Summary

The identification of prognostic factors and the appropriate selection of the patients more likely to benefit of anti-angiogenic therapies is a major area of research. Early experience with other molecular targeted drugs, such as imatinib and/or trastuzumab, has generated the perception that pre-treatment target assessment is a pre-requisite for therapy. However, emerging evidence suggests that presently we have no predictive biomarkers for anti-angiogenic agents. Despite considerable evidence for the association of intratumoral and/or plasma vascular endothelial growth factor (VEGF) levels with tumor progression and/or poor prognosis, pre-treatment VEGF levels are not predictive of response to angiogenesis inhibitors. This may possibly be due to the complexity of the angiogenic pathways and the limitations associated with current methods of VEGF detection and quantification; e.g. low assay sensitivity and lack of standardized methods could prevent detection of very small increases in VEGF, which may be clinically important. In addition to a general lack of agreement as to the relative clinical relevance of circulating versus tumor VEGF levels, the absence of a gold standard VEGF detection assay and the lack of a predefined, clinically relevant cut-off values pose a significant hindrance to the clinical utility of VEGF measurements for therapy selection. Several retrospective studies showed a promising important role of microvessel density and other pro-angiogenic factors (e.g. basic fibroblast growth factor, VEGF, thymidine phosphorylase, etc.) as independent prognostic markers in solid tumors, but these data have to be validated in prospective trials.

Key Words: Prognosis; predictive markers; angiogenesis.
1. PROGNOSTIC SURROGATE BIOMARKERS

1.1. Introduction

A number of studies, most of which are retrospective, correlated potential surrogate markers of angiogenesis with prognostic parameters in different solid tumor types (1). The morphological aspects of angiogenesis, such as microvessel density (MVD), total microvascular areas (TMA) or vascular patterns, as well as the overexpression of angiogenic factors have been correlated with disease outcome (2).

1.2. Microvessel Density

Most published prognostic studies are retrospective and are based on the measurement of intratumoral vascularity by counting microvessels identified by panendothelial or other angiogenesis-related markers using immunohistochemistry (IHC) techniques. The basic method for assessment of vascularity was first proposed by Weidner et al. (3). The first step of the method is the use of panendothelial markers, including factor VIII-related antigen (von Willebrand factor, fVIII-RA), monoclonal antibodies to CD31, CD34, or others, to immunostain blood microvessels. The second one is the identification of the single area of highest vascularization (“hot spot”) by scanning of the entire tumor section at low power (400× field) and then at higher power (200× field) to count each individual microvessel (any stained endothelial cell or separate clusters without vessel lumina is also an evaluable microvessel). To limit the subjectivity of tumor vascularity evaluation, two alternative techniques have been developed: the use of the Chalkley eye piece and multiparametric computerized imaging analysis systems, which evaluate vascular area, microvessel number and perimeter, and intensity of staining (4).

Panendothelial markers do not distinguish blood vessels from lymphatic vessels (as per fVIII-RA) or cross-stain other cells (plasma cells in the case of anti-CD31). The antibody to CD105 (endoglin) or LM609 to the integrin αvβ3 more selectively stain proliferating (activated) endothelium. The vascular parameters measured by CD105 appeared to better correlate with overall survival (OS) and disease-free survival (DFS) than panendothelial markers such as CD31 in breast, colon, lung, and prostate carcinomas (2).

1.2.1. Breast Cancer

Breast cancer (BC) is the widest studied tumor to define the relationship of angiogenesis with clinical outcome. The prognostic value of MVD in BC was first shown independently by two groups in 1992 (5,6). Other authors demonstrated that the degree of vascularization of the primary tumor correlates with the presence of bone marrow micrometastases at diagnosis, and that the degree of vascularity at “hot spots” in axillary lymph nodes is associated with outcome. The Chalkley count seems to be the preferable technique for estimating angiogenesis with regard to the prognostic stratification of BC patients, based on its acceptable reproducibility (7,8).

IHC staining of blood microvessels has been obtained by different markers, mainly with fVIII-RA, CD31, CD34, integrin αvβ3, CD105, or type IV collagen. In a series of 197 consecutive patients with invasive BC and long follow-up, the expression of integrin αvβ3 has been suggested as the single-most significant prognostic indicator for relapse-free survival (RFS) in both node-negative (N–) and node-positive (N+) BC