Am J Cancer Res 2016;6(6):1450-1457
www.ajcr.us /ISSN:2156-6976/ajcr0029088

Original Article

Functional impairment of activated protein C in breast cancer - relationship to survival outcomes

Mario Roselli[1*], Patrizia Ferroni[2*], Silvia Riondino[1,3], Sabrina Mariotti[1], Ilaria Portarena[1], Jhessica Alessandroni[3,4], Cristiano Ialongo[4], Renato Massoud[4], Leopoldo Costarelli[5], Francesco Cavaliere[6], Sergio Bernardini[4], Fiorella Guadagni[2,3]

[1]Department of Systems Medicine, Medical Oncology, Policlinico Tor Vergata Biospecimen Cancer Repository (PTV Bio.Ca.Re.), Tor Vergata University of Rome, Viale Oxford 81, 00133 Rome, Italy; [2]San Raffaele Roma Open University, Via di Val Cannuta 247, 00166 Rome, Italy; [3]Interinstitutional Multidisciplinary Biobank (BioBIM), IRCCS San Raffaele Pisana, Via di Val Cannuta 247, 00166 Rome, Italy; [4]Clinical Biochemistry and Molecular Biology, Department of Experimental Medicine and Surgery, Tor Vergata University of Rome, Italy; [5]Department of Pathology, San Giovanni Hospital-Addolorata, Via dell'Amba Aradan 9, 00184 Rome, Italy; [6]Department of Surgery, San Giovanni Hospital-Addolorata, Via dell'Amba Aradan 9, 00184 Rome, Italy. [*]Equal contributors.

Received March 24, 2016; Accepted April 1, 2016; Epub June 1, 2016; Published June 15, 2016

Abstract: An impairment of the activated protein C (APC) system has been occasionally reported in breast cancer (BC). However, the clinical significance and prognostic value of an impaired APC functionality in BC patients is still poorly understood. Thus, the present study was aimed at investigating the prognostic value of altered APC functionality for progression-free (PFS) and overall survival (OS) in a cohort study of BC patients. APC functionality was retrospectively analyzed by a coagulation inhibition assay (ThromboPath) in 290 consecutive patients with primary (n=246) or relapsing/recurrent (n=44) BC. All patients were prospectively followed for a median time of 3.5 years (14% recurrence rate). As control group, 145 age-matched healthy women were also investigated. The results obtained demonstrated that APC function was impaired in roughly 20% of all BC at baseline. BC women with stage I/II had a significantly lower rate of APC impairment (13%) than women with stage III (22%) or distant metastases (44%, p=0.001). At univariate analyses, an impairment of APC function had a negative prognostic impact in terms of PFS (5-year PFS rates 53% vs. 70%; HR=2.5; p<0.001) and OS (5-year OS rates 79% vs. 93%; HR=3.9; p=0.005). However, prognostic significance was retained in multivariate models only for PFS (HR=2.0; p=0.017). We may, thus, conclude that BC patients are in a prothrombotic condition, which could play a role in the progression of the disease. Monitoring coagulation changes in BC women could provide important prognostic information especially in patients with advanced stages.

Keywords: Breast cancer, prognosis, disease-free survival, activated protein C resistance, thrombophilia

Introduction

Nowadays, it is widely recognized that the host haemostatic system may play functional roles in breast cancer (BC) progression by shaping the tumor microenvironment through tissue factor (TF) expression, leading to thrombin generation and thrombin-dependent fibrin deposition [1, 2]. In this context, TF - expressed by either tumor or host vascular cells - has been proposed to regulate epithelial-to-mesenchymal transition, thus establishing the tumor cell premetastatic niche [2]. Furthermore, TF-induced thrombin generation and fibrin deposition have been directly implicated in BC cell proliferation, neoangiogenesis and metastatic spread [3, 4].

