Cellular Proteolysis and Oncology

Impact of pretreatment thrombocytosis on survival in primary breast cancer

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Summary

Platelet count has been reported to have predictive value in various cancer entities. In the case of breast cancer, evidence about involvement of platelets is still incomplete. Our objective was to assess the influence of pretreatment thrombocytosis on survival and establish its prognostic relevance for breast cancer patients.

We performed a retrospective, multivariate analysis of 4,300 patients with early-stage breast cancer. All subjects participated in one of five prospective, randomized, multicenter trials conducted by the Austrian Breast and Colorectal Cancer Study Group. Thrombocytosis was defined as a platelet count exceeding 400 G/L. Median follow-up was 52 months. Univariate and multiple Cox regression models were calculated for overall survival (OS), breast cancer-related survival and disease-free survival (DFS).

Pretreatment thrombocytosis was observed in 161 patients (3.7%). Estimated median OS, breast cancer-related survival and DFS for patients with versus those without thrombocytosis was 71.0 versus 99.5, 72.0 versus 100.9, and 80.4 versus 88.4 months, respectively (p = 0.0054, p = 0.0095, p = 0.0199). A multiple Cox regression model including tumor and nodal status, grading, age, hormone receptor status and pretreatment thrombocytosis identified pretreatment thrombocytosis as an independent predictive factor for OS (p = 0.0064) and breast cancer-related survival (p = 0.0162). Multivariate analysis failed to identify pretreatment thrombocytosis as an independent risk factor for DFS (p = 0.1355).

In our retrospective study, elevated platelet counts at time of diagnosis were associated with poor prognosis in breast cancer. We hypothesize that platelets may contribute to the pathophysiology of hematogenous metastasis.

Introduction

The majority of deaths in women with breast cancer is attributable to hematogenous metastasis. Malignant cells challenge hemostasis while approaching the site of metastasis. According to a revisited version of Virchow's triad (1), the three components of hemostasis are soluble coagulation factors, the endothelium and platelets. Some aspects of coagulation (2) and especially cancer cell-endothelium interactions (3) have in recent times been the goal of investigations in cancer and metastasis

research, and the impact of platelets in cancer angiogenesis and metastasis has for years been on debate (4-8). High pretreatment platelet count is reported to be associated with poor survival in malignant mesothelioma (9), lung cancer (10-14), gynecologic malignancies (15-24), renal cancer (25, 26) and colorectal cancer (14, 27). However, in the literature we found no evidence dealing with the influence of pretreatment platelet count on survival in breast cancer patients. We therefore performed a retrospective, multivariate analysis of women with stage I, II and IIIA breast cancer in an attempt to assess the influence of pretreatment platelet count on survival.

Material and methods

Study patients

The study population was pooled among stage I, II and IIIA breast cancer patients, participating as of 1990 in one of five prospective, randomized, multicenter trials initiated and implemented by the Austrian Breast and Colorectal Cancer Study Group (ABCSG). All trials were approved by local Ethics Committees and since 1995 were subject to continuous and independent monitoring according to Good Clinical Practice. Patients randomized before 1995 were monitored retrospectively. All patients in these five trials of the ABCSG had to give their informed consent before entering the trial according to the Helsinki declaration. Three trials have already been closed, the specific outcomes assessed have in part been published (28-30), and recruitment to two other trials is still ongoing. Study endpoints included death, breast cancer-related death, and first relapse of disease, respectively, for overall survival (OS), breast cancer-related survival , and disease-free survival (DFS).

A detailed description of these trials is given in Table 1. A total of 5,708 patients were randomized by May 2001. Pretreatment platelet counts and follow-up data were documented in 4,382 subjects, and those presenting pretreatment counts of less than 150 G/L were excluded. Finally, 4,300 patients (75% of total) were eligible for analysis. Patients were stratified in two groups according to the presence or absence of pretreatment thrombocytosis, which was defined as a platelet count exceeding 400 G/L. Detailed demographic characteristics are listed in Table 2.

Statistical analysis

We evaluated the impact of pretreatment thrombocytosis retrospectively. The proportions of patients with a given characteristic were compared by the chi-square test. Differences in the means of continuous variables were tested by Student's t test. All tests were two-tailed.

Table 1: Description of ABCSG multicenter trials in breast cancer patients*. Five different prospective, randomized, multicenter trials conducted by the Austrian Breast and Colorectal Cancer Study Group were described in detail, giving the trial number, total number of patients, the number of eligible patients, investigational objectives, stratification criteria and trial status.
* ABCSG = Austrian Breast and Colorectal Cancer Study Group
† CMF = Cyclophosphamide 600mg/m^2, Methotrexate 40mg/m^2, and 5-Fluorouracil 600mg/m^2 iv day 1+8, every 28 day;
‡ EC = Epirubicin 60mg/m^2 and Cyclophosphamide 600mg/m^2 every 21days

