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Role of P-selectin in thromboembolic events in patients with cancer

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Abstract. The objective of the present study was to evaluate the role of P-selectin in patients with cancer with suspected thromboembolic events (TEEs). Patients with cancer have a four times greater risk of developing TEEs. P-selectin is a glycoprotein that has the function of facilitating the interaction (adhesion) of leukocytes with the endothelium, or with platelets. There is a well-defined relationship between P-selectin and thrombosis; however, it is likely that the cut-off value of P-selectin for patients with cancer should be considered differently from that of the general population. In the present report, a prospective cross-sectional study was performed with patients of the Cancer Hospital of Barretos who were suspected of having TEEs. Among the 178 study participants, 167 (93.82%) were suspected of having deep vein thrombosis, while 59 of them (35.33%) were confirmed as such; and 11 (6.18%) were suspected of having pulmonary thromboembolism, while 3 of them were confirmed as such (27.69%). The mean results obtained were: P-selectin, 25.37 ng/ml; and D-dimer, 2,181.22 ng/ml. The P-selectin levels averaged 33.60 ng/ml with the confirmed TEE group compared with 20.40 ng/ml with the unconfirmed TEE group, with a standard deviation of 23.35 compared with 6.92 ($P<0.001$); and the level of D-dimer was 4,615.38 ng/ml compared with 977.52 ng/ml, with a standard deviation of 6,460.54 compared with 2,145.50 ($P<0.001$). Multiple logistic regression adjusted for distant metastases and the Eastern Cooperative Oncology Group (ECOG) score (2,3 and 4) were constructed. The cut-off value of P-selectin for patients with cancer was identified to be different from that reported in the literature for the general population, and the models using D-dimer and P-selectin therefore have been demonstrated to be a potentially useful tool to be used in a panel of tests to predict TEEs, either independently or in a prediction score.

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Introduction

The legendary pathologist, Rudolph Virchow, identified more than a century ago the three most important pathophysiological mechanisms for the development of thrombosis (i.e., hypercoagulability, blood stasis and endothelial injury) (1), parameters that are still relevant for, and of interest to, the scientific community. The incidence of deep vein thrombosis (DVT) in the United States is ~117 cases per 100,000 people/year (2); several published studies have included numbers that varied between 43 and 145 cases per 100,000 people/year; and pulmonary embolism (PE) has been identified in 20-65 cases per 100,000 people/year (2-7). In ~15% of these cases, the thromboembolic events (TEEs) are the first manifestation of a neoplasia (8). Patients with cancer have a four times greater risk of developing DVT compared with patients without cancer. If a person is on chemotherapy, this risk increases to up to six times compared with a person without cancer (9). Moser *et al* (10) have demonstrated that 43% of the patients treated for DVT without symptoms for TEEs had abnormal thorax scans, suggesting a high likelihood of lung embolism. Furthermore, ~70% of the patients had indications of PE, suggesting DVT without presenting symptoms had been identified (11,12). The rates of recurrence of idiopathic DVT are ~7.8% per year, but if a patient has active cancer, the recurrence rate will be much higher (~14% per year), reaching a percentage of 30.4% of recurrence in 8 years (9,13,14).

There are several risk factors for the development of TEEs, including an advanced age, obesity, any previous TEE, surgery, trauma, antiphospholipid antibody, thrombophilia, nephrotic syndrome, heart failure, chronic immobilization, central venous catheter and active neoplasia, predominantly in association with chemotherapy (15-18). Approximately 25-50% of cases are considered to be idiopathic, i.e. these cases do not have a well-defined etiological cause to explain

the occurrence of the TEE (9,19). TEE in patients with cancer is a major complication, and patients may be even worse off as a consequence of the TEE relative to the cancer itself.