Other factors, beside TF expression, might play a role in cancer-associated coagulation activation, i.e., an unbalance of host endogenous anticoagulants [5, 6]. An impairment of the activated protein C (APC) system, for instance, has been occasionally reported in cancer patients [7-10], possibly related to acquired defects in the PC anticoagulant system caused by cytokines either produced by cancer cells or generated by the acute-phase reaction associated

Table 1. Clinical characteristics of breast cancer (BC) patients

	BC (n=290)
Age (years)	
Mean ± SD (range)	57±13 (29-88)
Menopausal status	
Pre	132 (46%)
Post	158 (54%)
Histological diagnosis	
Ductal	255 (88%)
Lobular	27 (9%)
Other[a]	8 (3%)
Molecular type[*]	
Basal-like triple-negative	39 (13%)
Luminal-like A	104 (36%)
Luminal-like B	136 (47%)
HER2 pos	11 (4%)
Grading	
1	18 (6%)
2	95 (33%)
3	177 (61%)
Stage	
I	69 (24%)
II	105 (36%)
III	72 (25%)
IV[b]	4 (1%)
Metastatic[c]	40 (14%)
Receptor status	
ER+/PR+	205 (71%)
ER+/PR-	28 (10%)
ER-/PR+	6 (2%)
ER-/PR-	51 (17%)
HER2/NEU+	51 (18%)
Ki67 proliferation index ≥20%	197 (68%)
Follow-up (years)	
Median (range)	3.5 (0.11-7.95)

[a]Including: mucinous (n=5), papillary, tubular, comedocarcinoma (one each). [b]Single metastatic site radically resected. [c]Diagnosed in previously resected BC patients. [*]Clinicopathological determination was based on estrogen receptor, progesterone receptor, HER2, and Ki-67, according to St. Gallen Consensus Conference [25]. ER: estrogen receptors; PR: progesterone receptors; HER2: Human Epidermal Growth Factor Receptor 2.

with cancer [10]. However, the clinical significance and prognostic value of APC resistance (APCr) in BC patients is still poorly understood.

Therefore, we hypothesized that acquired APCr might be associated with a poor clinical outcome in BC patients. Accordingly, the present study was designed to investigate the prognostic significance of an impairment of the APC system for progression-free survival (PFS) and overall survival (OS) in a prospective cohort of BC women. Furthermore, since BC is a highly heterogeneous disease, characterized by different prognosis and treatment options, depending on hormone receptor (HR) status and human epidermal growth factor receptor 2 (HER2) expression [11], we analyzed the possible associations of APC functionality in subgroups of patients characterized by more aggressive tumor phenotypes, such as HER2-negative, or triple-negative BC (TNBC).

Patients and methods

Patients

The PTV Bio.Ca.Re. (Policlinico Tor Vergata Biospecimen Cancer Repository) and the Interinstitutional Multidisciplinary Biobank of the IRCCS San Raffaele Pisana (SR-BioBIM) are actively involved since January 2007 in the recruitment of out-patients with primary or metastatic cancer, who are prospectively followed under appropriate Institutional ethics approval, as part of a Clinical Database and Biobank project. Among these, a cohort of 290 consecutive BC patients, who were not receiving concomitant treatment with anticoagulant and/or antiplatelet drugs, was selected for this study.

BC was staged according to the TNM classification. Two hundred and fifty women with primary BC underwent surgery (29% mastectomy, 71% lumpectomy). The remaining 40 patients had relapsing/metastatic disease and entered the study prior to chemotherapy start. Neoadjuvant chemotherapy was instituted in 50 (17%) women. Adjuvant chemotherapy regimens - both anthracycline (n=138) and non-anthracycline (n=14) containing - were instituted in 152 (53%) women (87 with and 65 without lymph node involvement). Forty-five women with node-negative disease underwent adjuvant endocrine therapy only (tamoxifen or aromatase inhibitor). Fifty-nine patients underwent radiotherapy either alone or in combination. First-line chemotherapy was instituted in all metastatic patients. Patients with HER2/NEU positivity were all treated with trastuzumab-containing regimens. HER2/NEU positivity was defined according to the American Society of Clinical

Oncology - College of American Pathologists (ASCO-CAP) guidelines [12]. Immunohistochemical detection of Ki67 was performed as previously described [13]. A cut-off value of ≥20% was used in all association analyses, according to the latest recommendations of the St Gallen International Expert Consensus [14].

All patients were prospectively followed for a median time of 3.5 years. Patients' characteristics are summarized in **Table 1**. The study was performed in accordance with the principles embodied in the Declaration of Helsinki. All patients gave written informed consent, previously approved by our Institutional Ethics Committees.

Blood sampling and laboratory tests

Samples from all BC patients were obtained at baseline prior to any treatment. Citrated plasma (3.8%, 1:9, v:v) for APC functionality determination was obtained by a 10-min centrifugation at 1,500×g at 4°C. Samples were aliquoted and stored at -80°C in the facilities of the PTV Bio.Ca.Re. or of the SR-BioBIM. Storage conditions were carefully maintained and aliquots were limited to one freeze-thaw cycle at the time of batch analysis.