Trial number	Total patients	Eligible patients	Investigational objectives	Stratification criteria	Trial status
5	N=1,072	N=926 (86.4%)	Adjuvant chemotherapy (6xCMF)† vs. endocrine therapy (goserelin and tamoxifen) in premenopausal patients with receptor-positive disease	tumor stage, nodal status, type of surgery, hormone receptor status, grading, participating center	Closed, published[28]
6	N=1,991	N=1,645 (82.6%)	Adjuvant endocrine therapy (tamoxifen vs. tamoxifen + aminoglutethimide) in postmenopausal patients with receptor-positive disease	age, tumor stage, nodal status, type of surgery, hormone receptor status, grading, participating center	Closed, published[29]
7	N=372	N=304 (81.7%)	Preoperative vs. pre- and postoperative chemotherapy in high-risk disease	age, tumor stage, nodal status, hormone receptor status, participating center	Closed, abstract published[30]
8	N=2,089	N=1,257 (60.2%)	Tamoxifen vs. tamoxifen and anastrozole in postmenopausal patients with receptor-positive, high-grade disease	age, tumor stage, nodal status, hormone receptor status, type of surgery, participating center	open
9	N=184	N=168 (91.3%)	Tamoxifen vs. adjuvant chemotherapy (4xEC)‡ + tamoxifen in postmenopausal patients with receptor-positive, low-grade disease	age, tumor stage, nodal status, hormone receptor status, grading, type of surgery, participating center	open
total	N=5,708	N=4,300 (75.3%)			

* ABCSG = Austrian Breast & Colorectal Cancer Study Group

† CMF = Cyclophosphamide 600mg/m^2, Methotrexate 40mg/m^2, and 5-Fluorouracil 600mg/m^2 iv day 1+8, every 28 day; ‡ EC = Epirubicin 60mg/m^2 and Cyclophosphamide 600mg/m^2 every 21days

Table 2: Characteristics of 4,300 women with breast cancer eligible for analysis Patients characteristics were given in table 2 with detailed description of tumor stage, nodal stage, Grading, menopausal status, hormone-receptor status and platelets count in all patients and distributed in patients with and without thrombocytosis.

Characteristic	All patients data available	Without thrombocytosis	With thrombocytosis
No.	4,300	4,139 (96.3%)	161 (3.7%)
Age (yrs)		59.2 ± 11.4	57.2 ± 10.6*
Tumor stage†			
T1	2,541 (59.4%)	2,464 (59.8%)	77 (48.4%)
T2	1,607 (37.6%)	1,530 (37.1%)	77 (48.4%)
T3	132 (3.1%)	127 (3.1%)	5 (3.1%)
Nodal status†			
Negative	2,625 (61.1%)	2,540 (61.4%)	85 (53.1%)
Positive	1,670 (38.9%)	1,595 (38.6%)	75 (46.9%)
Grading†			
G1, G2, Gx	3,337 (77.8%)	3,227 (78.2%)	110 (68.8%)
G3	950 (22.2%)	900 (21.8%)	50 (31.3%)
Menopausal status†			
Pre	1,089 (25.3%)	1,036 (25.0%)	53 (32.9%)
Post	3,211 (74.7%)	3,103 (75.0%)	108 (67.1%)
Estrogen receptor†			
Negative	360 (8.4%)	349 (8.5%)	11 (6.9%)
+ or ++	3,012 (70.5%)	2,905 (70.6%)	107 (67.3%)
+++	899 (21.0%)	858 (20.9%)	41 (25.8%)
Progesterone receptor†			
Negative	923 (21.6%)	885 (21.5%)	38 (23.9%)
+ or ++	2,487 (58.2%)	2,393 (58.2%)	94 (59.1%)
+++	864 (20.2%)	837 (20.3%)	27 (17.0%)
Platelets			
Count (G/L)		258 ± 54	453 ± 50

* tested by chi-square, p < 0.05 compared to patients without pretreatment thrombocytosis,
† tested by Student's *t* test, p = 0.012 compared to patients without pretreatment thrombocytosis

Further analysis included well-known prognostic covariates in breast cancer: tumor stage, nodal status, grading, menopausal status, age, and estrogen receptor (ER) and progesterone receptor (PgR) status. Tumor stage (T1, T2, T3) was defined according to International Union Against Cancer (UICC) criteria, nodal status as negative or positive, and tumor grading according to Bloom and Richardson (G1, G2 and Gx versus G3). Premenopausal status was defined on the basis of known menstruation; menopausal status was otherwise assessed by serum hormone level. Age was included as a continuous variable. In the presence of immunohistochemistry, ER and PgR status were stratified as negative, + or ++, and +++. Performing quantitative, biochemical assays, these parameters were stratified in negative, less than 10 fmol/mg or positive, and more than 10 fmol/mg (31).

The univariate effect of the above-mentioned prognostic covariates and pretreatment thrombocytosis on OS, breast can-cer-related survival and DFS was described by applying the Cox regression model. Furthermore, the effect of pretreatment thrombocytosis on OS, breast cancer-related survival and DFS was checked in a multiple Cox regression model, also including the well known prognostic covariates, in order to describe the impact of pretreatment thrombocytosis in addition to the covariates.

OS was calculated for all patients from the date of randomization until death of any cause, breast cancer-related survival from randomization until death from breast cancer, and DFS from randomization until time of relapse. Breast cancer-related survival reports the percentage of patients surviving breast cancer (only deaths of breast cancer were counted), whereas DFS is defined as period after randomization with no evidence of disease, in particular without any relapse of breast cancer. Relapse was defined as occurrence of organ metastasis as well as distant lymph node metastasis. In the absence of events (death, breast

cancer-related death or relapse), calculations were based on the date when the patient was last known to be event-free. Cumulative OS, breast cancer-related survival and DFS curves were separately calculated according to Kaplan and Meier for patients with and without pretreatment thrombocytosis. The Mantel test was used to establish the significance of differences between the curves.