A plethora of biological markers have been studied in order to gain an improved understanding of TEE in patients with cancer. The D-dimer is such a biomarker. D-dimer is a product of the degradation of fibrin, and has proven to be very useful in excluding PE in patients with a low PE pretest probability score (20,21). However, in certain situations, such as in cases of surgery, acute myocardial infarction and septicemia, the D-dimer value may be shown to be reduced, as occurs in patients with cancer (22,23). P-selectin is a glycoprotein present in the endothelium and platelets (24,25) that, through the mediation of tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), binds to leukocytes, primarily to neutrophils (26), with the function of facilitating the interaction (adhesion) of leukocytes with the endothelium or with platelets (27), besides exerting different roles in various stages of inflammation (28). A well-defined association exists between elevated serum levels of P-selectin and thrombosis (29). An elevated level of P-selectin is a useful parameter to identify patients who are at risk of thrombotic events, showing an odds ratio of 2.6 for patients with serum levels of P-selectin >53.1 ng/dl after having been adjusted for age, sex, surgery, chemotherapy and radiotherapy. However, there are a group of patients who present abnormal values of P-selectin without TEEs (30-32). In all likelihood, the cut-off value of P-selectin for patients with cancer should be considered differently from that of the general population, given the important role of this protein in hematogenic metastasis (33).

Studies of TEEs in populations of cancer biomarkers remain scarce, particularly with respect to the role of P-selectin, where more accurate investigations are required to test the impact of P-selectin evaluation on TEE early diagnosis. The objective of the present study was to evaluate the role of P-selectin in patients with cancer suspected of TEE, and to compare these values with the classical parameters of hematological alterations in this population.

Patients and methods

The present study is a cross-sectional study with a prospective collection of data, performed with patients with cancer suspected of having TEEs, who were monitored or underwent treatment in the Cancer Hospital of Barretos (HCB) between November 2013 and June 2014. Patients over 18 years of age agreed to undergo imaging tests in order to confirm or exclude the diagnosis of TEEs. The Research Ethics Committee of the Barretos Cancer Hospital approved the present study.

Laboratory tests. Peripheral blood samples were collected from all the patients after signing a consent form that explained the proposals of this project. The following tests were performed: Fibrinogen (FBN) plasma levels were determined according to dosage by the Clauss method using an automated coagulation analyzer (Tcoag Destiny Max™; Trinity Biotech UK, Bray, Co. Wicklow, Ireland) and a specific kit (Tcoag TriniCLOT™; Trinity Biotech UK). Expected values ranged from 175-400 mg/dl (34). TNF-α levels were determined by quantitative measurement, using a solid-phase, sequential chemiluminescent immunometric assay (IMMULITE® 1000 Chemiluminescent Technology; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). Expected values were up to 8.1 pg/ml. Levels of activated Factor Xa (AFXa) were assessed using a chromogenic method (Tcoag Destiny Max™; Trinity Biotech UK) and a specific kit (Tcoag TriniCLOT™; Trinity Biotech UK). Ultra-sensitive C-reactive protein (hsCRP) was quantified using monoclonal antibodies with an immunonephelometric method on automated equipment (BN Systems II, incorporating a Cardio Phase hsCRP kit; Siemens Healthcare Diagnostics Products, Marburg, Germany). The reference range was <1.0 mg/l for risk assessment of vascular disease, and <5.0 mg/l for an evaluation of inflammatory/infectious processes. IL-6 levels were determined by quantitative measurement using a solid-phase, sequential chemiluminescent immunometric assay (IMMULITE® 1000 Chemiluminescent Technology; Siemens Medical Solutions Diagnostics). The expected values were up to 5.9 pg/ml. The D-dimer concentration was evaluated using an immunoturbidimetric method, by an agglutination test: Polystyrene microparticles with fibrin degradation products containing the D-dimer were quantitatively determined in human citrated plasma. The equipment used was a coagulation analyzer (Tcoag Destiny Max™; Trinity Biotech UK), and the reference value was 500 ng/ml. P-selectin levels were determined quantitatively using enzyme immunoassay/enzyme-linked immunosorbent assay, with an immune kit (IBL IMMUNO-Biological Laboratories, Co. Ltd., Fujioka, Japan). Expected values of P-selectin in the serum ranged from 67-233 ng/ml [in the plasma (EDTA) they range from 50-233 ng/ml; in the plasma (citrate), from 92-212 ng/ml; in the plasma (heparin), from 60-188 ng/ml. As our protocol was performed with plasma, a value of 233 ng/ml was accepted as being normal].