APC functionality was assessed by HemosIL ThromboPath (ThP, kindly provided by IL, Orangeburg, NY) using an ACL-TOP automated coagulometer (IL, Lexington, MA) as previously reported [15]. Test results are expressed as the Protac-Induced Coagulation Inhibition percentage (PICI%). A locally-defined cutoff value was determined by ROC analysis of the value distribution recorded in all patients and in 145 unrelated age-matched women recruited in a 2:1 ratio from apparently healthy individuals enrolled in the SR-BioBIM (mean age 57±12, ranging from 28 to 86 years, 53% postmenopausal). Cutoff was set at 80% PICI%. All samples with PiCI% below this value were categorized as pathologic. Measurements were ascertained while blinded to the sample origin.

Statistical analysis

Given the observed ThP means for patient (μ1) and control (μ2) groups and using a type I error probability of 0.05, the recruited population yielded a statistical power greater than 85%.

Data are presented as percentages, mean ± standard deviation (SD), or median and interquartile range (IQR). Student's unpaired t-test and ANOVA test were used for normally distributed variables. Appropriate non-parametric tests (Mann-Whitney U-test and Kruskal-Wallis ANOVA and median test) were employed for all the other variables. The cut-off values were estimated by receiver operating characteristic (ROC) curve analyses performed by MedCalc Statistical Software version 13.1.2 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2014).

Progression free survival (PFS) was calculated from the date of enrollment until relapse or progression of disease. If a patient had not progressed or died, PFS was censored at the time of the last follow-up. OS was calculated from the date of enrollment until disease-related death or study end. For administrative censoring follow-up was ended the date of March 31st, 2015. Survival curves were calculated by the Kaplan-Meier method and the significance level was assessed according to the log-rank test using a computer software package (Statistica 8.0, StatSoft Inc., Tulsa, OK). Cox-proportional hazards analysis was performed by a free web-based application (http://statpages.org/) to evaluate the association between clinical-pathological variables and PFS. All tests were two-tailed and only p-values <0.05 were regarded as statistically significant.

Results and discussion

Overall, an impairment of APC functionality was observed in all BC patients. Indeed, pre-treatment ThP values were lower in BC (83.8±8.0 PICI%) compared to controls (85.6±8.0 PICI%; Students t test; p=0.014), and gradually decreased with BC stage, being lower in metastatic cancer (79.5±11.0 PICI%, Anova test: F=5.14, p=0.0005). Specifically, APC functionality was impaired at baseline (ThP values below the cut-off) in roughly 13% of women with stage I/II BC, 22% of patients with stage III and 44% of patients with metastatic disease (Chi-square=17, p=0.0019). Furthermore, a significant association was found between pathological ThP values and larger tumor size (T1/2 vs. T3/4; 2-tailed Fisher test: p=0.05) or distant metastases (2-tailed Fisher test: p=0.0002), but none of the BC prognostic factors analyzed

