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Neovascularization in Clinical Stage A Testicular Germ Cell Tumor: Prediction of Metastatic Disease[1]

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ABSTRACT

Increased numbers of blood vessels (angiogenesis or neovascularization) in certain primary tumors correlates with an increased risk for metastatic disease. We therefore conducted a blinded review of the resected testicular germ cell tumors of 65 clinical stage A patients to evaluate the usefulness of angiogenesis in identifying those patients with clinically occult nodal metastases (pathological stage B).

Angiogenesis was assessed in the primary tumors using an immunohistochemical stain for factor VIII-related antigen assay for quantitation of microvessel counts. Of 65 clinical stage A patients, 43 had pathological stage B disease at retroperitoneal lymph node dissection. Eleven patients had microvessel counts >30 microvessels/×400 high powered field, and all of these patients had pathological stage B disease ($P = 0.02$ in univariate analysis). Multiple regression analysis using microvessel count and other histological findings found to be prognostic (venous invasion, lymphatic invasion, presence of embryonal carcinoma, and absence of yolk sac tumor) showed that only the absence of a yolk sac tumor component was significantly predictive of occult metastases.

This study shows that angiogenesis, as measured by quantitation of microvessel counts in the primary tumor of germ cell neoplasms, is significantly predictive of occult nodal metastatic disease by univariate analysis in clinical stage A patients. The prospective use of angiogenesis quantitation needs to be defined.

INTRODUCTION

The majority of testicular cancer patients are cured today, even in the presence of metastatic disease, with surgery or chemotherapy. Patients with clinical stage A disease (disease limited to the testicle) have a relapse rate of approximately 30% despite current clinical staging. In many medical centers, patients with clinical stage A disease undergo initial retroperitoneal lymph node dissection, which puts these patients through a procedure with some morbidity, but only benefits the fraction of those with pathological stage B disease. With the advent of successful chemotherapy and the availability of sensitive and specific tumor markers and noninvasive surveillance mechanisms, the need for primary retroperitoneal lymph node dissection in patients with clinical stage A nonseminomatous germ cell tumor has been questioned.

Ideally, prognostic factors could be used to select the group of patients who are at the highest risk for retroperitoneal metastasis and therefore are most likely to benefit from retroperitoneal lymph node dissection. A number of retrospective and prospective trials have been performed identifying several histopathological features of the primary tumor which are predictive of relapse. The Medical Research Council recently published a prospective study which identified venous and lymphatic invasion, the presence of embryonal carcinoma, and the absence of yolk sac tumor elements as prognostic factors for metastatic disease (1, 2).

These investigators were able to predict a subset of patients with a 54% relapse rate at 2 years who had three or four of the identified prognostic factors. Other prognostic factors identified in the literature include the percentage of teratoma in the primary tumor (3) and pathological T stage >1 (*i.e.*, tumor extending through the tunica albuginea, involving rete testis or epididymis, and invading the spermatic cord and scrotal wall) (4, 5).

The relatively poor ability to predict nodal metastasis led us to look at other possibilities. We have evaluated another histological technique, an immunohistochemical assessment of angiogenesis, in a group of clinical stage A patients with testicular germ cell tumor to determine its value as a new factor that is predictive of pathological stage.

Angiogenesis does play a role in cancer metastasis. The first published study that showed that intensity of neovascularization can be predictive of metastasis was performed in melanoma (6, 7). Similar studies have subsequently been performed in breast cancer (8, 9) and non-small cell lung cancer (10). We have also evaluated angiogenesis in relation to the known prognostic factors in germ cell tumors as published by the Medical Research Council (1).

PATIENTS AND METHODS

Patients. We studied tumor specimens from 65 patients with primary germ cell tumors seen at Indiana University Medical Center between 1983 and 1989. All patients had clinical stage A disease; most had their orchiectomy at local institutions and were referred to Indiana University for retroperitoneal lymph node dissection.

