

PRIMARY ANGIOSARCOMA OF THE BREAST: IS KI-67 proliferation index related to histologic grade? Does steroid hormone receptor expression play a role in the frequency of coexistent pregnancy?

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Bu çalışma XX. Avrupa Patoloji Kongresi'nde (Paris, 3-8 Eylül 2005) poster bildirisi olarak sunulmuştur.

AIMS: The aim of this study was to investigate the relationship between Ki-67 proliferation index and prognosis in primary angiosarcoma of the breast (PAB). Hormonal status of PAB was also discussed, since PAB sometimes can be seen with coexistent pregnancy.

METHODS: During a 17-year period 4 cases of PAB were established out of almost 5000 breast biopsies. Immunohistochemical analyzes were performed for antibodies against CD31, CD34, estrogen receptor (ER) and progesterone receptor (PR). Ki-67 proliferation index was also investigated.

RESULTS: All patients clinically presented with a palpable mass. The mean patient age at diagnosis was 40.8 years (29-56). Two patients were at the end of their first trimester in pregnancy. The mean score of Ki-67 index was 28.7% (2-55%). Two patients with Grade I tumor and with the lowest Ki-67 index were alive with no evidence of disease 7-8 years after surgery. Proliferation index was detected to be 50% in Grade II tumor, and the patient has been receiving radiotherapy and chemotherapy because of distant metastases. The patient with Grade III tumor died with disseminated disease one year after the initial diagnosis. Positive immunoreactivity for ER and PR could not be detected in any tumor.

CONCLUSIONS: Since, it was previously stated that grading is the most important prognostic indicator, we suggest that Ki-67 proliferation index may also be used for the same purpose. No evidence of an association with hormonal status could be drawn.

MEMENİN PRİMER ANJİYOSARKOMU: Ki-67 proliferasyon indeksi ile histolojik 'grad' ve gebelik ile steroid hormon reseptör ekspresyonu arasında bir ilişki var mı?

ÖZET

AMAÇ: Bu çalışmada, memenin oldukça nadir görülen primer anjiyosarkomlarında Ki-67 proliferasyon indeksinin prognoz ile olası ilişkisi ve ayrıca bu tümörlerin gebelik ile birlikteliğinden yola çıkarak steroid hormon reseptör ekspresyonu varlığı araştırıldı.

YÖNTEM: Onyediyedi yıl içerisinde Anabilim Dalımızda incelenmiş yaklaşık 5000 meme biyopsisine ait arşiv kayıtları gözden geçirildi. Dört adet primer anjiyosarkom olgusu saptandı. Parafin bloklara CD31, CD34, östrojen ve progesteron reseptörü immunohistokimyasal yöntem kullanılarak uygulandı. Ayrıca, Ki-67 proliferasyon indeksleri belirlendi.

BULGULAR: Tüm olgular palpe edilebilir kitle ile kliniğe başvurmuşlardı. Hastaların ortalama yaşları 40,8 olarak belirlendi (29-56). İki hasta gebeliklerinin ilk trimestrinin sonundaydı. Ortalama Ki-67 proliferasyon indeksi %28,7 olarak saptandı (%2-55). Histolojik olarak Grade I tümöre sahip iki hasta en düşük Ki-67 proliferasyon indeksi ile cerrahiden 7-8 yıl sonra sağ ve sağlıklı idi. Grade II tümörde Ki-67 proliferasyon indeksi %50 olup, hastanın 12 ay sonunda uzak metastazlar nedeni ile onkolojik tedavi almakta olduğu belirlendi. Grade III tümörlü hastanın ise 1 yıl içerisinde diseminat hastalık ile öldüğü belirlendi. Tümörlerden hiçbirinde östrojen ve progesteron reseptörleri ile pozitif immün reaktivite saptanmadı.