Results

Comparison of groups

Thrombocytosis was observed in 161 patients (3.7%). Patients with thrombocytosis were more likely to be younger (p = 0.018), to present with T2 and T3 stages (chi-square: 8.54, p = 0.014), N1 status (chi-square: 4.47, p = 0.035), G3 grading

(chi-square: 7.96, p = 0.005) and to be premenopausal (chi-square: 5.10, p = 0.024) as compared to women with normal-range platelet counts. Detailed data are shown in Table 2. With respect to the trial therapy arms, no significant difference was identified in distribution between subjects with or without pretreatment thrombocytosis (data not shown).

Survival analysis

At a median follow-up of 52.0 months (95% confidence interval, 49.3 – 54.4 months), 367 (8.5%) patients had died. A higher proportion of subjects presenting with pretreatment thrombocytosis (16.8%) died as compared to those without thrombocytosis (8.2%). Breast cancer-related deaths occurred in 327 (7.6%) women, 14.9% in patients with as compared to 7.3% in patients without pretreatment thrombocytosis. Recurrence

Figure 1: Overall survival with and without thrombocytosis. Kaplan-Meier estimates and significance levels in patients with and without thrombocytosis concerning overall survival were shown in figure 1.
– – – with thrombocytosis, —— without thrombocytosis.
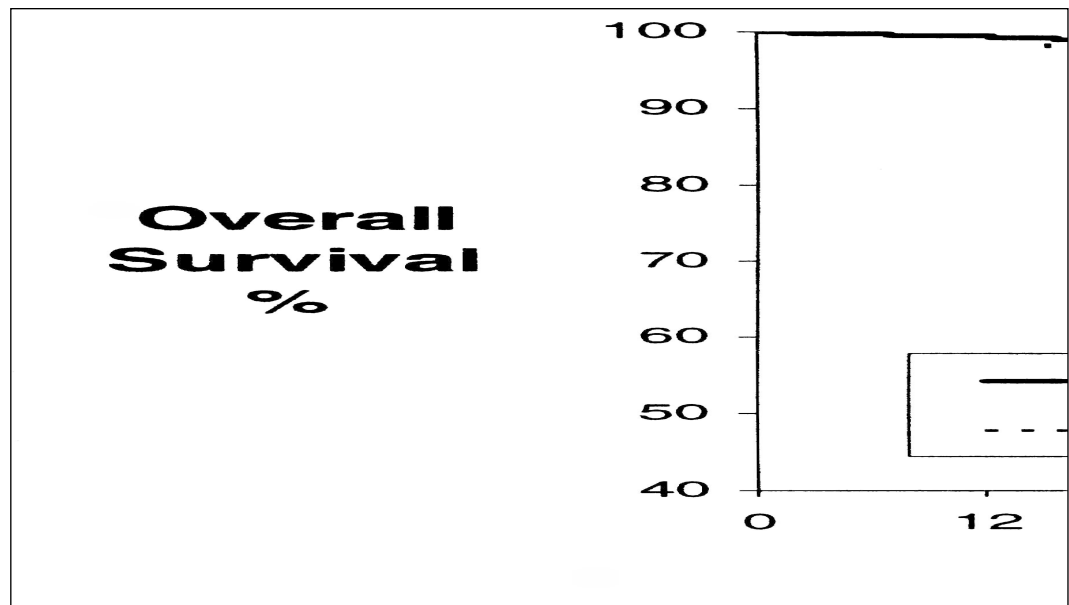
Figure 2: Breast cancer-related survival with and without thrombocytosis. Kaplan-Meier estimates and significance levels in breast cancer patients with and without thrombocytosis concerning breast cancer-related survival were shown in figure 2.
– – – with thrombocytosis, —— without thrombocytosis.
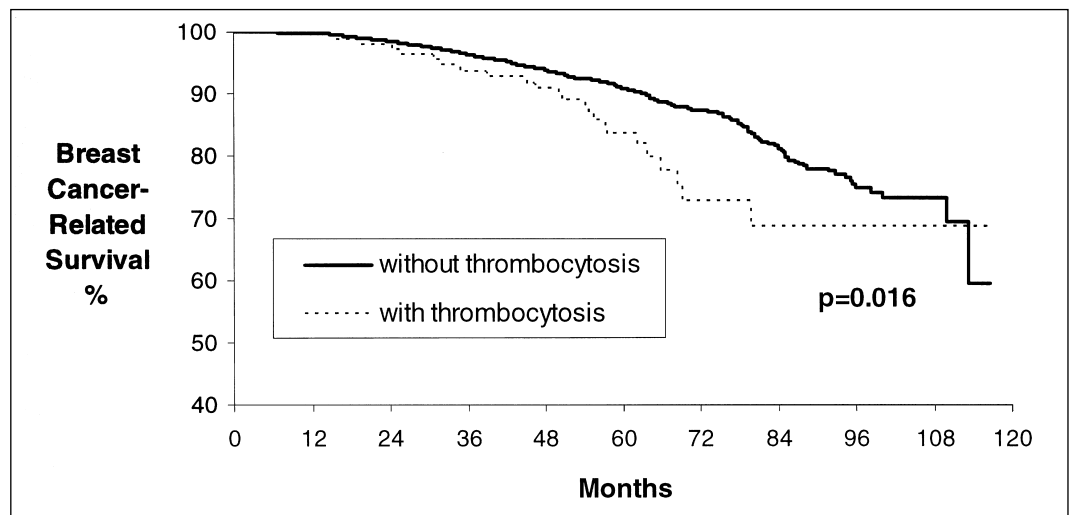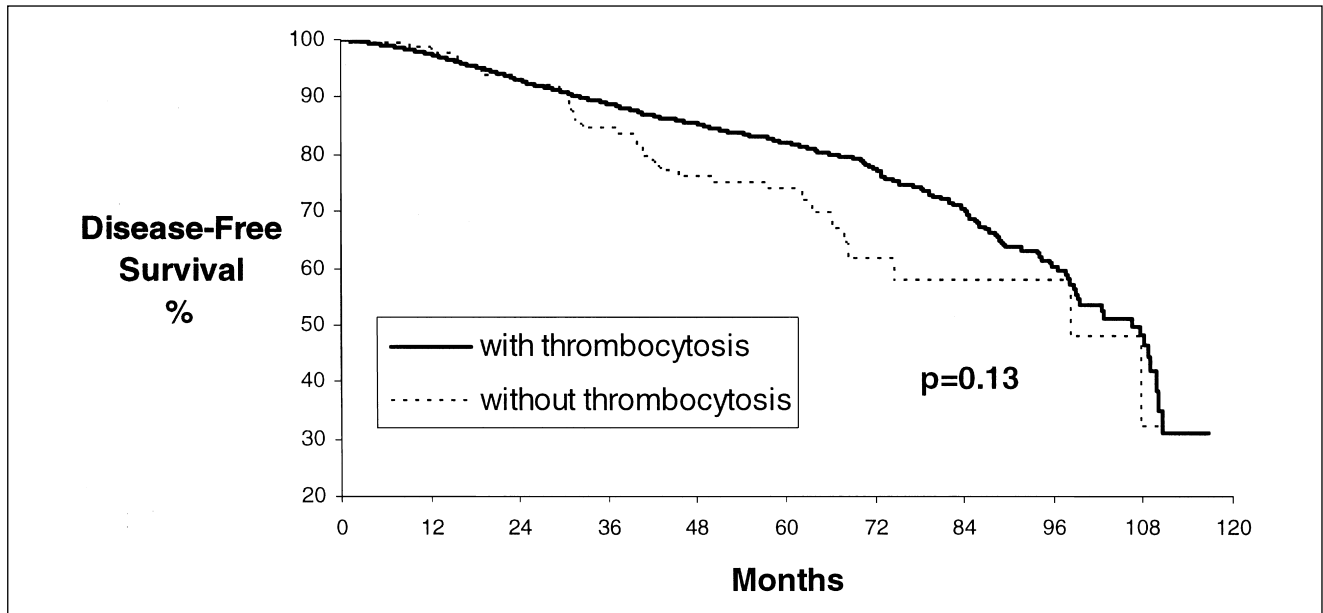
Figure 3: Disease-free survival in patients with and without thrombocytosis
Kaplan-Meier estimates and significance levels in breast cancer patients with and without thrombocytosis concerning disease-free survival were shown in figure 3.
− − − with thrombocytosis, —— without thrombocytosis

