NOWOTWORY Journal of Oncology • 2006 • volume 56
Number 3 • 249–253

Original papers

Expression of selected coagulation factors in breast cancer within the primary tumor and axillary node metastases

Marek Z. Wojtukiewicz[1], Zbigniew Sawicki[1], Ewa Sierko[1], Lech Zimnoch[2]

Introduction. Breast cancer is the most frequent cause of morbidity and mortality due to malignancy in women in Poland. Thromboembolic episodes are common complication of the disease. The aim of this study was to evaluate the expression of selected coagulation factors in loco within the primary tumor and lymph node metastases in patients with breast cancer.
Material and methods. Breast cancer specimens obtained during surgery were examined. Immunohistochemical procedure according to the ABC method was employed.
Results. The expression of tissue factor (TF), prothrombin fragment 1+2 and fibrin was demonstrated in both primary tumors and lymph node metastases.
Conclusions. The results indicate that there exists local activation of blood coagulation in the primary tumor and metastatic lesions of breast cancer and suggest that drugs interfering with the hemostatic system may by of value in the therapy of breast cancer patients.

Key words: breast cancer, blood coagulation

Breast cancer is the most common cause of malignancy among women in Poland. It accounts for 20% of overall cancer morbidity and for some 15% of cancer deaths. Treatment of breast cancer patients is a multidisciplinary and long-lasting process. The choice of the treatment method depends, apart from other factors, upon the stage of clinical advancement. Unfortunately, a significant number of patients present with disseminated disease at the time of diagnosis, and therefore cannot be offered radical treatment. What is more, the absence of distant metastases at the time of diagnosis does not guarantee therapeutic success; a number of such patients develop recurrent disease despite combined antineoplastic treatment.

Neoplastic dissemination is possible only after the cells within the primary tumor undergo phenotype alteration, which in turn permits local invasion and separation from main tumor mass, penetration of the vascular basal membrane, transportation and survival within the vessels and, finally, proliferation at a distant site. In the interactions between the cancer cell and its surrounding environment the hemostatic system play an important role. The effects of coagulation activation include both clinically evident thromboembolic complications, such as deep vein thrombosis, *thrombophlebitis migrans*, nonbacterial thrombotic endocarditis, cerebral arterial thrombosis and thrombosis of digital arteries, but also disorders which may be only found in laboratory analyses, such as increased levels of the thrombin-antithrombin III complex, fibrinogen, fibrinogen degradation products, increased factor XIII activity and decreased protein C activity [1-4].

Coagulopathies may be initiated by cancer cells, iatrogenic [5-7] or may arise from other pathologies, such as obesity, immobilization or inflammation [8]. Thromboembolic complications appear in all stages of cancer disease. They may proceed cancer diagnosis by a number of years [9], appear during the clinically evident stages [10] and may even be the direct cause of cancer death [11]. The coagulation activation takes place not only within the blood vessels, but also in the extravascular compartment of the tumor [2, 12].

The concept of monoclonal development of cancer suggests that the malignant tumor is in fact a single clone derived from a primary cell. However, a number of clinical observations have failed to support this theory. Cancer cells may vary as to their phenotype both within one tumor mass and between different cancer foci. The cells are often heterogeneous in regard to their morphology [13]. This, in turn, causes the cells to differ as to their antigens, metabolism and proliferation even within the same tumor [14]. Cancer cells within both the primary site and metastatic foci in the axillary lymph nodes have been found to have similar expressions of receptors for EGFR [15] and c-erbB2 [15, 16] as well as protein p53

[1] Department of Oncology
[2] Department of Medical Pathomorphology
Medical University in Białystok, Poland

[15, 16], Ki-67 [16] and S-phase cell ratio [17]. In turn, significant quantitative differences have been observed regarding the expression of hormone receptors [18, 19]. An analysis of 54 cases of bilateral metachronous breast cancers has shown, that the expression of the p53 protein and HER2 is comparable, but the cancer cells differ as to the degree of hormone receptor expression [20].

Until today the expression of hemostatic proteins has been analyzed within a number of primary malignant tumors [21-26], but there is no data concerning the expression of coagulation factors within metastatic breast cancer lesions. A comparison between the expression of hemostatic proteins in the metastatic lesion in relation to the primary tumor may help to determine the role of hemostatic system in the development of breast cancer.

