DYNAMICS OF HEMOSTATIC MARKERS THROUGHOUT SYSTEMIC TREATMENT IN PATIENTS WITH BREAST CANCER

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Abstract: Breast cancer is the most commonly diagnosed cancer among women globally and is increasingly managed with complex multimodal systemic therapy. Despite advances in oncologic treatment, venous thromboembolism (VTE) is still a major health problem and the second leading cause for cancer-related mortality. Patients with breast cancer have roughly a fourfold higher risk of developing VTE compared with age- and sex-matched healthy controls, and this risk is further amplified by systemic chemotherapy and other adjuvant treatments. The prothrombotic tendency in breast cancer arises from a complex interaction between tumor biology, host response, and anticancer therapy. Tumor cells can directly activate coagulation by expressing tissue factor (TF) and releasing TF-bearing extracellular vesicles, thereby initiating thrombin generation and fibrin formation. Prothrombin fragment 1+2 (F1+2) and thrombin-antithrombin complexes (TAT) are clinical-laboratory parameters, which provide a direct measure of in vivo thrombin formation. D-dimer is widely used in clinical practice as an indicator of ongoing coagulation and fibrinolysis. In cancer patients, the coagulation system is often chronically activated and AT III, a natural anticoagulant, may be consumed through ongoing inhibition of thrombin and factor Xa or down-regulated. Objective: To investigate longitudinal changes in hemostatic markers – F1+2, TAT, D-dimer, TF, fibrinogen and Antithrombin III in patients with breast cancer undergoing systemic treatment. Materials and methods: We investigated 38 women with breast cancer. A control group of 34 age-matched healthy women was also studied. TAT, F1+2, TF, D-dimer, AT III and fibrinogen levels were determined prior to therapy initiation – Visit 1; Visit 2 – monitoring of therapeutic response; Visit 3 – after completing treatment. ELISAs were used to quantify F1+2, TF and TAT. Fibrinogen levels were determined by the Claus clotting method, AT III activity by chromogenic assay, and plasma D-dimer concentration by immunoturbidimetry. Results: Levels of F1+2, TAT, TF and D-dimer declined throughout systemic therapy, with baseline values being significantly higher than those at visits 2 and 3 ($P<0.05$). ATIII activity showed a statistically significant increase at visit 3 compared to visit 2 ($P=0.023$). Conclusion: Systemic treatment in breast cancer patients significantly influenced the dynamics of hemostatic markers. A hypercoagulable state persists during the applied therapy, despite the tendency for lower levels of TAT, F1+2, TF, D-dimer and increased AT III activity. This highlights the importance of an individualized anticoagulation strategy aimed at preventing thrombotic events and effectively managing cancer-related venous thromboembolism.

Keywords: coagulation, fibrinolysis, breast cancer, thrombosis

1. INTRODUCTION

Breast cancer is the most commonly diagnosed cancer among women globally and is increasingly managed with complex multimodal systemic therapy. Despite advances in oncologic treatment, venous thromboembolism (VTE) is still a major health problem and the second leading cause of cancer-related mortality. Patients with breast cancer have roughly a fourfold higher risk of developing VTE compared with age- and sex-matched healthy controls, and this risk is further amplified by systemic chemotherapy and other adjuvant treatments (Crichi et al, 2023). The prothrombotic tendency in breast cancer arises from a complex interaction between tumor biology, host response and anticancer therapy. Tumor cells can directly activate coagulation by expressing tissue factor (TF) and releasing TF-bearing extracellular vesicles, thereby initiating thrombin generation and fibrin formation (Koizume et al., 2022). Systemic treatments can further disturb the endothelial function, activate platelets, enhance inflammatory and procoagulant signaling, and thus influence the balance between coagulation and fibrinolytic systems. Recent longitudinal data in women undergoing adjuvant therapy for breast cancer demonstrate a tendency toward hypercoagulability combined with post-treatment hypofibrinolysis. The prothrombotic state is characterized by increased plasma levels of tissue factor (TF), increased TF activity, and low levels of tissue plasminogen activator (t-PA) (Wrzeszcz et al., 2023). These hemostatic alterations may contribute not only to VTE but also to disease progression and poorer survival.