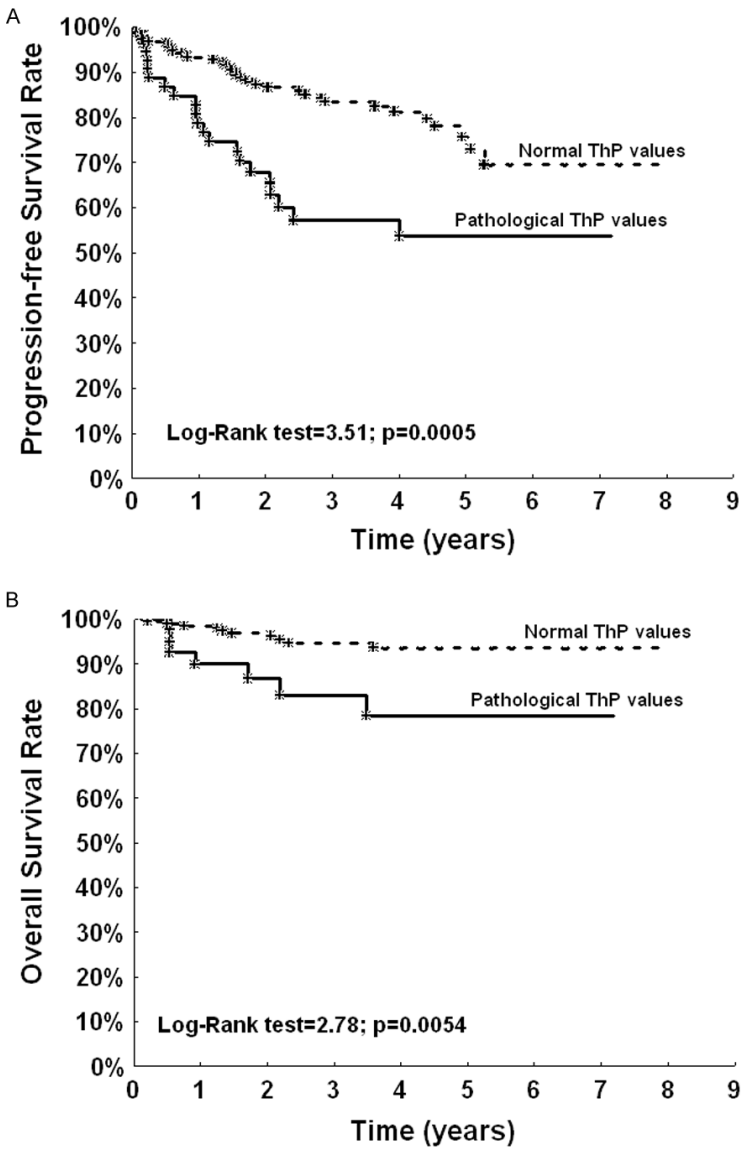

Figure 1. Kaplan-Meier curves of (A), progression-free and (B), overall survival of patients with breast cancer. Comparison between patients with normal (dotted line) or pathologic (solid line) APC function evaluated by ThrombPath assay.

in this study. These results are in agreement with preliminary findings highlighting a link between the APC pathway and BC [7, 13], and suggest that tumor burden, rather than other prognostic factors, might be responsible for an impairment of APC functionality in BC patients, possibly arising from tumor cell released products.

APC - the main protease of the protein C anticoagulant pathway [16] - may play an important role in balancing the pro-metastatic effects of thrombin generation initiated by tumor cell-expressed TF. Indirect evidence of an active involvement of the PC pathway in cancer cells invasive behavior stems from experimental evidences that BC cells expressing PC inhibitor have decreased invasive activity *in vitro* [17]. Furthermore, using in vivo animal models, Bezuhly et al. demonstrated that treatment with recombinant human APC resulted in a 44% reduction in lung metastases [5], and van Sluis et al. reported that endogenous APC could be effective in limiting cancer cell extravasation and metastasis [18]. Of interest, it has been advocated that, since endothelial PC receptor deficiency has minimal effects on metastasis, direct neutralization of thrombin should represent the dominant mechanism by which metastatic spread is prevented once tumor cells have entered the bloodstream [6]. Although there is still much to be done to unravel the mechanisms underlying the role of APC in cancer progression, the finding of an impairment of the APC function in BC is of particular interest in the clinical setting, as hypercoagulability might be associated with unfavorable prognosis [19].

Therefore, based on the hypothesis that acquired APCr might be associated with a poor clinical outcome in BC patients, we investigated the prognostic significance of APC functionality for PFS and OS in our cohort of BC women. During a median follow-up of 3.5 years, 216 of 250 (86%) primary BC patients remained clinically free of disease, while 34/249 (14%) had recurrence or new primary breast cancer (one second primary BC after one year). Among patients with relapsing metastatic BC, 10/40 (25%) had a complete/partial response during chemotherapy, while 30/40 (75%) had BC progression. An

Table 2. Cox proportional hazards survival regression analysis of the predictive value of clinical-pathological variables and impaired activated protein C (APC) functionality on progression-free (PFS) and overall survival (OS) of breast cancer patients

