87, 88, 93, 102) using factor VIII as the immunohistochemical marker could be included in a separate meta-analysis for RFS (Fig. 5). There was an important heterogeneity between studies. Only 6 studies using CD31 were available for meta-analysis and 5 studies using CD34.

We performed a sensitivity testing by subtracting the study with the highest sample size (25), first from the global analysis, then from the node-negative subgroup. This testing did not modify the estimated RRs. Because we had decided not to assess the quality of each study by scoring, we did not perform sensitivity testing by subtracting studies of lower quality. Anyway, such assessment is controversial in meta-analyses of observational studies (12).
Discussion

Our overview and meta-analysis of all published studies from which statistical data could be obtained or calculated shows that MVD, a marker of angiogenesis, does indeed predict poor survival in women with invasive breast cancer, especially in node-negative patients. Our findings are in agreement with a recent meta-analysis on the link between MVD and survival in patients with non-small cell lung cancer (11). However, our conclusions should be tempered for several reasons. First, the overall statistical link we elicited between MVD and survival was rather weak, with a global RR of 1.54 for OS and RFS [even lesser than the hazard ratios found by Meert et al. (11) in lung cancer: 1.80–1.99]. As a rule of the thumb, a prognostic factor with a RR <2 is of limited practical use (2).

In addition, we relied on published reports of aggregate data and
were unable to do a survival analysis making full use of the time-to-relapse of individual patients. Finally, the studies included in our meta-analysis were observational studies, more prone to many biases than prospective randomized controlled studies (107). We attempted to minimize publication bias by making our literature search as complete as possible. However, we could not take into account the few studies published in abstract form only or in Japanese language; this selection might have favored the "positive" studies, because they are more often published in English whereas the "negative" ones tend to be reported more often in native languages (108). Obviously, we could not account for unpublished studies. However, the discrepancies in the conclusions of various studies could have encouraged researchers to publish their data whatever the results, thus limiting such publication bias. Duplication of patients between studies from the same group was sometimes difficult to avoid. Studies may have differed in the baseline characteristics of patients included (age, menopausal status, tumor type, and size), the adjuvant treatment they might have received for their cancer, the number of patients, the duration of follow-up, the immunohistochemical marker used to assess MVD, or the method of microvessel count itself. All of these sources of variability represent potential selection biases. Observers were not blinded to outcome data in all of the studies (information bias). Multivariate analyses tried to control for different factors, but the factors controlled for were few and differed between studies. Finally, the discrepancy between the conclusions of a meta-analysis of epidemiological studies relating the use of hormone replacement therapy to the risk of coronary heart disease (halved, a statistically robust conclusion; Ref. 109) and the findings of three recent randomized controlled trials showing no benefit of this therapy should be kept in mind (110).

Obviously, the choice of the endothelial marker for immunohistochemical staining may modify the conclusions. In his first study (1992) relating MVD to survival, Weidner et al. (106) used an antibody against factor VIII-related antigen, also termed von Willebrand’s factor, staining mainly mature vessels and cross-reacting with lymphatic endothelium. This marker remains the most used in the studies we reviewed. Some recent studies used antibodies directed against platelet endothelial cell adhesion molecule (also known as CD31) or CD34. JC-70, a monoclonal antibody against CD31, has the advantage over F. VIII Ag of being present also on immature blood vessels. Consequently, counts using this marker are ~30% higher than those using F. VIII Ag. However, CD31 can react mildly with fibroblasts and some plasma cells, and is rarely expressed quite strongly: staining failure can reach 20% in routinely fixed breast specimens. CD34 has the same characteristics as CD31, but without the high rate of staining failure (16). The optimal marker has not been identified yet. One study compared F. VIII Ag, CD31, and CD34, and was positive only for CD34 (64). Standardization with quality control programs involving multicenter studies is mandatory before use of MVD as a surrogate marker of angiogenesis for prognostic or predictive purposes (111, 112).