Some circulating hemostatic biomarkers have emerged as promising tools for identifying prothrombotic state. Among markers of thrombin generation, prothrombin fragment 1+2 (F1+2) and thrombin–antithrombin complexes (TAT) provide a direct measure of in vivo thrombin formation. A recent systematic review across solid and hematologic malignancies supports their role in predicting cancer-associated VTE (Gyldenholm et al., 2024). D-

dimer, a degradation product of cross-linked fibrin, is widely used in clinical practice as an indicator for ongoing coagulation and fibrinolysis. Increased D-dimer levels have been associated with increased VTE risk, shortened survival, early recurrence of disease, and therapeutic response in patients at high risk and with metastatic breast cancer (Alkhoder et al., 2024), (Wang et al., 2022). Coagulation system is often chronically activated in patients with cancer and AT III, a natural anticoagulant, may be consumed through ongoing inhibition of thrombin and factor Xa or down-regulated. This, in turn, further shifts the balance towards hypercoagulability (Hong et al., 2010), (Sun et al., 2015). Hemostatic markers reflecting the initiation of coagulation are also of interest. TF expressed by tumor cells contributes not only to thrombogenesis but also to tumor invasion, metastasis and angiogenesis (Hisada et al., 2019). TF antigen levels and the activity of circulating TF-bearing extracellular vesicles, originating from tumor or platelet microparticles, have been strongly linked to cancer-associated thrombosis (Koizume et al., 2022).

Several recent studies have highlighted that breast cancer patients undergoing systemic treatment exhibit significant alterations in global coagulation profiles compared with healthy controls and with their own pre-treatment baselines (Ayana et al., 2025), (Mandoj et al., 2018), (Lu et al., 2025). However, data specifically detailing the dynamic changes in TAT, F1+2, TF, D-dimer, AT III, and fibrinogen throughout the therapy in breast cancer remain limited and heterogeneous. A better understanding of how these hemostatic markers change over time, and how they relate to thrombotic events, could help with the decisions for individualized approach regarding thromboprophylaxis in these patients.

The present study aims to evaluate longitudinal changes in hemostatic markers - prothrombin fragment 1+2 (F1+2), thrombin–antithrombin complexes (TAT), D-dimer, tissue factor (TF), fibrinogen, and Antithrombin III in patients with breast cancer undergoing systemic treatment.

2. MATERIAL AND METHODS

Thirty-eight women with histologically confirmed breast cancer treated at the Clinic of Medical Oncology, University Hospital "St. George" in Plovdiv, were enrolled between September 2017 and May 2019. Thirty clinically healthy women were also included as a control group. The study received approval from the Scientific Ethics Committee of the Medical University of Plovdiv and was carried out in compliance with the principles of the Declaration of Helsinki. Clinical-laboratory analyses were performed in the hospital's Clinical Laboratory.

The inclusion criteria required participants to be aged 18 years or older with histologically confirmed, newly diagnosed breast cancer, either localized or advanced stage, who were scheduled to receive systemic treatment. Individuals were excluded if they had NYHA class III/IV heart failure, an ECOG performance status ≥4, diabetes mellitus, acute bacterial or viral infection within two weeks before evaluation, hepatic or renal failure, or if they had taken vitamin K antagonists or other oral anticoagulants in the previous three months. All participants provided written informed consent.

Patients were prospectively evaluated at three predefined time points:

Visit 1 – baseline (prior to therapy initiation)
Visit 2 – during treatment (monitoring of therapeutic response)
Visit 3 – after completing treatment

Healthy controls underwent a single assessment.

Venous blood samples were collected according to standardized pre-analytical requirements. Platelet-poor plasma was used for markers of coagulation and fibrinolysis and screening coagulation tests. If longer-term storage of plasma is required, it is stored under certain conditions until analysis (-20°C or -70°C).

AT III activity, D-dimer, fibrinogen, prothrombin time (PT), and activated partial thromboplastin time (aPTT) were measured using the Sysmex CS-2000i coagulation analyzer (Siemens Diagnostics). Fibrinogen levels were determined by the Claus clotting method, AT III activity by a chromogenic assay, plasma D-dimer concentration by immunoturbidimetry, and PT and aPTT by one-step coagulation methods. Plasma concentrations of TF, F1+2, and TAT were quantified by ELISA following manufacturer protocols.

Statistical analyses were performed using IBM SPSS Statistics v.26 and Microsoft Excel 2010. A significance threshold of $\alpha < 0.05$ was applied. Variables with normal distribution (Shapiro-Wilks test) were presented as mean ± SD and analysed with parametric tests, including t-test and repeated-measures ANOVA. Variables that did not follow normal distribution were evaluated using non-parametric methods such as Mann-Whitney U test, Friedman test, and Wilcoxon signed-rank test.