Sample size. The sample size was calculated taking into account a study by Ramacciotti *et al* (35) that evaluated 178 patients suspected of DVT; the authors of that study identified that, in 62 cases, there was confirmation of the event. Comparing the serum levels of P-selectin from the groups with or without DVT, a significant difference was observed between them. Considering a significance of 5% and a test power of 90%, and clinical relevance with values ranging from 20-30 ng/dl between the groups, the present authors calculated that there were 24-55 cases of TEEs, as shown in Table I. Statistical analyses were performed using IBM-SPSS software, version 21 (IBM SPSS, Inc., Armonk, NY, USA). $P<0.05$ was considered to indicate a statistically significant value.

Results

Among the 178 patients, 108 (60.70%) were female and 70 (39.30%) were male. Detailed socio-demographic information concerning the patients is shown in Table II.

An examination of the clinical history of the patients revealed that 79 (44.38%) participants had hypertension, 27 (15.25%) had diabetes mellitus, 56 (54.24%) had varicose veins, 80 (44.94%) declared that they had never smoked, 89 (50.00%) had no restrictions on mobility, which permitted the inclusion of the Eastern Cooperative Oncology Group (ECOG) classification (36), and 86 (48.31%) and 72 (40.45%)

Table I. Frequency of the clinical variables of the patients enrolled in the present study.

Variable	Specification	Frequency	%
Sex	Female	108	60.70
	Male	70	39.30
Hypertension	No	99	55.62
	Yes	79	44.38
Diabetes mellitus	No	150	85.75
	Yes	27	15.25
Varicose veins	No	81	45.76
	Yes	96	54.24
Smoking	Never	80	44.94
	Refrained from smoking for >6 months	72	40.45
	Current smoker	26	14.61
Mobility	No restriction on locomotion	89	50.00
	Walks unaided, but with difficulty	56	31.46
	Walks only with help from others	21	11.80
	Cannot walk, but sits out of bed	5	2.81
	Confined to bed	7	3.93
ECOG	0	31	17.42
	1	86	48.31
	2	30	16.85
	3	21	11.80
	4	10	5.62
Body mass index	Underweight	12	6.74
	Normal	72	40.45
	Obesity grade I	51	28.65
	Obesity grade II	25	14.04
	Obesity grade III	12	6.74
Primary site of the cancer	Breast	34	19.10
	Uterine cervix	19	10.64
	Lung	11	6.18
	Rectum	11	6.18
	Prostate	9	5.06
	Others	94	52.81
Chemotherapy	Not administered to date	48	26.97
	Ongoing	87	48.88
Clinical hypothesis	Deep vein thrombosis	167	93.82
	Pulmonary thromboembolism	11	6.18
Deep vein thrombosis	Negative	108	64.67
	Positive	59	35.33
Pulmonary thromboembolism	Negative	8	72.73
	Positive	3	27.27

ECOG, Classification according to the Eastern Cooperative Oncology Group.

of the participants had a body mass index (BMI) within the normal range, according to the Brazilian Guidelines for Obesity, 2011 (37,38). Among the participants, 34 (19.10%) had been diagnosed with breast cancer, 19 (10.64%) had uterine cervical cancer, 11 (6.18%) had lung cancer, 11 (6.18%) had rectal cancer, 9 (5.06%) had prostate cancer and 99 (52.81%) had been diagnosed with other cancers; 175 (98.31%) of the patients had active cancer, 90 (50.56%) had distant metastases, 87 (8.88%) were under chemotherapy treatment, and 96 (32.32%) had completed chemotherapy; 77 (59.23%) of the chemotherapy treatments were palliative, and 107 (60.11%) patients were not submitted to radiotherapy; regarding those

Table II. Detailed description of the laboratory findings.

Variable	n	Mean	Standard deviation	Minimum	1st quartile	Median	3rd quartile	Maximum
Hemoglobin (g/dl)	170	10.79	2.04	4.00	9.00	11.00	12.00	16.00
Hematocrit (%)	170	32.78	5.68	11.00	28.75	34.00	47.00	44.00
Platelets (k/mm^3)	170	245.62	252.00	1.00	153.00	228.00	280.75	3,026.00
Creatinine (mg/dl)	159	1.08	1.22	0.20	0.70	0.80	1.02	10.00
FBN (mg/dl)	146	427.14	145.48	45.00	331.00	413.00	514.75	830.00
TNF-α (pg/ml)	136	16.76	16.23	4.00	9.43	11.90	17.00	109.00
AFXa (%)	146	105.43	28.76	18.00	85.50	103.00	128.00	179.00
hsCRP (mg/l)	138	52.62	66.86	0.29	6.36	20.20	82.43	334.00
IL-6 (pg/ml)	120	22.86	41.75	2.00	4.28	8.85	23.45	298.00
D-dimer (ng/ml)	136	2,181.22	4,429.92	46.00	166.25	576.50	1,693.75	23,796.00
P-selectin (ng/ml)	117	25.37	16.52	3.00	16.50	20.80	27.90	120.70