SONUÇLAR: Memenin primer anjiyosarkomlarında grade bilinen önemli prognostik belirleyici olmakla birlikte Ki-67 proliferasyon indeksi de yardımcı prognostik parametre olarak kullanılabilir. Memenin primer anjiyosarkomlarında hormon reseptör ekspresyonu ile bir ilişki olmadığı sonucuna varılmıştır.

Angiosarcoma (ASA) of the breast is a rare and highly aggressive vascular tumor. It may present as a secondary tumor, which includes postmastectomy and postradiation ASAs as well as a primary form (1,2). The incidence of secondary ASA in breast increases with the widespread use of conservative surgery treatments and adjuvant therapies. However, the incidence of primary angiosarcoma of the breast (PAB) has remained constant (1). The incidence of PAB is about 0.05% of all primary malignancies of the organ (3,4).

If well-differentiated ASAs (Grade I) were excluded, PAB is usually lethal (4). Grading has been shown to be the most important prognostic indicator in PAB (1,6). PAB can be subdivided into three categories, Grade I to III or well to poorly differentiated, according to two most used grading systems (6,7). Both grading systems are almost identical. The main difference is the quantitative approach of Merino et al. (6). Besides, the system by Donnell et al.(7) has gained wide impact as it was tested in a large number of patients with adequate follow-up (1).

Table 1. Antibodies used for immunohistochemical studies

Antibodies	Source	Clone	Dilution	Incubation
CD31	NeoMarkers, CA, USA	JC/70A	1/50	1 h
CD34	NeoMarkers, CA, USA	QBEnd/10	1/50	1 h
ER	NeoMarkers, CA, USA	SP1	1/100	2 h
PR	NeoMarkers, CA, USA	SP2	1/200	2 h
Ki-67 index	NeoMarkers, CA, USA	SP6	1/200	1 h

The age at diagnosis ranges from 17 to 70 years with a median value of 38 years (8). Coexistent pregnancy was reported in 6% of cases, which may be related with the young age of the patients (8).

Reproductive steroid hormones play a role in carcinogenesis in the breast by altering the kinetics of proliferation, differentiation and atrophy (9). Estrogens promote growth and proliferation, whereas progestines cause both growth and differentiation (10). Because PAB is seen mostly in reproductive age, sometimes with coexistent pregnancy, it is of our interest that whether PAB is associated with the hormonal receptor status.

The Ki-67 nuclear antigen is associated with cell proliferation and is detectable in the nuclei of cycling cells. It is widely accepted that active tumors express high levels of Ki-67 nuclear antigen (11,12).

We, here report 4 cases of PAB with clinicopathologic features and clinical follow-up. Immunohistochemical studies for steroid hormone receptor expression were performed. Also, Ki-67 proliferation indices were investigated for their possible relation with grade and prognosis.

Materials and methods

Review of pathology records of primary breast malignancies revealed 4 cases of PAB out of almost 5000 surgical breast materials, that were diagnosed at Istanbul University, Istanbul Faculty of Medicine, Department of Pathology between years 1987 and 2004. The results of gross examinations and histologic grades of cases were obtained from the pathology reports. Hematoxylin and eosin-stained (H&E) slides of 4 cases of PAB were examined for a representative paraffin block to per-

form immunohistochemical analysis (Table 1). Immunohistochemical studies were carried out by using the standard avidin-biotinylated-peroxidase complex technique. Antibodies against CD31, CD34, estrogen receptor (ER), progesterone receptor (PR) and Ki-67 were used (Table 1). Nuclear localization for ER, PR and Ki-67, and cytoplasmic immunoreactivity for CD31 and CD34 were accepted as positive. The proliferation index was given as ratio of percent Ki-67-positive cells of 1000 tumor cells per section. Negative controls in which the primary antibody was omitted, and positive controls on tissues with known positive immunoreaction for each antibody were used. Clinical data were obtained from the patients' charts and surgery reports.