3. RESULTS

A total of 38 women with breast cancer and a control group of 30 clinically healthy women were studied. The patient and control groups were comparable in terms of gender and age. The average age of the patient group was 56.47±9.64 years, and of the control group 59.20±11.74 years, p=0.297.

The distribution of women with breast cancer in a localized stage (I and II stage) is 60.5% (n=23) and advanced stage (III and IV stage) – 39.5% (n=15). Two of all patients (5.3%) have body mass index ≥35, three women (7.9%) have hemoglobin <100 g/l, seven (18.4%) are with leucocytes ≥11 G/l and ten patients with breast cancer (26.3%) have platelets ≥350 G/l.

Table 1 presents hemostatic markers in patients with breast cancer (n=38) at the start of therapy (visit 1) and in the healthy control group (n=30). Breast cancer patients showed significantly elevated levels of TF, F1+2, TAT, fibrinogen, and D-dimer compared with healthy individuals, whereas AT III activity was markedly reduced ($p<0.05$).

Table 1. Hemostatic markers in patients with breast cancer and control group

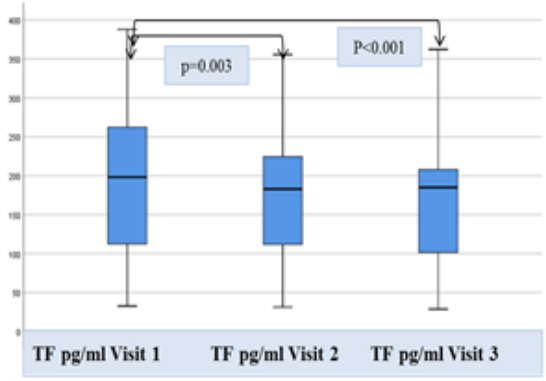
Parameter	Group	N	Mean	Standard deviation	Median	25 percentile	75 percentile	P-value
D-dimer mg/l Visit 1	breast cancer	38	1.13	0.91	0.87*	0.54	1.34	p<0.001
	controls	30	0.35	0.14	0.33*	0.23	0.42	
TAT ng/ml Visit 1	breast cancer	38	10.03	4.87	8.52*	6.28	12.64	p<0.001
	controls	30	5.43	2.23	4.77*	3.81	6.74	
F 1+2 ng/ml Visit 1	breast cancer	38	19.70	8.27	21.10*	11.73	25.93	p<0.001
	controls	30	9.97	4.94	10.60	5.33	12.45	
fibrinogen g/l Visit 1	breast cancer	38	3.61	1.09	3.23	2.85	4.37	p=0.003
	controls	30	2.97	0.57	2.88	2.60	3.23	
AT III % Visit 1	breast cancer	38	87.30	8.21	87.40	81.80	93.73	p=0.001
	controls	30	93.65	6.93	93.85	89.00	98.53	
TF pg/ml Visit 1	breast cancer	38	196.17	92.08	198.15	112.15	263.68	p<0.001
	controls	30	138.81	54.41	140.45	101.25	169.28	

* Variable is not with normal distribution Source: Author's research

The dynamics of hemostatic markers were examined throughout the applied therapeutic strategy. The factor "time" (visit) had a significant influence on the plasma levels of all monitored markers. The results are summarized below and graphically illustrated in Figures 1–6.

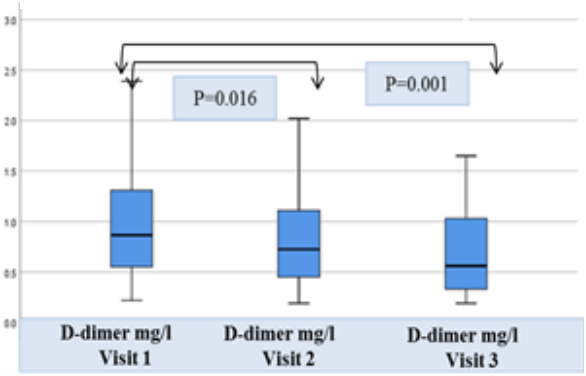
Mean TF values in patients with breast cancer differed significantly across visits ($p<0.001$). TF levels at visit 2 were 167.44±73.75 pg/ml and 164.66±71.90 pg/ml at visit 3. They were significantly lower compared to visit 1 - 196.17±92.08 pg/ml, with $p=0.003$ and $p<0.001$, respectively (Fig. 1). Levels of D-dimer also decreased during therapy. D-dimer were 0.87 mg/l (0.54–1.34 mg/l) at visit 1, 0.73 mg/l (0.45–1.11 mg/l) at visit 2 and 0.56 mg/l (0.33–1.03 mg/l) at visit 3, $p=0.016$ and $p=0.001$ (Fig. 2).