Although most studies used a technique similar to that of Weidner et al. (8), many variations to the MVD assessment exist. The size of the area examined varied between studies. Some authors considered the mean or the highest value among three or more determinations of MVD at different fields of the same hot spot. Some measured MVD as the mean or highest value at several hot spots. The cutoff value for MVD varied among studies. Many authors used the median value of MVD as cutoff. Some divided the values of MVD by tertiles. Some measured MVD at different fields of the same hot spot. Some measured MVD as a continuous variable in their multivariate analysis (22, 74).

The Chalkley count is considered as a simple and acceptable procedure for daily clinical use. Often, the Chalkley count for one tumor is taken as the mean value of three graticule counts. Its use is said to increase the reproducibility of counts within a given hot spot and to reduce considerably the time needed to evaluate a hot spot. Although intended to be a surrogate estimate of MVD, it is actually a relative area estimate of immunostained vessels expressed by an index without unit. The earlier studies analyzed the survival data with cutoff points at Chalkley counts 5 and 7, splitting at the tertiles.

To conclude, our meta-analysis found a statistically significant inverse relationship between angiogenesis, assessed by MVD, and survival, confirming that human invasive breast cancer is an angiogenesis-dependent malignancy. In view of our findings, the following recommendations should be made to future authors, include a large series of consecutive patients from a single cohort, stratify by lymph node status, describe the clinical characteristics of the study population, use antibodies directed against either CD31 or rather CD34 for immunostaining, express the results both in the global population and separately by nodal status, and both as comparison of survival curves and as multivariate regression analysis, and give a full description of survival events to allow future calculations.

In the future, a better answer to the question raised would be difficult to provide, because this epidemiological issue cannot be answered directly by a randomized controlled trial (only intervention studies, using antiangiogenic drugs, would provide an answer). A meta-analysis of individual patient data would be hardly conceivable given the important dispersion of studies, and the time and resources needed. The degree of vascularization has prognostic value both in node-negative and node-negative patients. We could not assess the prognostic role of MVD in the subgroup of lymph node-negative patients with no initial chemotherapy, because most studies in node-negative patients did not stratify their results by the nature of the treatment prescribed initially. We must already think of the best methodology to validate the approach of treating or not with adjuvant chemotherapy or specific antiangiogenic drugs node-negative patients according to their MVD. To choose the patients to be treated by new specific antiangiogenic drugs, and monitor such treatments, reliable, standardized, and useful predictive markers of angiogenesis are needed. Some authors argued that MVD is possibly one of them. Conversely, Hlatky et al. (114) stated that it may not be a predictor of treatment efficacy, because it is not a measure of the angiogenic dependence of a tumor, but rather reflects the metabolic burden of the supported tumor cells. Consequently, MVD is not predictive of the response to antiangiogenic drugs, and, therefore, not useful for stratifying patients in clinical trials. However, MVD can now be added rather confidently to the limited list of demonstrated prognostic factors in invasive breast cancer, but how much it adds to classical prognostic factors is debatable.

Acknowledgments

We thank all authors whose publications could be included in our meta-analysis, and especially those who provided us with complementary data allowing meta-analytic calculations. We especially thank Professor Daniel F. Hayes and the Cancer and Leukemia Group B group for both completing their data concerning protocols 8541/8869 and 8082, and reviewing our manuscript.

Appendix

Appendix 1. Complete list of 237 publications retrieved by our electronic search (PubMed, indexed for MEDLINE). All articles include related articles and links. For more information, write to the Help Desk National Center for Biotechnology Information, National Library of Medicine, NIH.