Characteristic	Overall survival	Breast cancer-related survival	Disease-free survival
Tumor stage	2.30 (1.96 – 2.72)	2.40 (2.02 – 2.84)	2.26 (2.00 – 2.56)
P value	0.0001	0.0001	0.0001
Nodal status	2.79 (2.25 – 3.47)	3.15 (2.49 – 3.98)	2.57 (2.19 – 3.00)
P value	0.0001	0.0001	0.0001
Grading	1.61 (1.30 – 2.00)	1.75 (1.40 – 2.19)	1.79 (1.52 – 2.10)
P value	0.0001	0.0001	0.0001
Age	1.00 (0.99 – 1.01)	0.99 (0.99 – 1.00)	0.98 (0.97 – 0.98)
P value	0.5891	0.1993	0.0001
Menopausal status	0.90 (0.72 – 1.11)	0.81 (0.65 – 1.02)	0.66 (0.56 – 0.77)
P value	0.3188	0.0736	0.0001
Estrogen receptor status	0.71 (0.60 – 0.85)	0.71 (0.59 – 0.85)	0.73 (0.64 – 0.83)
P value	0.0001	0.0002	0.0001
Progesterone receptor status	0.72 (0.62 – 0.84)	0.72 (0.62 – 0.84)	0.68 (0.61 – 0.76)
P value	0.0001	0.0001	0.0001
Thrombocytosis	1.73 (1.17 – 2.57)	1.72 (1.14 – 2.61)	1.47 (1.06 – 2.03)
P value	0.0054	0.0095	0.0199

Table 3: Risk ratios concerning survival (univariate Cox regression model) Univariate Cox regression model revealed tumor stage, nodal status, grading, age, menopausal status, ER status, PgR status and pretreatment thrombocytosis as significant factors influencing OS, breast cancer-related survival and DFS.