Fig. 1 TF pg/ml in patients with breast cancer throughout treatment



Source: Author's research

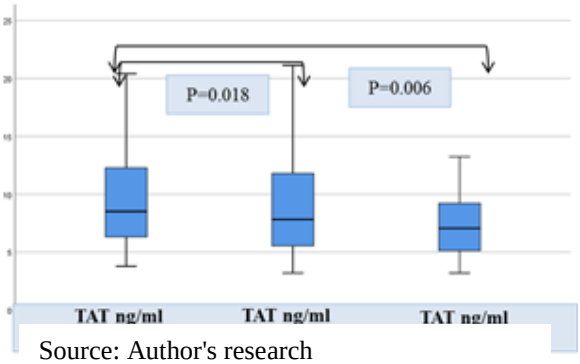
Fig. 2 D-dimer mg/l in patients with breast cancer throughout treatment



Source: Author's research

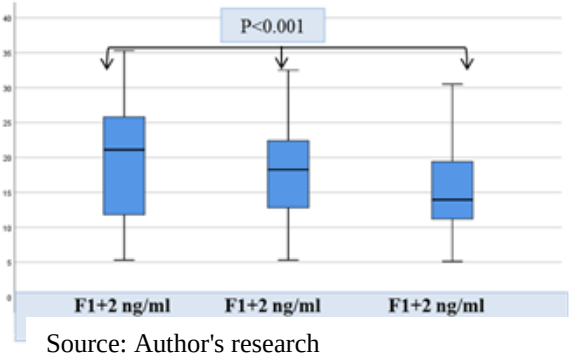
TAT levels were significantly lower at Visit 2 - 7.83 ng/ml (5.54–11.8 ng/ml) and visit3 - 7.06 ng/ml (5.12–9.26 ng/ml), compared with Visit 1, 8.52 ng/ml (6.28–12.64 ng/ml), p=0.018 and p=0.006 (Fig. 3). F1+2 levels progressively declined across the three assessments: first visit (19.70 ± 8.27 ng/ml), second visit (17.75 ± 6.35 ng/ml), and third visit (15.80 ± 6.48 ng/ml). This downward trend was statistically significant, $p < 0.001$ (Fig. 4). Plasma fibrinogen levels did not differ significantly across the three assessment points: visit 1 (3.61 ± 1.09 g/L), visit 2 (3.46 ± 0.82 g/L), and visit 3 (3.42 ± 0.96 g/L), with a p-value of 0.438 (Fig. 5).
Antithrombin III (ATIII) activity showed a significant increase at visit 3 (88.15 ± 6.99%) compared with visit 2 (86.72 ± 7.13%), with a p-value of 0.023 (Fig. 6).

Fig. 3 TAT ng/ml in patients with breast cancer throughout treatment



Source: Author's research

Fig. 4 F1+2 ng/ml in patients with breast cancer throughout treatment



Source: Author's research

Fig. 5 Fibrinogen g/l in patients with breast cancer throughout treatment

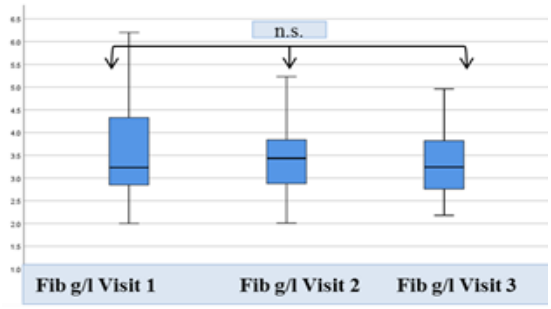
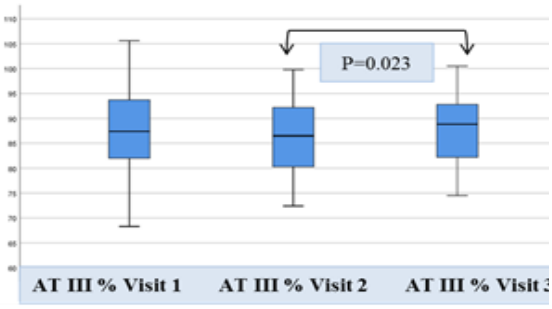


Fig. 6 AT III % in patients with breast cancer throughout treatment



4. DISCUSSION

In our study, we evaluate longitudinal changes in markers of coagulation and fibrinolysis in 38 women with breast cancer throughout the course of systemic treatment. Our findings demonstrate that hemostatic markers - TF, TAT, F1+2 and D-dimer decreased significantly across treatment visits, indicating a gradual reduction in hypercoagulability as therapy progressed. These results are consistent with the established concept that effective oncological treatment can mitigate tumor-induced coagulation activation and contribute to reducing thrombotic risk in this patient population (Tinholt et al., 2024) (Moik et al., 2022).