Vessel Staining. Paraffin sections (4 μm thick) were cut from tissue blocks of the original orchiectomy specimen. Blood vessels were identified by staining endothelial cells for Factor VIII-related antigen (DAKO polyclonal, DAKO Corp., Santa Barbara, CA) with the use of a standard avidin-biotin complex technique (Vectastain, Vector Laboratories, Inc., Burlingame, CA) as described by Weidner *et al.* (8, 9). A section from one representative tumor block per tumor was stained. With the use of a light microscope, the slides were scanned at low power (×40 or 100), and the areas of most intense neovascularization within viable tumor were identified. These areas tended to be at the margins of the tumor and tended to include normal stromal elements surrounded by malignant tissue. After the areas with the highest neovascularization within the germ cell tumor were identified, the microvessels were counted. Any brown-staining single endothelial cell or cluster of endothelial cells were counted. A vessel lumen was not necessary for a microvessel to be counted. If a microvessel was cut longitudinally it was counted as one vessel. Each count was expressed as the highest number of microvessels identified within a ×400 high power field. The use of a ×400 high power field was arbitarily chosen for ease of seeing and accurately counting microvessels. All counts were performed by the same investigator, and many were reviewed by a second investigator. Fig. 1 shows a representative section of germ cell tumor stained with Factor VIII-related antigen.

Statistics. Microvessel counts were first evaluated in a univariate analysis using Fisher's exact testing. To examine whether microvessel counts were an independent prognostic factor, multiple logistic regression was performed.

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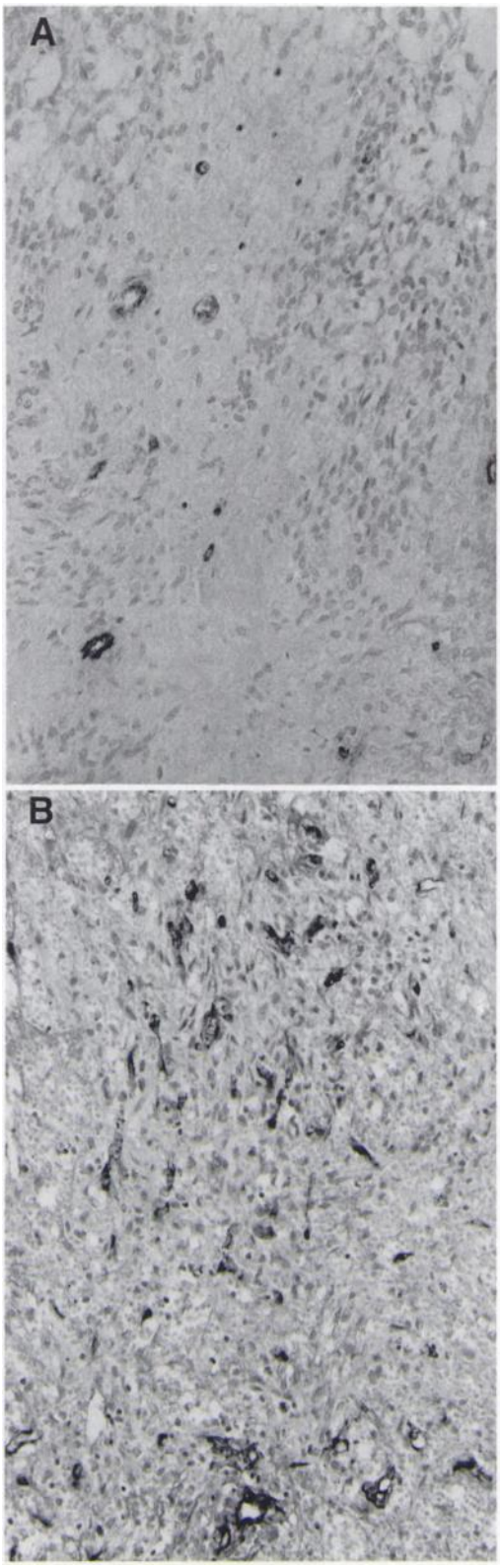

Fig. 1. Factor VIII antigen-stained microvessels. *A*, low microvessel density; *B*, high microvessel density.

RESULTS

Each patient has a minimum follow-up of at least 3 years without evidence of relapse. Twenty-two patients had pathological stage A disease and 43 had pathological stage B disease. Histological review revealed 12 embryonal carcinomas, 50 mixed germ cell tumors, 2 yolk sac tumors, and 1 mature and immature teratoma.