			PFS		OS	
Variable	Code	N.	HR (95% C.I.)	*p*-Value	HR (95% C.I.)	*p*-Value
Molecular type	Basal like TN	39				
	LLA	104				
	LLB	136				
	HER2 pos	11	0.51 (0.31-0.81)	*0.0051*	0.55 (0.15-1.93)	*0.347*
Stage of disease	I	69				
	II	105				
	III	72				
	IV	4				
	Metastatic	40	1.76 (1.10-2.81)	*0.018*	4.22 (1.58-11.2)	*0.004*
Estrogen receptors	Negative	56				
	Positive	234	0.71 (0.31-1.64)	*0.422*	1.84 (0.24-14.4)	*0.561*
Progesterone receptors	Negative	79				
	Positive	211	1.78 (0.79-4.00)	*0.162*	0.85 (0.21-3.45)	*0.822*
HER2/NEU status	Negative	239				
	Positive	51	0.70 (0.32-1.51)	*0.361*	0.56 (0.11-2.95)	*0.496*
Ki67 proliferation index	Low	93				
	High	197	2.08 (1.04-4.16)	*0.040*	1.53 (0.32-7.45)	*0.597*
Type of treatment						
	Neoadjuvant	50	6.32 (2.08-19.2)	*0.001*	0.68 (0.05-8.84)	*0.767*
	Hormone CT	45	1.84 (0.69-4.90)	*0.223*	0.38 (0.04-3.91)	*0.413*
	Adjuvant CT	152	2.05 (0.72-5.85)	*0.181*	0.00 (0.00-3.97)	*0.960*
	Metastatic	44	9.57 (1.83-49.9)	*0.007*	0.37 (0.02-7.68)	*0.521*
Impaired APC function	No	232				
	Yes	58	2.71 (1.51-4.88)	*0.001*	1.53 (0.45-5.25)	*0.499*

C.I.: Confidence interval; HR: Hazard ratio; ER: estrogen receptors; PR: progesterone receptors; HER2: Human Epidermal Growth Factor Receptor 2. Numbers in parentheses represent percentages. CT: chemotherapy.

impaired APC function (as evidenced by pathological ThP values) was found in a higher percentage of patients who underwent disease progression compared to patients with stable disease (36% vs. 15%, 2-tailed Fisher test: p=0.001).

Univariate Cox proportional hazards survival analyses showed that pathological pre-treatment ThP values had a negative prognostic value in terms of both PFS (HR=2.5; 95% C.I.: 1.48-4.26; p=0.0007) and OS (HR=3.7; 95% C.I.: 1.41-9.73; p=0.008). The Kaplan-Meier PFS and OS curves for the 290 BC patients stratified on the basis of ThP values are depicted in **Figure 1**, demonstrating that an impairment of APC functionality had a negative prognostic impact in terms of both PFS (5-year PFS rates 53% vs. 70%; log-rank test=3.51; p=0.0005) and OS (5-year OS rates 79% vs. 93%; log-rank test=2.78; p=0.0054) (**Figure 1A** and **1B**, respectively). However, after adjustment for known prognostic variables, significance was retained only for PFS (HR=2.71, 95% C.I.: 1.51-4.88) (**Table 2**).

The negative prognostic value of impaired APC functionality for PFS was further confirmed in a subgroup analysis of primary non-metastatic BC patients (log-rank test=1.94, p=0.051) (**Table 3**). Of interest, patients with pathological pre-treatment ThP values had worse 5-year survival rates mostly in ER/PR positive, HER2/NEU negative patients and, accordingly, in luminal-like A (LLA) molecular sub-types (**Table 3**). Clinically, LLA disease has a better prognosis than other subtypes, at a point that the relative benefit from adjuvant chemotherapy would

Table 3. 5-year progression-free survival rates (%) of breast cancer patients according to main prognostic clinical-pathological variables

		APC function			
Variable	Code	Normal	Pathologic	Log-rank test	*p*-Value
Molecular type	Basal like TN	69%	100%	-1.40	*0.162*
	LLA	83%	50%	3.86	*0.0001*
	LLB	64%	58%	1.54	*0.123*
	HER2 pos	100%	0%	2.39	*0.017*
Stage of disease	Localized	72%	80%	1.94	*0.051*
	Metastatic	20%	5%	0.85	*0.395*
Hormone receptor status	ER+/PR+	71%	52%	3.82	*0.0001*
	ER-/PR-	74%	45%	0.33	*0.739*
HER2/NEU status	Negative	72%	58%	3.05	*0.002*
	Positive	37%	31%	1.54	*0.124*
Ki67 proliferation index	Low	81%	53%	2.72	*0.007*
	High	63%	54%	2.37	*0.018*

ER: estrogen receptors; PR: progesterone receptors; HER2: Human Epidermal Growth Factor Receptor 2.