Tissue factor - a molecule, often overexpressed in tumor cells, showed a significant decrease at the second and third visits compared with baseline. This decrease likely reflects reduced tumor burden or reduced tumor-associated inflammation during therapy. Recent studies have highlighted that TF expression in cancer may not only promote thrombosis but also tumor progression and metastasis, making its decline throughout systemic treatment clinically relevant (Ahmadi et al., 2023). D-dimer as an indicator of active coagulation and fibrinolysis also decreased significantly across time points. The initial elevation of this biomarker is consistent with previous studies demonstrating increased fibrin turnover and fibrin accumulation in cancer patients. The subsequent decrease may suggest attenuated activation of coagulation and decreased fibrinolytic activity as therapy progresses. Various studies of hemostatic biomarkers in cancer patients have reported that a decrease in D-dimer is frequently observed with effective therapy and may have prognostic implications (Moik et al., 2022), (Alkhoder et al., 2024). TAT and F1+2, both sensitive markers of thrombin generation, also decreased significantly across visits. This further supports the assumption that the activation of coagulation system attenuated throughout systemic treatment. Taken together, the lower levels of TF, TAT, D-dimer, and F1+2 after completing therapy provide a consistent picture of decreasing thrombin generation in cancer patients (Gyldenholm et al., 2024). Fibrinogen levels remained stable across all time points in breast cancer patients. As both a factor of coagulation and an acute-phase reactant, fibrinogen is influenced by inflammation, liver function, and other systemic processes. Its stability throughout treatment suggests that therapy did not substantially alter these broader physiological determinants. ATIII activity increased significantly by the third visit. As a natural anticoagulant, antithrombin plays a key regulatory role by inhibiting thrombin and factor Xa. The increase in ATIII activity may represent a compensatory normalization of natural anticoagulant mechanisms as the overall activation of coagulation decreases. Englisch et al. (2022) reported that cancer patients with very low levels of AT III had poorer overall survival. In the prospective Vienna CATS study, significantly higher levels of P-selectin, F1+2, D-dimer and factor VIII were observed before therapy initiation in cancer patients who later developed VTE (Königsbrügge et al., 2014).

Several limitations should be taken into account when evaluating these findings. To begin with, the study involved a relatively small cohort (n = 38), which may limit the ability to identify more subtle alterations in hemostatic parameters. Additionally, the follow-up consisted of only three assessment points, potentially missing longer-term changes or late effects of treatment. Third, the study did not assess clinical outcomes such as thrombotic events and therapeutic response in parallel with the changes in biomarkers, which limits the ability to assess the prognostic or clinical significance of the observed tendency. Future studies with larger cohorts, longer follow-up, detailed stratification of therapy, and integration of clinical outcomes are needed to improve the interpretation of these findings.

Although coagulation activation markers declined over the course of follow-up, F+2, TAT, TF, and D-dimer levels still remained elevated after systemic therapy compared with the control group. These persistent, subtle coagulation abnormalities are typical of patients with breast cancer, indicating that a prothrombotic state is present both before therapy and during systemic treatment.

5. CONCLUSION

In this study, we demonstrated that patients with breast cancer exhibit marked activation of coagulation at the time of initiation of therapy, as reflected by increased levels of TF, TAT, F1+2, and D-dimer, along with decreased ATIII activity. Although systemic treatment resulted in significant reduction in key hemostatic markers, their levels remained higher than those of healthy controls, indicating that a prothrombotic state persists throughout the course of treatment.

The observed reduction in markers of thrombin generation suggests a gradual attenuation of tumor-associated hypercoagulability during therapy. However, the persistence of subclinical coagulation disorders highlights the ongoing hemostatic imbalance characteristic of cancer, even in the context of active antitumor treatment. These findings highlight the importance of monitoring coagulation parameters in breast cancer patients, as they may help identify individuals at increased risk for thrombotic complications.

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