Results

All the patients clinically presented with a palpable mass and additionally with bluish-red discoloration in one patient. Two of the patients (50%) were at the end of their first trimester of pregnancy. The mean patient age at diagnosis was 40.8 years (ranged from 29 to 56). The initial diagnosis was done on excisional biopsy for 3 patients, incisional biopsy for one patient and all the patients underwent a simple mastectomy afterwards. None of the patients has received prior radiotherapy which would have predisposed them to the formation of PAB. Grossly, all tumors had hemorrhagic cystic spaces with solid, white colored areas and ill-defined border. The mean tumor size was 8.37 cm (ranged from 2.5 cm to 12 cm). One case revealed two masses of PAB, 9 cm and 1.3 cm in diameter. Of all tumors, two were Grade I, one was Grade II and the other one was Grade III on microscopic examination (Figure 1-2), and all were positive for CD31 and three for CD34 on immunohistochemical analysis (Figure 3-4). The mean score of Ki-67 index was 28.7% (ranged from

Table 2. Clinicopathologic features and immunohistochemical findings of 4 cases of primary angiosarcoma of the breast

	Case 1	Case 2	Case 3	Case 4
Age (years)	29	56	43	35
Multifocality	none	none	none	present (two foci)
Tumor size (cm)	12	2.5	10	9 and 1.3
Histologic grade	I	I	III	II
CD31	+	+	+	+
CD34	+	+	---	+
ER	---	---	---	---
PR	---	---	---	---
Ki-67 index (%)	8	2	55	50
Pregnancy	present	none	none	present
Follow-up	FOD (84 mo)	FOD (96 mo)	DOD (12 mo)	AWD (12 mo)

ER: estrogen receptor, PR: progesterone receptor, FOD: free of disease, DOD: dead of disease, AWD: alive with disease

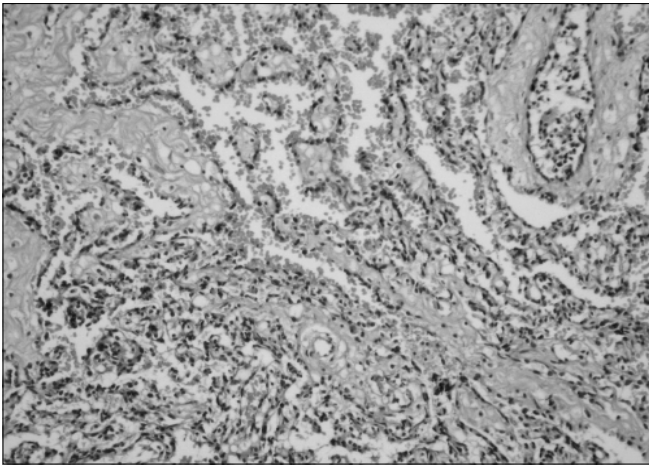


Figure 1. Histologic features of a well-differentiated (Grade I) angiosarcoma displaying interanastomosing channels containing red blood cells (H&E, x100).

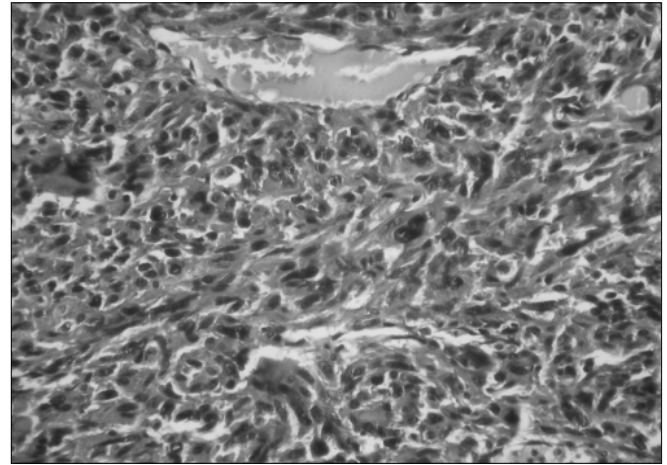


Figure 2. Pleomorphic endothelial cells surrounding vascular channels in a solid area of a poorly differentiated (Grade III) angiosarcoma (H&E, x200).