Aim

The aim of the study was to evaluate the expression of selected coagulation system proteins (tissue factor – TF; prothrombin fragment 1+ 2 – F1+2; fibrin) within primary breast cancer and axillary lymph node metastases.

Material and methods

The study was performed on invasive ductal adenocarcinoma tissue obtained from the primary site and from axillary node metastases of 10 patients undergoing surgery for breast cancer. Tissue specimens were preserved in buffered formaldehyde and then embedded in paraffin of low melting temperature. The cancer specimens underwent immunohistochemical staining according to ABC method using *Vectastatin Kits* (Vector Laboratories, Burlingame, CA, USA) [27]. The method bases upon the formation of avidin-biotin complexes with diaminobenzidine as a substrate. Protein expression had been assessed with the aid of the following, unique and highly specific antibodies:

- antibody T2G1 reacting with fibrin II. We used a mixture of two murine monoclonal antibodies: intact and $F(ab')_2$ provided by Doctor Bohdan Kudryk of the Lidsay F. Kimball Research Blood Centre, New York, NY, USA;
- rabbit policlonal antibody against human recombined tissue factor provided by Professor Walter Kisiel of the Department of Pathology of the University of New Mexico School of Medicine in Albuquerque, NM, USA;
- rabbit policlonal antibody against prothrombin fragment 1+2 (F1+2) provided by Professor Walter Kisiel of the Department of Pathology of the University of New Mexico School of Medicine in Albuquerque, NM, USA.

The examined antigens were visualized as a brown reaction product.

Results

TF

A strong cancer-cell associated TF expression was found both within the primary site of the breast and within the sites of the axillary lymph node metastases. TF antigens were also found on small blood vessel walls and macrophages – both within the primary tumor and within the metastases (Figures 1, 2).

Prothrombin fragment 1+2 (F1+2)

Non-uniform, intensive antigen expression of the F1+2 was found within the cancer cells of the primary tumor and in the axillary lymph node metastases. The expression of this particular peptide was non-uniform, i.e. not all the cancer cells were found to show the presence of F1+2, The presence of F1+2 was also discerned within macrophages and small vessel walls –

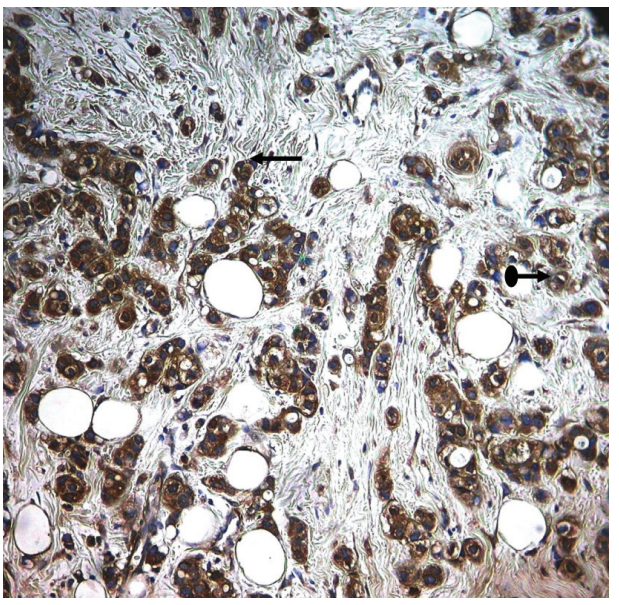

Figure 1. Breast cancer – primary site. Staining of breast cancer tumor cells (⟶) and of small blood vessels wall (⟶) for tissue factor. Magnification about 200x