35: Faridi A, Rudlowski C, Biesterfeld S, Schuh S, Rath W. Long-term follow-up and prognostic significance of angiogenic basic fibroblast growth factor (bFGF) expression in pa-
p53, cathepsin D, hormone receptors and prognosis. Oncology 2001;60:72–80. PMID: 1150912
97: Takei H, Iino Y, Li J. The correlation between tumor angiogenesis and lymph node metastasis in primary breast carcinoma. PMID: 10678042
98: Gasparini G. Prognostic value of vascular endothelial growth


angiogenesis and tumor cell shedding into effluent venous blood

PMID: 8679461


Prechtl K, Prechtl D. Breast carcinoma and breast saving therapy—a critical comment from the viewpoint of the pathologist. Geburtshilfe Frauenheilkd 1996;56:184–9. German. PMID: 8682283


Siitonen SM, Haapasalo HK, Rantalai IS, Helin HJ, Isola JJ. Comparison of different immunohistochemical methods in the assessment of angiogenesis: lack of prognostic value in a group of 77 selected node-negative breast carcinomas. Mod Pathol 1995;8:745–52. PMID: 8539232


Toi M, Inada K, Suzuki H, Tominaga T. Tumor angiogenesis


222: Vartanian RK, Weidner N. Correlation of intratumoral endothelial cell proliferation with microvessel density (tumor angiogenesis) and tumor cell proliferation in breast carcinoma. Am J Pathol 1994;144:1188–94. PMID: 7515558


Appendix 2. List of the 17 publications excluded (from the 87 selected in view of meta-analysis) for not mentioning survival data or being out of the scope of our review.

<table>
<thead>
<tr>
<th>First author—year of publication (Reference)</th>
<th>Reason(s) for rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kato—2002 (31)</td>
<td>Parameter assessed: blood vessel invasion (and not MVD)*</td>
</tr>
<tr>
<td>Tynninen—2002 (32)</td>
<td>Metastasized breast cancer</td>
</tr>
<tr>
<td>Ryska—2001 (36)</td>
<td>Out of the scope of the review</td>
</tr>
<tr>
<td>Kato—2000 (39)</td>
<td>Out of the scope of the review</td>
</tr>
<tr>
<td>Nakamura—1999 (47)</td>
<td>No survival data (OS, RFS)</td>
</tr>
<tr>
<td>Lampe—1998 (57)</td>
<td>MVD not assessed</td>
</tr>
<tr>
<td>Marinho—1997 (63)</td>
<td>Out of the scope of the review</td>
</tr>
<tr>
<td>Paulsen—1997 (65)</td>
<td>No survival data</td>
</tr>
<tr>
<td>Aranda—1996 (67)</td>
<td>Prediction by MVD of response to doxorubicin</td>
</tr>
<tr>
<td>Karatissi—1996 (70)</td>
<td>No survival data (OS, RFS)</td>
</tr>
<tr>
<td>Leek—1996 (72)</td>
<td>No survival data (OS, RFS)</td>
</tr>
<tr>
<td>Miliaras—1995 (86)</td>
<td>Out of the scope of the review</td>
</tr>
<tr>
<td>Veermeulen—1995 (89)</td>
<td>No survival data (OS, RFS)</td>
</tr>
<tr>
<td>Vartanian—1994 (97)</td>
<td>No survival data (OS, RFS)</td>
</tr>
<tr>
<td>Sahin—1992 (104)</td>
<td>No survival data (OS, RFS)</td>
</tr>
<tr>
<td>Weidner—1991 (8)</td>
<td>Abstract</td>
</tr>
</tbody>
</table>

* MVD, microvascular density; OS, overall survival; RFS, relapse-free survival.

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MVD, A PROGNOSTIC FACTOR IN BREAST CANCER

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and other conventional features in operable breast cancer: subanalysis in node-


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immunohistochemical methods in the assessment of angiogenesis: lack of 
prognostic value obtained in a group of 77 selected node-negative breast carcinomas. 

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membrane structure in breast cancer as related to tumour histology and prognosis. 


Microvessel Density as a Prognostic Factor in Women with Breast Cancer: A Systematic Review of the Literature and Meta-Analysis

Bernard Uzzan, Patrick Nicolas, Michel Cucherat, et al.

*Cancer Res* 2004;64:2941-2955.

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