2% to 55%) (Figure 5A). The tumor without CD34 immunoreactivity was Grade III and exhibited the highest Ki-67 index (55%) (Figure 5B). Two patients, one of whom was pregnant, with Grade I tumors and with the lowest Ki-67 indices were alive with no evidence of disease 7-8 years after surgery. One of the pregnant patients, having two foci of Grade II tumor, presented with distant metastases 5 months after surgery. She is still alive 12 months after the initial diagnosis, and has been receiving radiotherapy and chemotherapy. Only the Grade III patient died with disseminated disease one year after the initial diagnosis. Clinicopathologic features and immunohistochemical results of our cases are summarized in Table 2.

Discussion

PAB is an unusual neoplasm with an adverse outcome. The incidence of PAB was reported as 0.05% (3,4) in comparison with our study displaying an incidence of 0.08% during a 17-year period. However, the frequency of coexistent pregnancy, which is written as 6% in literature (8), is much higher in our study. This fact might be due to rapid population increase in our country and the frequent occurrence of PAB at the reproductive period. Nevertheless, the present study is restricted to a limited number of PAB cases.

Estrogens and progestins have an important role on tumorigenesis in breast cancer(9,10). Both steroid hormones balance the proliferation, differentiation and atrophic processes (10). We could not demonstrate a relationship between hormonal status of PAB and pregnancy, although PAB is seen among women in reproductive age. The coexistence of pregnancy and PAB should be related to the patients' young age (2).

Histologic grade is the most important prognostic indicator in cases of PAB (6,7). Our results are keeping with those in literature, for instance the patient who had Grade III tumor died of disseminated disease in 1-year period subsequent to surgery. Therefore, the critical point in the evaluation of PAB cases is the adequate sampling that should be performed carefully to determine the poorly differentiated areas of the tumor.

Disease-free survival was detected to be correlated with tumor grade. Despite the fact that low-grade ASAs have a relatively favorable outcome, these patients can develop local and systemic recurrences (7,8). Ki-67 proliferation index is widely used for detecting highly aggressive tumors of various kinds (13-15). Regar-

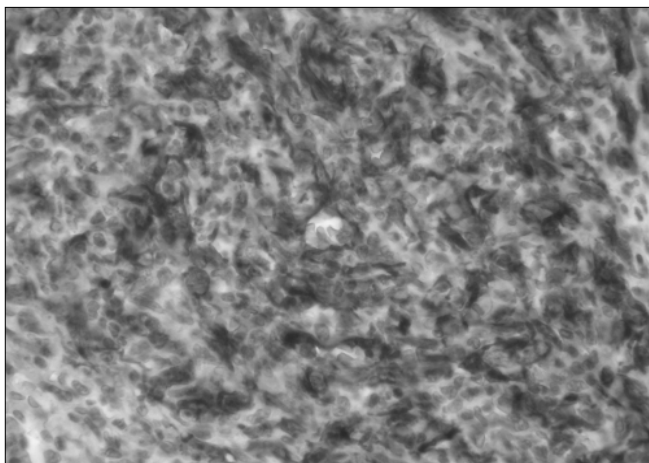


Figure 3. CD31 was strongly positive in tumor cells of an angiosarcoma (anti-CD31, x200).