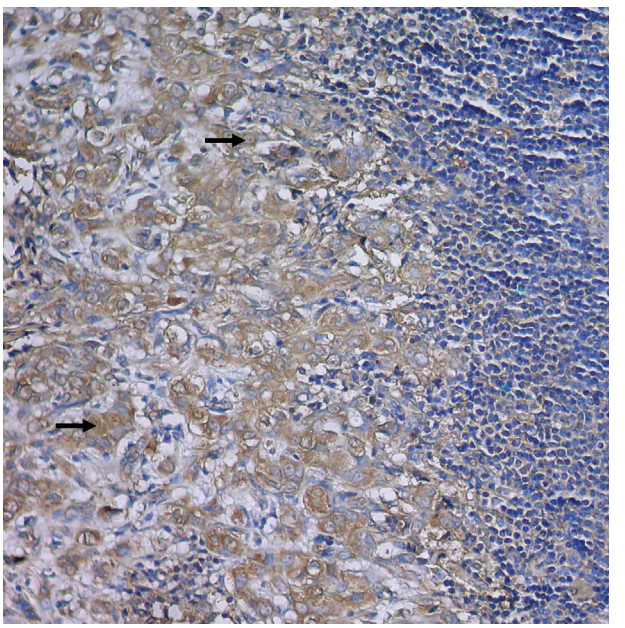

Figure 2. Breast cancer – axillary lymph node. Staining of breast cancer tumor cells for tissue factor (⟶). Magnification about 200x

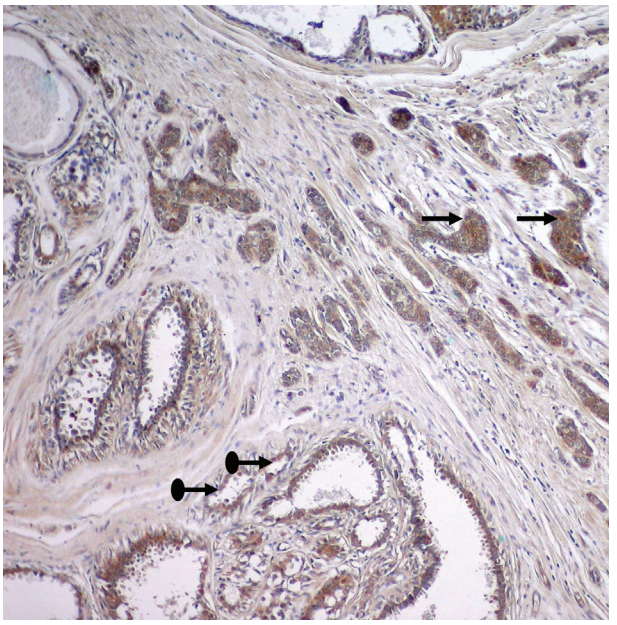

Figure 3. Breast cancer – primary site. Staining of breast cancer tumor cells (→) and of small blood vessels wall (●→) for prothrombin fragment 1 + 2. Magnification about 200x

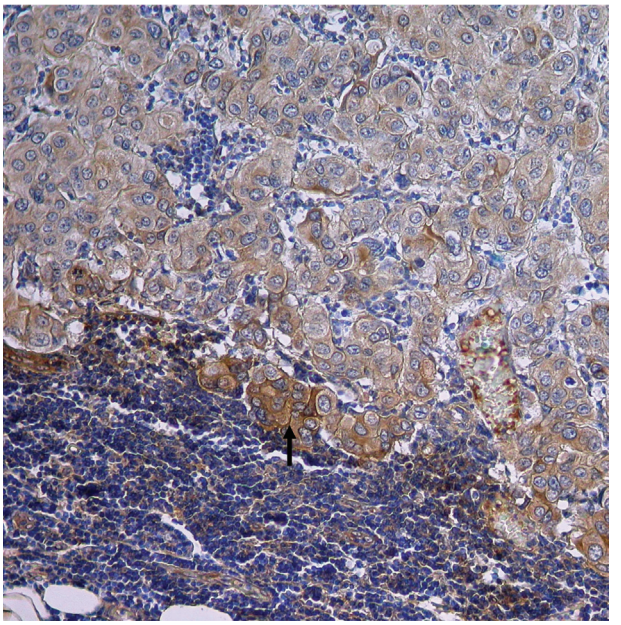

Figure 4. Breast cancer – axillary lymph node. Staining of breast cancer tumor cells (→) for prothrombin fragment 1 + 2. Magnification about 200x

both within the primary tumor and within the metastases (Figures 3, 4).