Fig. 2 shows the results of microvessel count broken down by pathological stage A *versus* B patients. Eleven patients had microvessel count >30/×400 high powered field. All 11 of these patients had pathological stage B disease, whereas none of 22 patients with pathological stage A disease had microvessel counts exceeding 30/×400 high power field (P = 0.011 by Fisher's exact univariate analysis).

We also looked at other established prognostic factors for metastatic disease in clinical stage A germ cell tumor using univariate analysis. These are vascular invasion (either venous or lymphatic), presence of embryonal carcinoma, and absence of yolk sac tumor in the primary tumor specimen. We found vascular invasion and the absence of yolk sac tumor elements to be significant prognostic factors (Table 1).

Multiple logistic regression was also performed, and microvessel count no longer significantly predicted pathological stage after accounting for venous or lymphatic invasion, absence or presence of embryonal carcinoma, and absence or presence of yolk sac tumor elements. In this analysis, only yolk sac tumor elements achieved statistical significance (Table 2). We are unaware of any biological basis for this result, although similar results have been obtained in other studies (1, 2).

DISCUSSION

Growth of solid tumors beyond 1–2 mm in diameter depends on the induction of a functional microcirculation from surrounding host tissue. Abundant evidence supports the concept that tumors induce

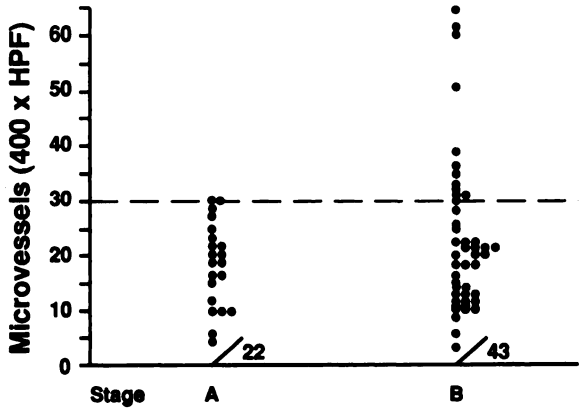

Fig. 2. Microvessel counts obtained in stage A and B patients. *HPF*, high power field.

Table 1 *Univariate analysis of prognostic histological variables and pathological stage B germ cell tumor (Fisher's exact statistic)*

Variable	P value
Microvessel count >30/×400 HPF[a]	0.011
Vascular invasion	0.03
Presence of embryonal carcinoma elements	1.00
Absence of yolk sac tumor elements	0.001

[a] HPF, high power field.

Table 2 *Logistic regression multivariate analysis results in predicting pathological stage B germ cell tumor*

Variable	P value
Microvessel count >30/×400 HPF[a]	0.1826
Vascular invasion	0.0867
Presence of embryonal carcinoma elements	0.5095
Absence of yolk sac tumor elements	0.0150

[a] HPF, high power field.

angiogenesis though a variety of soluble factors (11, 12). The tumors themselves may not be the only source of angiogenesis factors. They may recruit macrophages or mast cells to secrete or amplify angiogenic molecules. Neovascularization takes place by a process of capillary sprouting from preexisting normal microvessels.

Angiogenesis plays a role in the process of cancer metastasis. The subset of cells destined to enter circulation and metastasize are aided by the immature nature of these neovessels. They have markedly reduced basement membranes and increased permeability and lack vascular pericytes compared to normal vessels. As well, metastatic cells are aided by the increased surface area of the newly formed vessels (13).

We evaluated angiogenesis with the goal of finding a new prognostic factor in clinical stage A germ cell tumor to help determine which of these patients are at highest risk for pathological stage B disease and who would therefore benefit most from retroperitoneal dissection. Neovascularization has been shown to be predictive of metastatic disease in other tumors and was therefore studied in germ cell tumors. We did find that microvessel counts >30/×400 high power field using a Factor VIII antigen stain can predict for pathological stage B germ cell tumor. When examined in relation to other established prognostic variables in multivariable analysis, microvessel count did not retain its significance. However this loss of significance may reflect the small sample size. To determine future usefulness of neovascular quantitation in clinical stage A germ cell tumors as a prognostic tool, a larger prospective study needs to be undertaken.

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