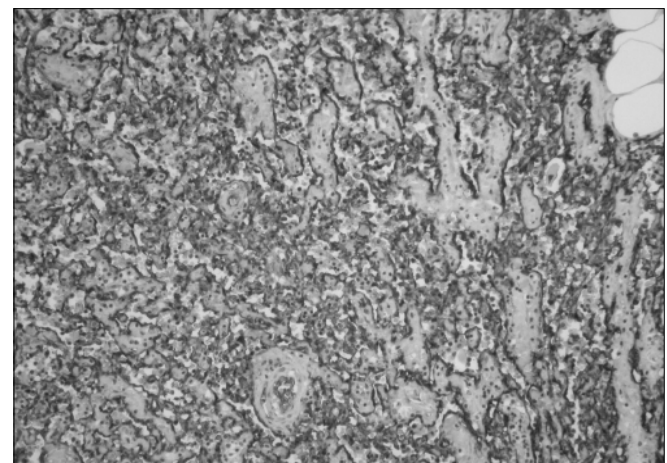


Figure 4. Diffuse and strong positive immunoreactivity for CD34 (anti-CD34, x100).

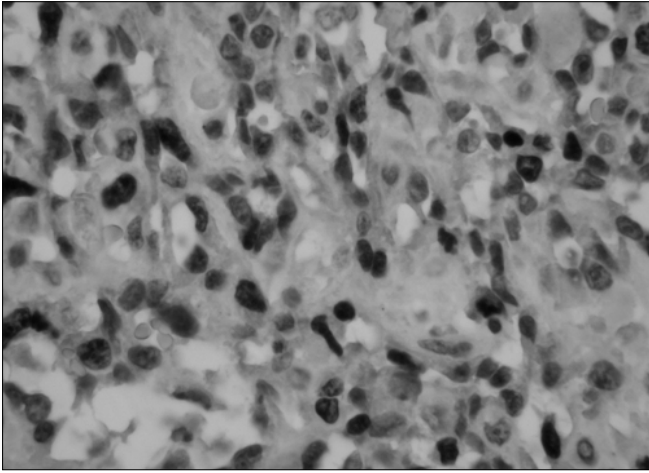


Figure 5A. A well-differentiated (Grade I) angiosarcoma displaying low Ki-67 proliferation index (anti-Ki-67, x200).

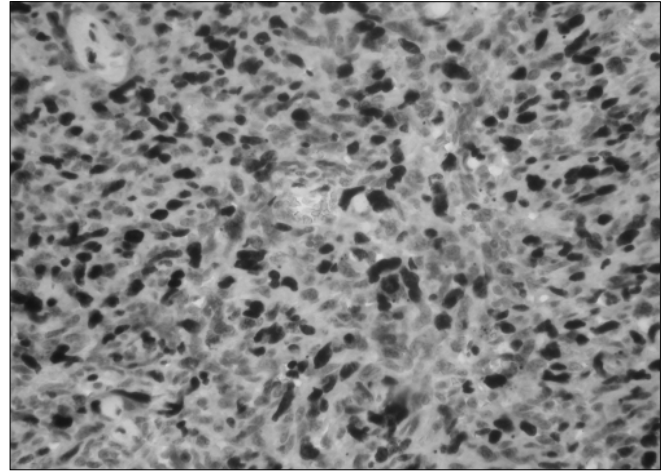


Figure 5B. Ki-67 proliferation index was detected to be high in a poorly-differentiated (Grade III) angiosarcoma.(anti-Ki-67, x200).

ding the relationship between high proliferation index and poor prognosis in a Grade II tumor in the present study, we suggest that Ki-67 proliferation index should be used to predict nonhigh-grade tumors with adverse outcome. Low proliferation index of two grade I tumors in our study also supports the theory of relationship between Ki-67 antigen and aggressiveness of PAB.

ASAs exhibit reactivity for CD34 and CD31 especially in distinguishing epithelioid forms of ASA from carcinomas and other neoplasms (16,17). Three of our cases were positive for both anti-

bodies, CD31 and CD34, but the poorly differentiated tumor was positive only for CD31. To our knowledge, the expression of CD31 and CD34 may be lost in more poorly differentiated areas (1). We recommend to use at least two endothelial markers in the differential diagnosis of poorly differentiated tumors.

In summary, we suggest that Ki-67 proliferation index should also be used to predict outcome of PAB patients as well as grading. No evidence of an association with hormonal status could be drawn, although coexistent pregnancy could be seen.

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