Fibrin

A strong immunohistochemical staining for fibrin was observed in association with cancer cells within the primary tumor, while its expression was significantly weaker in cancer cells localized within the axillary lymph node metastases. In some cases the fibrin expression was detected in all cancer cells, while in others it was non-uniform and was observed only in some of the cells (Figures 5, 6)

Discussion

As far back as in the year 1865 Armand Troussau had described increased incidence of thrombosis in patients with cancer of the digestive tract. At present, it is well known that hemostatic abnormalities are a common complication of malignancy. It has also been observed that occurrence of venous thromboembolic episodes in

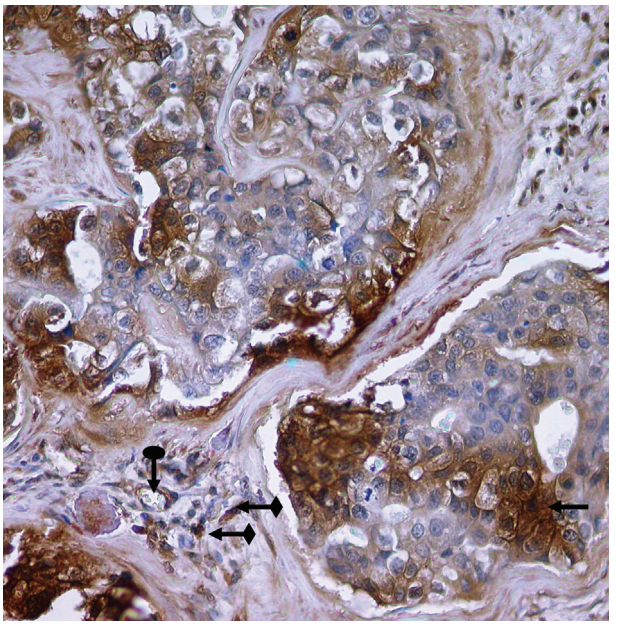

Figure 5. Breast cancer – primary site. Staining of breast cancer tumor cells (→), macrophages (●→) and of small blood vessels wall (◆→) for fibrin. Magnification about 200x

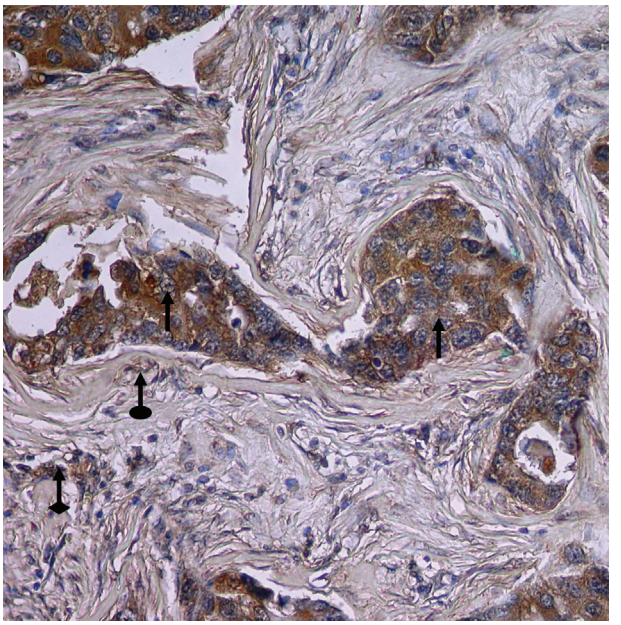

Figure 6. Breast cancer – axillary lymph node. Staining of breast cancer tumor cells (→), macrophages (◆→) and of small blood vessels wall (●→) for fibrin. Magnification about 200x

the course of adjuvant chemotherapy of breast cancer patients is a negative prognostic risk factor [28]. It has to be noted that the activation of blood coagulation is not just an epiphenomenon of malignancy, but is an integral part of pathomechanism of primary tumor growth and metastatic spread [29, 30]. The main procoagulant responsible for the activation of blood coagulation in cancer patients is tissue factor (TF) [31]. Under physiological conditions TF does not contact with blood. However, during the vessel wall disruption and due to the activity of such factors as TNF, C5a, endotoxins, microbial mucopolysaccharides, FGF, PDGF and VEGF, TF expression is induced and, in consequence, the blood coagulation cascade becomes activated [32].