Table 4: Results of multivariate analysis concerning survival (Cox model). Multivariate analysis of survival showed pretreatment thrombocytosis to have a significant predictive value for OS (risk ratio: 1.73, p = 0.0064) and breast cancer-related survival (risk ratio: 1.67, p = 0.0162), but not for DFS (risk ratio 1.29, p = 0.1355). Detailed data including risk ratios, levels of significance and 95% confidence intervals are listed in Table 4. *since age and menopausal status interact, these covariates were tested in combination

Characteristic	Overall survival	Breast cancer-related survival	Disease-free survival
Tumor stage	1.96 (1.66 – 2.33)	1.98 (1.66 – 2.37)	1.92 (1.69 – 2.19)
P value	0.0001	0.0001	0.0001
Nodal status	2.33 (1.86 – 2.93)	2.57 (2.01 – 3.30)	2.10 (1.78 – 2.48)
P value	0.0001	0.0001	0.0001
Grading	1.11 (0.88 – 1.39)	1.19 (0.94 – 1.51)	1.25 (1.05 – 1.48)
P value	0.3756	0.1494	0.0103
Age*	1.02 (1.01 – 1.04)	1.01 (0.99 – 1.02)	0.98 (0.97 – 0.99)
P value	0.0100	0.6791	0.0001
Menopausal status*	0.72 (0.50 – 1.04)	0.88 (0.60 – 1.29)	1.10 (0.85 – 1.43)
P value	0.0100	0.6791	0.0001
Estrogen receptor status	0.73 (0.60 – 0.88)	0.75 (0.61 – 0.91)	0.90 (0.78 – 1.04)
P value	0.0009	0.0043	0.1460
Progesterone receptor status	0.81 (0.69 – 0.95)	0.80 (0.67 – 0.95)	0.70 (0.62 – 0.80)
P value	0.0098	0.0096	0.0001
Thrombocytosis	1.73 (1.17 – 2.57)	1.67 (1.10 – 2.54)	1.29 (0.93 – 1.79)
P value	0.0064	0.0162	0.1355

*since age and menopausal status interact, these covariates were tested in combination

occurred in 658 (15.3%) of all subjects, 24.2% in patients with as compared to 15.0% in those without pretreatment thrombocytosis.

The estimated median OS time was 71.0 months (with thrombocytosis) versus 99.5 months (without thrombocytosis), the estimated median breast cancer-related survival was 72.0 months (with thrombocytosis) versus 100.9 months (without thrombocytosis), and the estimated median DFS was 80.4 months (with thrombocytosis) versus 88.4 months (without thrombocytosis). Kaplan-Meier estimates and significance levels for both groups concerning OS, breast cancer-related survival and DFS are shown in Figures 1-3.

Univariate analysis revealed tumor stage, nodal status, grading, ER status, PgR status and pretreatment thrombocytosis as significant factors influencing OS, breast cancer-related survival and DFS (Table 3). Remarkably thrombocytosis was associated with a risk ratio similar to tumor grading (approximately 1.7).

Multivariate analysis of survival showed pretreatment thrombocytosis to have a significant predictive value for OS (risk ratio: 1.73, p = 0.0064) and breast cancer-related survival (risk ratio: 1.67, p = 0.0162), but not for DFS (risk ratio 1.29,

p = 0.1355). Detailed data including risk ratios, levels of significance and 95% confidence intervals are listed in Table 4.

Discussion

Thrombocytosis is a finding associated either with essential thrombocytosis or chronic myeloproliferative syndromes (termed primary), or more frequently with trauma, acute and chronic infections, iron deficiency anemia, surgical interventions with or without splenectomy and in 6 - 62.5% of cancer patients (termed secondary) (32-40).

The present retrospective analysis is the first to assess the influence of pretreatment thrombocytosis on prognosis in women with stage I, II and IIIA breast disease. To our knowledge, no data on pretreatment thrombocytosis have been reported in patients with breast cancer. We found a rather low incidence of thrombocytosis in our study population (3.7%). Percentages of pretreatment thrombocytosis in other cancer entities are reported at 10-39% in cervical cancer (15, 17, 19, 34), 24-63% in ovarian cancer (20, 35, 36), 15-27% in vulvar cancer (23, 24), 25-32% in lung cancer (13, 37, 38), 27% in malignant peritoneal mesothelioma (39), 48% in malignant

pleural mesothelioma (40), 33-57% in renal cancer (25, 26), and finally at 56% in patients with a malignant pelvic mass (16).

This retrospective analysis clearly demonstrates the independent negative prognostic value of pretreatment thrombocytosis for survival in a large series of breast cancer patients. The effect was seen in multivariate analysis of both OS and breast cancer-related survival. With regard to DFS, multivariate analysis failed to identify pretreatment thrombocytosis as prognostic factor. One may ask why such a simple question was not answered earlier. We argue that a very large patient cohort is required to observe a significant effect in a factor that is only present in a very small percentage of patients. The present retrospective analysis does not discriminate between primary and secondary thrombocytosis. There is some possibility that primary breast cancer, in a small number of patients, is coincidentally associated with primary thrombocytosis. This is as likely as a small minority of cases in which breast cancer-inducing thrombocytosis is seen as a secondary effect. One path of thrombocytosis induction is the production of thrombopoietin.

Thrombopoietin stimulates platelet production (41-43) and seems to be regulated by a positive feedback from platelet alpha granular contents released during activation (44). However, the role of thrombopoietin in reactive thrombocytosis is discussed controversially (45, 46). In patients with advanced carcinoma associated with thrombocytosis, blood thrombopoietin levels were observed to be high, possibly caused, at least in part, by carcinoma-cell thrombopoietin (47). Furthermore, thrombopoietin concentrations are not strictly inversely related to platelet counts, arguing for the existence of additional mechanisms of thrombopoietin regulation (48). One possible candidate is interleukin 6, since blood levels are reported to be high in malignant disease (36, 49, 50) and interleukin 6 is produced by various non-hematological cancer entities (51-55). Schuler et al. reported profound regulatory effects of recombinant human interleukin 6 on hematopoiesis and inflammatory response (56). Interleukin 6, thrombopoietin and/or their interaction thus appear to be involved in cancer-associated thrombocytosis, and thrombocytosis may secondarily reflect the challenge of hemostasis by malignant cells.