translate into very small absolute benefits, which may not outweigh the risks [20]. However, the results here reported demonstrated a 5-year PFS of 50% for LLA patients with pathological ThP values prior to treatment, compared to 83% of LLA patients with a normal ThP (log-rank test=3.86; p<0.0001). These finding was also confirmed in a sub-set analysis of early stage LLA (5-year PFS rates 64% vs. 90%; log-rank test=2.76; p=0.006). Thus, whilst adjuvant chemotherapy would not be generally indicated in early stage LLA, it may still be considered in those individuals with large tumors and impaired APC functionality at time of diagnosis. While evidence for basing chemotherapy choices on subtype remains insufficient, particularly the case for the LLA subtype [20], nonetheless, the results here reported demonstrate, for the first time to our knowledge that the evaluation of APC functionality might be of prognostic value in predicting PFS in BC cancer patients. On the other hand, the finding of an unbalance in the endogenous anticoagulant pathway has important clinical implications in BC care and treatment, given that chemotherapy [21-23] or hormone-therapy [24] could further contribute to acquired APCr in BC patients.

There are, of course, some limitations to acknowledge. First of all, this study was a retrospective analysis, although all eligible consecutive patients within the designated timeframe were included. Moreover, recruitment was mono-institutional, which might have posed further limitation as the primary and most obvious shortcoming of single-centre studies is their potentially limited external validity. Nonetheless, the data here reported demonstrate that BC patients are in a prothrombotic condition possibly due to an imbalance of the APC pathway, which might foster the progression of the disease. Monitoring APC function in BC women could also provide important prognostic information especially in particular subset of patients. Whether anticoagulation might limit cancer progression and prolong the life expectancy of BC patients, still remains a matter for further debate, as does the potential therapeutic use of recombinant APC, or protein C concentrates, in selected subgroups of cancer patients.

Acknowledgements

The team expresses deep gratitude to all patients and their families for providing the opportunity to conduct the present research project. Authors also wish to thank the nursery staff of the Day Hospital of the Medical Oncology Unit, Tor Vergata Clinical Center who have contributed and supported the researchers to the overall success of the project. This work was partially supported by European Social Fund, under the Italian Ministry of Education, University and Research PON03PE_00146_1/10 BIBIOFAR (CUP B88F12000730005) and PhD program in Systems Medicine - XXVII Cycle.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Patrizia Ferroni, San Raffaele Roma Open University, IRCCS San Raffaele Pisana, Via di Val Cannuta 247, 00166 Rome, Italy. Tel: +39 06 52253733; Fax: +39 06 52255668; E-mail: patrizia.ferroni@sanraffaele.it

References

[1] Yokota N, Zarpellon A, Chakrabarty S, Bogdanov VY, Gruber A, Castellino FJ, Mackman N, Ellies LG, Weiler H, Ruggeri ZM and Ruf W. Contributions of thrombin targets to tissue factor-dependent metastasis in hyperthrombotic mice. J Thromb Haemost 2014; 12: 71-81.

[2] Versteeg HH. Tissue Factor: Old and New Links with Cancer Biology. Semin Thromb Hemost 2015; 41: 747-755.

[3] Lal I, Dittus K and Holmes CE. Platelets, coagulation and fibrinolysis in breast cancer progression. Breast Cancer Res 2013; 15: 207.

[4] Chaari M, Ayadi I, Rousseau A, Lefkou E, Van Dreden P, Sidibe F, Ketatni H, Galea V, Khaterchi A, Bouzguenda R, Frikha M, Ghorbal L, Daoud J, Kallel C, Quinn M, Gligorov J, Lotz JP, Hatmi M, Elalamy I and Gerotziafas GT. Impact of breast cancer stage, time from diagnosis and chemotherapy on plasma and cellular biomarkers of hypercoagulability. BMC Cancer 2014; 14: 991.

[5] Bezuhly M, Cullen R, Esmon CT, Morris SF, West KA, Johnston B and Liwski RS. Role of activated protein C and its receptor in inhibition of tumor metastasis. Blood 2009; 113: 3371-3374.

[6] Ruf W and Schaffner F. Role of the protein C receptor in cancer progression. Thromb Res 2014; 133: S85-S89.

[7] Nijziel MR, van Oerle R, Christella M, Thomassen LG, van Pampus EC, Hamulyák K, Tans G, and Rosing J. Acquired resistance to activated protein C in breast cancer patients. Br J Haematol 2003; 120: 117-122.