Increased TF expression has been discerned within the cancer cells of a number of primary malignant tumors, e.g. in pancreatic cancer [22, 25], gastric cancer [21], laryngeal cancer [23, 25], ovarian cancer [25], lung cancer [24, 25] and breast cancer [26, 33]. A number of experimental studies (on animal models) have shown that the degree of TF expression within the cancer cells correlates with their ability to form distant metastases [29]. *In vitro* studies have shown that the presence of TF antigens on cancer cells may be a predictive factor – doxorubicin resistance is more common in those cases where cancer cells do not express TF [34].

It appears that the presence of TF within the cancer cells may also have some prognostic value. Breast cancer patients, in whom cancer cells within the primary tumor were found to be TF positive, achieved shorter overall survival than patients without TF expression in cancer cells [26]. It has been also noted that the risk of developing liver metastases is greater in patients with TF expression within the breast cancer cells [26].

It is, therefore, likely that TF may play a role in the natural course of breast cancer. Tissue factor may influence the progression of cancer in two different ways – directly, through increasing intracellular signaling, and indirectly – by activating extrinsic coagulation cascade. The activation of intracellular signaling may occur either via TF and factor VIIa binding, or may be VIIa-independent and then occur via the cytoplasmatic domain of TF. This cytoplasmic domain of TF is considered to play the main role in the development of metastases. Cancer cell lines with truncated TF expression have a significantly lower metastasizing capacity than cancer cells with a complete TF molecule [35]. Tissue factor plays a very important role in the process of angiogenesis, mainly through the stimulation of VEGF synthesis [36]. The processes listed above may allow cancer cells to acquire new characteristics which, in turn, allow for the formation of distant metastases.

The final stage of blood coagulation is the formation of fibrin. The presence of thrombin is necessary in the process of conversion of fibrinogen into fibrin. This latter enzyme is formed when the F1+2 fragment breaks off from a molecule of prothrombin. Therefore the degree of coagulation activity may be indirectly assessed from prothrombin fragment 1+2 expression [12]. Thrombin, a serin protease with a molecular mass of 36 kD, not only is a catalyst of the conversion of fibrinogen into fibrin, but also exerts multiple biological effects on normal cells, i.e. it activates platelets, has a mitogenic effect on fibroblasts and smooth muscle cells and may affect endothelial cells and leukocytes [37]. The cellular effects of thrombin are exerted via a specific receptor, which in turn activates protein kinase C, alters the metabolism of phosphoinositol and induces the mobilization of calcium ions. Receptors with a high affinity to thrombin have been identified not only on the surface of healthy cells, but also on cancer cells [37]. The presence of a thrombin-specific receptor on the surface of cancer cells (the so-called TLTR – tethered ligand thrombin receptor, also known as the PAR-1 – protease activated receptor) may explain the proangiogenic, mitogenic and promigratory thrombin activity, which it exerts towards cancer cells. It has been shown *in vitro* that an increase in the expression of this receptor correlates with increased distant metastases formation, while its blocking inhibits the invasive potential of breast cancer cells [37]. It has been observed, that thrombin stimulation of malignant cells of melanoma and colon cancer induces tumor growth and promotes metastatic potential under the experimental conditions [38]. It has been also shown that thrombin mediates tumor cell-induced platelet aggregation (TCIPA) and causes increased expression of adhesion molecules (P-selectin and ICAM-1) in endothelial cells, thus facilitating the development of cancer metastases [39]

Fibrin does not only play a role in hemostasis, but it also promotes tumor growth. It creates a three-dimensional structure, which not only forms a framework for the growing tumor mass, but which also acts as a mechanical barrier preventing an effective reaction of the immunological system against cancer antigens. Fibrin increases TF expression which, through a vicious circle of feedback, increases the production of fibrin. It stimulates endothelial cell proliferation and migration, and thus may promote angiogenesis [40].

In examined material the expression of TF, fibrin and prothrombin fragment 1+2 has been observed both within the primary tumor and within nodal metastases. This data indicates that breast cancer cell – initiated blood coagulation is activated both within the primary tumor and within the metastatic lesions. Due to the fact that such hemostatic components as TF, thrombin and fibrin may facilitate the development and dissemination of malignant tumors. It seems that compounds interfering with blood coagulation system may be of value in the treatment of breast cancer patients.

Conclusions

The observed expression of TF, prothrombin fragment 1+2 and fibrin, both within the primary tumor and axillary lymph node metastases of breast cancer, indicates that in breast cancer patients there occurs the

extravascular activation of coagulation dependent on the cancer cell-associated TF.