The question remains unresolved as to why thrombocytosis is an independent prognostic factor in breast cancer patients, regardless of whether it is a primary or secondary phenomenon. It cannot be ruled out that this phenomenon is simply mechanistic. Sticky platelets bind circulating tumor cells by entrapment, leading to the origin of metastasis. Other possible explanations are the production of growth factors derived from platelets that result in enhanced proliferation of tumor cells.

Platelets are known to contain platelet-derived endothelial cell growth factor (PD-ECGF), and there is evidence for vascular endothelial growth factor (VEGF) storage in platelets (57-59). The clinical significance arising from the angiogenic effects of VEGF and PD-ECGF in breast cancer has been reviewed by Locopo et al (60). Platelets of cancer patients show VEGF values that are some three times as high as those present in healthy controls, whereas very little or no VEGF was found in the plasma (61). In breast cancer patients, serum VEGF concentrations correlate with platelet counts during chemotherapy (57). Platelets seem to prevent circulating VEGF from inducing the development of new blood vessels except at sites where coagulation takes place. Apart from its thrombopoietic effect, interleukin 6 also seems to affect the amount of VEGF stored in the platelets. Interaction of interleukin 6 with the angiogenic pathways in cancer might explain the stimulation of tumor growth occasionally observed in the course of interleukin 6 administration. Such interaction conforms to the worst outcome associated with high interleukin 6 levels and with thrombocytosis in several tumor types (62).

Taken together, we suggest that different compounds produced in tumor cells are challenging hemostasis and lead to thrombocytosis. High concentrations of VEGF are stored in the platelets of cancer patients, and VEGF levels correlate with platelet counts. VEGF is released at the site of metastasis during tumor cell-induced platelet aggregation. Furthermore, PD-ECGF is released during platelet activation, resulting in a locally high angiogenic environment.

In conclusion, our retrospective study describes pretreatment thrombocytosis as an independent risk factor for survival in women with breast cancer. Given, that platelets contribute to the pathophysiology of hematogenous metastasis, the underlying mechanism needs to be elucidated, preferably in a prospective study design.

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Appendix

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References

1. Blann AD, Lip GY. Virchow's triad revisited: the importance of soluble coagulation factors, the endothelium, and platelets. Thromb Res 2001; 101: 321-327.

2. Hejna M, Raderer M, Zielinski CC. Inhibition of metastases by anticoagulants. J Natl Cancer Inst 1999; 91: 22-36.

3. Honn KV, Tang DG, Chen YQ. Platelets and cancer metastasis: more than an epiphenomenon. Semin Thromb Hemost 1992; 18: 392-415.

4. Gasic GJ, Gasic TB, Stewart CC. Antimetastatic effects associated with platelet reduction. Proc Natl Acad Sci U S A 1968; 61: 46-52.

5. Gasic GJ. Role of plasma, platelets, and endothelial cells in tumor metastasis. Cancer Metastasis Rev 1984; 3: 99-114.

6. Karpatkin S, Pearlstein E. Role of platelets in tumor cell metastases. Ann Intern Med 1981; 95: 636-41.

7. Pearlstein E, Ambrogio C, Karpatkin S. Effect of antiplatelet antibody on the development of pulmonary metastases following injection of CT26 colon adenocarcinoma, Lewis lung carcinoma, and B16 amelanotic melanoma tumor cells into mice. Cancer Res 1984; 44: 3884-7.

8. Pinedo HM, Verheul HM, D'Amato RJ, Folkman J. Involvement of platelets in tumour angiogenesis? Lancet 1998; 352: 1775-7.

9. Herndon JE, Green MR, Chahinian AP, Corson JM, Suzuki Y, Vogelzang NJ. Factors predictive of survival among 337 patients with mesothelioma treated between 1984 and 1994 by the Cancer and Leukemia Group B. Chest 1998; 113: 723-31.

10. Levin J, Conley C. Thrombocytosis associated with malignant disease. Arch Intern Med 1964; 114: 497-500.

11. Silvis SE, Turkbas N, Doscherholmen A. Thrombocytosis in patients with lung cancer. Jama 1970; 211: 1852-3.

12. Engan T, Hannisdal E. Blood analyses as prognostic factors in primary lung cancer. Acta Oncol 1990; 29: 151-4.

13. Pedersen LM, Milman N. Prognostic significance of thrombocytosis in patients with primary lung cancer. Eur Respir J 1996; 9: 1826-30.

14. Costantini V, Zacharski LR, Moritz TE, Edwards RL. The platelet count in carcinoma of the lung and colon. Thromb Haemost 1990; 64: 501-5.

15. Hernandez E, Lavine M, Dunton CJ, Gracely E, Parker J. Poor prognosis associated with thrombocytosis in patients with cervical cancer. Cancer 1992; 69: 2975-7.