FBN, fibrinogen, TNF-α, tumoral necrosis factor-α; AFXa, activated factor Xa; hsCRP, ultrasensitive C-reactive protein; IL-6, interleukin-6.

patients for whom radiotherapy was performed, 66 (61.11%) had palliative radiotherapy indication, and 110 (61.80%) among all participants had undergone surgical treatment proposed for the treatment of cancer. A total of 167 participants (93.82%) were suspected of DVT: 59 of them (35.33%) were confirmed as such, and 11 (6.18%) were suspected of PE, with 3 of them being confirmed as such (27.69%). Six (3.41%) patients were treated for DVT, and one (0.57%) was receiving treatment for PE; one (0.56%) was receiving an oral anticoagulant treatment, 5 (3.13%) were receiving injectable anticoagulants, 15 (9.38%) were receiving an antiplatelet medicament and 5 (5.26%) had been administered oral contraceptives (Table I). Upon the suspicion of a TEE, the average age of the patients was 56.39 years.

The mean results of the blood examinations are shown in Table II: Hemoglobin was 10.79 g/dl; hematocrit, 32.78%; platelets, 245.62 k/mm^3; creatinine, 1.08 mg/dl; FBN, 427.14 mg/dl; TNF-α, 16,76 pg/ml; activated factor X (AFXa), 105.43%; hsCRP, 52.62 mg/l; IL-6, 22.86 pg/ml; D-dimer, 2,181.22 ng/ml; and P-selectin was 25.37 ng/ml.

Among the cases positive for a TEE, 30 (48.39%) of the participants were women compared with 78 (67.24%) in the negative group (P=0.014) (Table III). Comparisons of the clinical background between the groups of the 62 positive cases and the 116 cases negative for the TEEs were as follows: Hypertension, 28 (45.16%) compared with 51 (43.97%; P=0.878); diabetes, 11 (18.03%) compared with 16 (13.79%; P=0.456); never used tobacco, 27 (43.55%) compared with 53 (45.69%; P=0.527); currently smoking, 7 (11.29%) compared with 19 (16.38%); had no restrictions on locomotion or walking with difficulty, but without assistance, 47 (75.81%) compared with 98 (84.48%); not walking, or walking only with the help of others or was confined to bed, 10 (16.13%) compared with 15 (13.80%; P=0.020); had an ECOG score of 1, 25 (40.32%) compared with 61 (52.59%; P=0.002); normal BMI, 32 (51.61%) compared with 40 (34.48%; P=0.045). Further details concerning the data regarding treatment of the patients are shown in Table III.

As shown in Table IV, the mean value of hemoglobin was 10.29 g/dl in the positive group compared with 11.05 g/dl in the negative group (P=0.007); the mean value of the hematocrit test was 31.38% compared with 33.51% (P=0.007); platelets were 220.14 k/mm^3 compared with 258.83 k/mm^3; creatinine was 1.19 mg/dl compared with 1.01 mg/dl; FBN was 41,146 mg/dl compared with 435.81 mg/dl; TNF-α was 14.76 pg/ml compared with 17.85 pg/ml; AFXa was 102.94% compared with 106.81%; hsCRP was 68.42 mg/l compared with 43.92 mg/l (P=0.056); IL-6 was 24.83 pg/ml compared with 21.72 pg/ml, with a standard deviation of 27.02 compared with 48.4 (P=0.032). The mean level of D-dimer was 4,615.38 ng/ml compared with 977.52 ng/ml, with a standard deviation of 6,460.54 compared with 2,145.50 (P<0.001), and the P-selectin averaged 33.60 ng/ml compared with 20.40 ng/ml, with a standard deviation of 23.35 compared with 6.92 (P<0.001). Further details are shown in Table IV.