Professor Marek Z. Wojtukiewicz MD, PhD
Department of Oncology, Medical University
12 Ogrodowa St., 15-027 Białystok, Poland
e-mail: mwojtuk@amb.edu.pl

References

1. Falanga A, Rickles FR. Pathophysiology of the thrombophilic state in the cancer patient. *Semin Thromb Hemost* 1999; 25: 173-82.
2. Wojtukiewicz MZ, Rucińska M. Aktywacja krzepnięcia krwi u chorych na nowotwory: implikacje kliniczne. *Nowotwory* 1999; 49: 381-91.
3. Wojtukiewicz MZ, Rucinska M, Kloczko J et al. Profiles of plasma serpins in patients with advanced malignant melanoma, gastric cancer and breast cancer. *Haemostasis* 1998; 28: 7-13.
4. Mielicki WP, Tenderenda M, Rutkowski P, Chojnowski K. Activation of blood coagulation and the activity of cancer procoagulant (EC 3.4.22.26) in breast cancer patients. *Cancer Lett* 1999; 146: 61-6.
5. Caine GJ, Stonelake PS, Rea D, Lip GY. Coagulopathic complication in breast cancer. *Cancer* 2003; 98: 1578-86.
6. Lee AY, Levine MN. Venous thromboembolism and cancer: risk and outcomes. *Circulation* 2003; 107: 17-21.
7. Pitchard KI, Paterson AH, Paul NA et al. Increased thromboembolic complications with concurrent tamoxifen and chemotherapy in a randomized trial of adjuvant therapy for women with breast cancer. *J Clin Oncol* 1996; 14: 2731-7.
8. Luzzatto G, Schafer AI. The prethrombotic state in cancer. Semin Oncol 1990; 17: 147-59.
9. Hettiarachchi RJ, Lok J, Prins MH et al. Undiagnosed malignancy in patients with deep vein thrombosis: incidence, risk indicators, and diagnosis. *Cancer* 1998; 83: 180-5.
10. Hillen HF. Thrombosis in cancer patients. *Ann Oncol* 2000; Supl. 3: 273-6.
11. Nand S. Hemostasis and cancer. *Cancer J* 1993; 6: 54-8.
12. Wojtukiewicz MZ, Rucińska M, Zimnoch L et al. Expression of prothrombin fragment 1+2 in cancer tissue as an indicator of local activation of blood coagulation. *Thromb Res* 2000; 97: 335-42.
13. Jannink I, Risberg B, Van Diest PJ, Baak JP. Heterogeneity of mitotic activity in breast cancer. *Histopathology* 1996; 29: 421-8.
14. Fidler IJ. Tumor heterogeneity and the biology of cancer invasion and metastasis. *Cancer Res* 1978; 38: 2651-60.
15. Tsutsui S, Ohno S, Murakami S et al. EGFR, c-erbB2 and p53 protein in the primary lesions and paired metastatic regional lymph nodes in breast cancer. *Eur J Surg Oncol* 2002; 28: 383-7.
16. Briffod M, Hacene K, Le Doussal V. Immunohistochemistry on cell blocks from fine-needle cytopunctures of primary breast carcinomas and lymph node metastases. *Mod Pathol* 2000; 13: 841-50.
17. Goodson WH 3rd, Ljung BM, Moore DH 2nd et al. Tumor labeling indices of primary breast cancers and their regional lymph node metastases. *Cancer* 1993; 15: 3914-9.
18. Reameakers JM, Beex LV, Koenders AJ et al. Concordance and discordance of estrogen and progesterone receptor content in sequential biopsies of patients with advanced breast cancer: relation to survival. *Eur J Cancer Clin Oncol* 1984; 20: 1011-8.
19. Cianga C, Cianga P, Cozma L et al. Loss of hormonal receptors expression from primary breast carcinoma to axillary lymph node metastases. Rev Med Chir Soc Med Nat Iasi 2003; 107: 540-4.
20. Matsuo K, Fukutomi T, Tsuda H et al. Differences in estrogen receptor status, Her2, and p53 comparing metachronous bilateral breast carcinoma. *J Surg Oncol* 2001; 77: 31-4.
21. Wojtukiewicz MZ, Sierko E, Zacharski LR et al. Tissue factor-dependent coagulation activation and impaired fibrinolysis in situ in gastric cancer. *Semin Thromb Hemost* 2003; 29: 291-300.