16. Chalas E, Welshinger M, Engellener W, Chumas J, Barbieri R, Mann WJ. The clinical significance of thrombocytosis in women presenting with a pelvic mass. Am J Obstet Gynecol 1992; 166: 974-7.

17. Lopes A, Daras V, Cross PA, Robertson G, Beynon G, Monaghan JM. Thrombocytosis as a prognostic factor in women with cervical cancer. Cancer 1994; 74: 90-2.

18. Nathan FE, Hernandez E, Dunton CJ, Treat J, Switalska HI, Joseph RR, Tuszynski GP. Plasma thrombospondin levels in patients with gynecologic malignancies. Cancer 1994; 73: 2853-8.

19. Rodriguez GC, Clarke-Pearson DL, Soper JT, Berchuck A, Synan I, Dodge RK. The negative prognostic implications of thrombocytosis in women with stage IB cervical cancer. Obstet Gynecol 1994; 83: 445-8.

20. Menczer J, Schejter E, Geva D, Ginath S, Zakut H. Ovarian carcinoma associated thrombocytosis. Correlation with prognostic factors and with survival. Eur J Gynaecol Oncol 1998; 19: 82-4.

21. Kerpsack JT, Finan MA. Thrombocytosis as a predictor of malignancy in women with a pelvic mass. J Reprod Med 2000; 45: 929-32.

22. Scholz HS, Petru E, Gucer F, Haas J, Tamussino K, Winter R. Preoperative thrombocytosis is an independent prognostic factor in stage III and IV endometrial cancer. Anticancer Res 2000; 20: 3983-5.

23. Lavie O, Comerci G, Daras V, Bolger BS, Lopes A, Monaghan JM. Thrombocytosis in women with vulvar carcinoma. Gynecol Oncol 1999; 72: 82-6.

24. Hefler L, Mayerhofer K, Leibman B, Obermair A, Reinthaller A, Kainz C, Tempfer C. Tumor anemia and thrombocytosis in patients with vulvar cancer. Tumour Biol 2000; 21: 309-14.

25. Symbas NP, Townsend MF, El-Galley R, Keane TE, Graham SD, Petros JA. Poor prognosis associated with thrombocytosis in patients with renal cell carcinoma. BJU Int 2000; 86: 203-7.

26. O'Byrne KJ, Dobbs N, Propper D, Smith K, Harris AL. Vascular endothelial growth factor platelet counts, and prognosis in renal cancer. Lancet 1999; 353: 1494-5.

27. Monreal M, Fernandez-Llamazares J, Pinol M, et al.. Platelet count and survival in patients with colorectal cancer—a preliminary study. Thromb Haemost 1998; 79: 916-8.

28. Jakesz R, Hausmaninger H, Kubista E, et al.. Randomized adjuvant trial of tamoxifen and goserelin versus cyclophosphamide, methotrexate, and Fluorouracil: evidence for the superiority of treatment with endocrine blockade in premenopausal patients with hormone-responsive breast cancer-austrian breast and colorectal cancer study group trial 5. J Clin Oncol 2002; 20: 4621-7.

29. Schmid M, Jakesz R, Samonigg H, et al. Randomized trial of tamoxifen versus tamoxifen plus aminoglutethimide as adjusted treatment in postmenopausal breast cancer patients with hormone receptor positive disease: Austrian Breast and Colorectal Cancer Study Group Trial 6. J Clin Oncol 2003; 21: 924-90.

30. Jakesz R. Comparison of pre- vs postoperative chemotherapy in breast cancer patients: four-year results of Austrian Breast and Colorectal Cancer Study Group (ABCSG) Trial 7. Proc Am Soc Clin Oncol; 2001; 32a, abstract 125.

31. Reiner A, Neumeister B, Spona J, Reiner G, Schemper M, Jakesz R. Immunocytochemical localization of estrogen and progesterone receptor and prognosis in human primary breast cancer. Cancer Res 1990; 50: 7057-61.

32. Santhosh-Kumar CR, Yohannan MD, Higgy KE, al-Mashhadani SA. Thrombocytosis in adults: analysis of 777 patients. J Intern Med 1991; 229: 493-5.

33. Griesshammer M, Bangerter M, Sauer T, Wennauer R, Bergmann L, Heimpel H. Aetiology and clinical significance of thrombocytosis: analysis of 732 patients with an elevated platelet count. J Intern Med 1999; 245: 295-300.

34. Hernandez E, Heller PB, Whitney C, Diana K, Delgado G. Thrombocytosis in surgically treated stage IB squamous cell cervical carcinoma (A Gynecologic Oncology Group study). Gynecol Oncol 1994; 55: 328-32.

35. Zeimet AG, Marth C, Muller-Holzner E, Daxenbichler G, Dapunt O. Significance of thrombocytosis in patients with epithelial ovarian cancer. Am J Obstet Gynecol 1994; 170: 549-54.

36. Gastl G, Plante M, Finstad CL, Wong GY, Federici MG, Bander NH, Rubin SC. High IL-6 levels in ascitic fluid correlate with reactive thrombocytosis in patients with epithelial ovarian cancer. Br J Haematol 1993; 83: 433-41.

37. Ascensao JL, Oken MM, Ewing SL, Goldberg RJ, Kaplan ME. Leukocytosis and large cell lung cancer. A frequent association. Cancer 1987; 60: 903-5.