[8] Green D, Maliekel K, Sushko E, Akhtar R and Soff GA. Activated-protein-C resistance in cancer patients. Haemostasis 1997; 27: 112-118.

[9] Haim N, Lanir N, Hoffman R, Haim A, Tsalik M and Brenner B. Acquired activated protein C resistance is common in cancer patients and is associated with venous thromboembolism. Am J Med 2001; 110: 91-96.

[10] Ferroni P, Riondino S, Portarena I, Formica V, La Farina F, Martini F, Massimiani G, Palmirotta R, Guadagni F and Roselli M. Association between increased tumor necrosis factor alpha levels and acquired activated protein C resistance in patients with metastatic colorectal cancer. Int J Colorectal Dis 2012; 27: 1561-1567.

[11] Dai X, Li Y, Bai Z and Tang XQ. Molecular portraits revealing the heterogeneity of breast tumor subtypes defined using immunohistochemistry markers. Sci Rep 2015; 5: 14499.

[12] Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM and Hayes DF; American Society of Clinical Oncology/College of American Pathologists. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med 2007; 131: 18-43.

[13] Ferroni P, Roselli M, Portarena I, Formica V, Riondino S, LA Farina F, Costarelli L, Melino A, Massimiani G, Cavaliere F, Palmirotta R and Guadagni F. Plasma plasminogen activator inhibitor-1 (PAI-1) levels in breast cancer - relationship with clinical outcome. Anticancer Res 2014; 34: 1153-1161.

[14] Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B and Senn HJ; Panel members. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol 2013; 24: 2206-2223.

[15] Ferroni P, La Farina F, Palmirotta R, Martini F, Raparelli V, Nigro C, Riondino S, Rampini MR, Basili S and Guadagni F. Predictive value of thrombopath determination in women with infertility and pregnancy complications. Clin Chim Acta 2010; 411: 37-42.

[16] Dahlbäck B and Villoutreix BO. Anticoagulant protein C pathway. FEBS Lett 2005; 579: 3310-3316.

[17] Asanuma K, Wakabayashi H, Okamoto T, Asanuma Y, Akita N, Yoshikawa T, Hayashi T, Matsumine A, Uchida A and Sudo A. The thrombin inhibitor, argatroban, inhibits breast cancer metastasis to bone. Breast Cancer 2013; 20: 241-246.

[18] Van Sluis GL, Niers TMH, Esmon CT, Tigchelaar W, Richel DJ, Buller HR, Van Noorden CJ and Spek CA. Endogenous activated protein C limits cancer cell extravasation through sphingosine-1-phosphate receptor 1-mediated vascular endothelial barrier enhancement. Blood 2009; 114: 1968-1973.

[19] Spek CA, Versteeg HH and Borensztajn KS. Anticoagulant therapy of cancer patients: Will patient selection increase overall survival? Thromb Haemost 2015; 114: 530-536.

[20] Hart CD, Sanna G, Siclari O, Biganzoli L and Di Leo A. Defining optimal duration and predicting benefit from chemotherapy in patients with luminal-like subtypes. Breast 2015; 24: S136-S142.
[21] Woodley-Cook J, Shin LY, Swystun L, Caruso S, Beaudin S and Liaw PC. Effects of the chemotherapeutic agent doxorubicin on the protein C anticoagulant pathway. Mol Cancer Ther 2006; 5: 3303-3311.
[22] Rogers JS, Murgo AJ, Fontana JA and Raich PC. Chemotherapy for breast cancer decreases plasma protein C and protein S. J Clin Oncol 1988; 6: 276-281.
[23] Soliman AA, Csorba R, Ullrich A, Tsikouras P, Rath W and von Tempelhoff GF. Antiphopholipid antibodies and functional activated protein C resistance in patients with breast cancer during anthracycline-based chemotherapy administered through an intravenous port-catheter device. Clin Appl Thromb Hemost 2014; 20: 338-340.
[24] Rühl H, Schröder L, Müller J, Fimmers R, Sukhitashvili S, Welz J, Kuhn WC, Oldenburg J, Rudlowski C and Pötzsch B. Tamoxifen induces resistance to activated protein C. Thromb Res 2014; 133: 886-891.
[25] Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B and Senn HJ; Panel members. Strategies for subtypes - dealing with the diversity of breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol 2011; 22: 1736-1747.