22. Wojtukiewicz MZ, Rucinska M, Zacharski LR et al. Localization of blood coagulation factors in situ in pancreatic carcinoma. *Thromb Haemost* 2001; 86: 1416-20.
23. Wojtukiewicz MZ, Zacharski LR, Rucińska M et al. Expression of tissue factor and tissue factor pathway inhibitor in situ in laryngeal carcinoma. *Thromb Haemost* 1999; 82: 1659-62.
24. Wojtukiewicz MZ, Sierko E, Zimnoch L et al. Immunohistochemical localization of tissue factor pathway inhibitor-2 in human tumor tissue. *Thromb Haemost* 2003; 90: 140-6.
25. Clahsen PC, van de Velde CJ, Julien JP et al. Thromboembolic complication after perioperative chemotherapy in women with early breast cancer. *J Clin Oncol* 1994; 12: 1266-71.
26. Ueno T, Toi M, Koike M et al. Tissue factor expression in breast cancer tissues: its correlation with prognosis and plasma concentration. *Br J Cancer* 2000; 83: 164-70.
27. Sato Y, Mukai K, Watanabe S et al. The AMeX method. A simplified technique of tissue processing and paraffin embedding with improved preservation of antigens for immunostaining. *Am J Pathol* 1986; 125: 431-5.
28. von Tempelhoff GF, Schönmann N, Heilmann L. Thrombosis – a clue of poor prognosis in primary non-metastatic breast cancer? *Breast Cancer Res Treat* 2002; 73: 275-7.
29. Rickles FR, Shoji M, Abe K. The role of the hemostatic system in tumor growth, metastasis, and angiogenesis: tissue factor is a bifunctional molecule capable of inducing both fibrin deposition and angiogenesis in cancer. *Int J Hematol* 2001; 73: 145-50.
30. Varna JA, Stang MT, Grande JP, Getz MJ. Expression of tissue factor in tumor stroma correlates with progression to invasive human breast cancer: paracrine regulation by carcinoma cell-derived members of the transforming growth factor beta family. *Cancer Res* 1996; 56: 5063-70.
31. Gordon SG. Cancer cell procoagulants and their role in malignant disease. *Semin Thromb Hemost* 1992; 18: 424-33.
32. Mann KG. Biochemistry and physiology of blood coagulation. *Thromb Haemost* 1999; 82: 165-74.
33. Wojtukiewicz MZ, Sierko E, Rak J. Contribution of the hemostatic system to angiogenesis in cancer. *Semin Thromb Hemost* 2004; 30: 5-20.
34. Koomagi R, Volm M. Tissue-factor expression in human non-small-cell lung carcinoma measured by immunohistochemistry: correlation between tissue factor and angiogenesis. *Int J Cancer* 1998; 79: 19-22.
35. Bromberg ME, Sundaram R, Homer RJ et al. Role of tissue factor in metastasis: functions of the cytoplasmic and extracellular domains of the molecule. *Thromb Haemost* 1999; 82: 88-92.
36. Fernandez PM, Patierno SR, Rickles FR. Tissue factor and fibrin in tumor angiogenesis. *Semin Thromb Hemost* 2004; 30: 31-44.
37. Wojtukiewicz MZ, Tang TG, Ben-Josef E et al. Solid tumor express functional "tethered ligand" thrombin receptor. *Cancer Res* 1995; 55: 698-704.
38. Wojtukiewicz MZ, Tang TG, Nelson KK et al. Thrombin enhances tumor cell adhesive and metastatic properties via increased αIIbβ3 expression on the cell surface. *Thromb Res* 1992; 68: 233-45.
39. Wojtukiewicz MZ, Tang TG, Ciarelli JJ et al. Thrombin increases the metastatic potential of tumor cell. *Int J Cancer* 1993; 54: 793-806.
40. Wojtukiewicz MZ, Sierko E, Klement P, Rak J. The hemostatic system and angiogenesis in malignancy. *Neoplasia* 2001; 3: 371-384.

Paper received: 12 December 2005
Accepted: 20 February 2006