38. Olesen LL, Thorshauge H. Thrombocytosis in patients with malignant pleural mesothelioma. Cancer 1988; 62: 1194-6.

39. Eltabbakh GH, Piver MS, Hempling RE, Recio FO, Intengen ME. Clinical picture, response to therapy, and survival of women with diffuse malignant peritoneal mesothelioma. J Surg Oncol 1999; 70: 6-12.

40. Nakano T, Chahinian AP, Shinjo M, Tonomura A, Miyake M, Togawa N, Ninomiya K, Higashino K. Interleukin 6 and its relationship to clinical parameters in patients with malignant pleural mesothelioma. Br J Cancer 1998; 77: 907-12.

41. Alexander WS. Thrombopoietin. Growth Factors 1999; 17: 13-24.

42. Caen JP, Han ZC, Bellucci S, Alemany M. Regulation of megakaryocytopoiesis. Haemostasis 1999; 29: 27-40.

43. George JN. Platelets. Lancet 2000; 355: 1531-9.

44. Sungaran R, Chisholm OT, Markovic B, Khachigian LM, Tanaka Y, Chong BH. The role of platelet alpha-granular proteins in the regulation of thrombopoietin messenger RNA expression in human bone marrow stromal cells. Blood 2000; 95: 3094-101.

45. Wang JC, Chen C, Novetsky AD, Lichter SM, Ahmed F, Friedberg NM. Blood thrombopoietin levels in clonal thrombocytosis and reactive thrombocytosis. Am J Med 1998; 104: 451-5.

46. Hsu HC, Tsai WH, Jiang ML, Ho CH, Hsu ML, Ho CK, Wang SY. Circulating levels of thrombopoietic and inflammatory cytokines in patients with clonal and reactive thrombocytosis. J Lab Clin Med 1999; 134: 392-7.

47. Sasaki Y, Takahashi T, Miyazaki H, Matsumoto A, Kato T, Nakamura K, Iho S, Okuno Y, Nakao K. Production of thrombopoietin by human carcinomas and its novel isoforms. Blood 1999; 94: 1952-60.

48. Uppenkamp M, Makarova E, Petrasch S, Brittinger G. Thrombopoietin serum concentration in patients with reactive and myeloproliferative thrombocytosis. Ann Hematol 1998; 77: 217-23.

49. Estrov Z, Talpaz M, Mavligit G, Pazdur R, Harris D, Greenberg SM, Kurzrock R. Elevated plasma thrombopoietic activity in patients with metastatic cancer-related thrombocytosis. Am J Med 1995; 98: 551-8.

50. Blay JY, Rossi JF, Wijdenes J, Menetrier-Caux C, Schemann S, Negrier S, Philip T, Favrot M. Role of interleukin-6 in the paraneoplastic inflammatory syndrome associated with renal-cell carcinoma. Int J Cancer 1997; 72: 424-30.

51. Rawle FC, Shields J, Smith SH, Iliescu V, Merkenschlager M, Beverley PC, Callard RE. B cell growth and differentiation induced by supernatants of transformed epithelial cell lines. Eur J Immunol 1986; 16: 1017-9.

52. Watson JM, Sensintaffar JL, Berek JS, Martinez-Maza O. Constitutive production of interleukin 6 by ovarian cancer cell lines and by primary ovarian tumor cultures. Cancer Res 1990; 50: 6959-65.

53. Van Meir E, Sawamura Y, Diserens AC, Hamou MF, de Tribolet N. Human glioblastoma cells release interleukin 6 in vivo and in vitro. Cancer Res 1990; 50: 6683-8.

54. Yanagawa H, Sone S, Takahashi Y, Haku T, Yano S, Shinohara T, Ogura T. Serum levels of interleukin 6 in patients with lung cancer. Br J Cancer 1995; 71: 1095-8.

55. Schmitter D, Lauber B, Fagg B, Stahel RA. Hematopoietic growth factors secreted by seven human pleural mesothelioma cell lines: interleukin-6 production as a common feature. Int J Cancer 1992; 51: 296-301.

56. Schuler M, Bruntsch U, Spath-Schwalbe E, et al.. Lack of efficacy of recombinant human interleukin-6 in patients with advanced renal cell cancer: results of a phase II study. Eur J Cancer 1998; 34: 754-6.

57. Verheul HM, Hoekman K, Luykx-de Bakker S, et al.. Platelet: transporter of vascular endothelial growth factor. Clin Cancer Res 1997; 3: 2187-90.

58. Banks RE, Forbes MA, Kinsey SE, Stanley A, Ingham E, Walters C, Selby PJ. Release of the angiogenic cytokine vascular endothelial growth factor (VEGF) from platelets: significance for VEGF measurements and cancer biology. Br J Cancer 1998; 77: 956-64.

59. Wartiovaara U, Salven P, Mikkola H, et al.. Peripheral blood platelets express VEGF-C and VEGF which are released during platelet activation. Thromb Haemost 1998; 80: 171-5.

60. Locopo N, Fanelli M, Gasparini G. Clinical significance of angiogenic factors in breast cancer. Breast Cancer Res Treat 1998; 52: 159-73.

61. Salven P, Orpana A, Joensuu H. Leukocytes and platelets of patients with cancer contain high levels of vascular endothelial growth factor. Clin Cancer Res 1999; 5: 487-91.

62. Salgado R, Vermeulen PB, Benoy I, et al.. Platelet number and interleukin-6 correlate with VEGF but not with bFGF serum levels of advanced cancer patients. Br J Cancer 1999; 80: 892-7.