Receiver operating characteristic (ROC) curves were used to determine the best cut-off point for each test, which allowed a comparison to be made between the two groups: Positive and negative for TEE. The best cut-off point identified for the hemoglobin was 10 g/dl, that discriminated 23 positive participants (39.66%) from 71 (63.39%) negative ones (P=0.003); for hematocrit, a value >32% was the preferred cut-off, with 20 (34.48%) positive participants compared with 74 (66.07%) negative (P<0.001). Similarly, the cut-off points, and numbers of positive and negative participants for the further variables, were as follows: Platelet >240 k/mm^3, 20 (34.48%) positive compared with 38 (65.52%) negative (P=0.166); creatinine >0.8 mg/dl, 32 (54.24%) positive compared with 40 (39.60%) negative (P=0.081); FBN >393 mg/dl, 23 (44.23%) positive compared with 58 (61.7%) negative (P=0.042); TNF-α >12.9 pg/ml, 25 (51.02%) positive compared with 29 (32.58%) negative (P=0.034); AFXa, >104%, 20 (38.46%) positive compared with 51 (54.26%) negative (P=0.067); hsCRP >20.6 mg/l, 31 (60.78%) positive compared with 36 (40.00%) negative (P=0.018); IL-6 >11.3 pg/ml, 28 (58.33%) positive compared with 25 (28.09%) negative (P=0.001); and levels

Table III. Comparison of the groups with or without thromboembolic events.

Variable	Specification	Positive		Negative		P-value
		n	(%)	n	(%)	
Sex	Female	30	48.39	78	67.24	0.014
	Male	32	51.61	38	32.76	
Mobility	No restrictions on locomotion	26	41.94	63	54.31	0.216
	Walks unaided, but with difficulty	21	33.87	35	30.17	
	Walks only with help from others	8	12.90	13	11.21	
	Cannot walk, but sits out of bed	2	3.23	3	2.59	
	Confined to bed	5	8.06	2	1.72	
ECOG score	0	5	8.06	26	22.41	0.002
	1	25	40.32	61	52.59	
	2	6	25.81	14	12.07	
	3	9	14.52	12	10.34	
	4	7	11.29	3	2.59	
Body mass index	Low weight	5	8.06	7	6.03	0.047
	Normal	32	51.61	40	34.48	
	Overweight	13	20.97	38	32.76	
	Obesity I	9	14.52	16	13.79	
	Obesity II	1	1.61	11	9.48	
	Obesity III	2	3.23	4	3.45	
Primary site of cancer	Breast	5	8.06	29	25.0	0.047
	Uterine cervix	5	8.06	14	12.07	
	Lung	4	6.45	7	6.03	
	Rectum	3	4.84	8	6.90	
	Prostate	4	6.45	5	4.31	
	Others	41	66.13	53	45.69	
Chemotherapy	Not performed to date	20	32.26	28	24.14	0.463
	Ongoing	27	43.55	60	51.72	
	Treatment concluded	15	24.19	28	24.14	
Surgical treatment	No	22	35.48	46	39.66	0.585
	Yes	40	64.52	70	60.34	
Clinical hypothesis	Deep vein thrombosis	59	95.16	108	93.10	0.587
	Pulmonary thromboembolism	3	4.84	8	6.90	

ECOG, Eastern Cooperative Oncology Group.

of D-dimer >633 ng/ml, 39 (76.47%) positive compared with 33 (35.11%) negative (P<0.001). Finally, the cut-off point for P-selectin was >21.6 ng/ml, and there were 28 (63.6%) positive participants compared with 23 (31.51%) negative ones (P<0.0015).

In order to identify the association between the variables that might exert an influence on the occurrence of TEEs, multiple logistic regression adjusted for distant metastases and the ECOG score (2,3 and 4) were used. All variables with a P-value <0.2 were selected: Mobility, need for hospitalization, height, time elapsed between the first consultation and the TEE, body mass, hematocrit, hemoglobin, FBN, TNF-α, hsCRP, IL-6, D-dimer levels, and P-selectin levels. Additional multiple logistic regressions were constructed. The first (regression 1) had, as variables, distant metastasis, ECOG (scores 2, 3 and 4), sex, and D-dimer. For this regression model, the following results (odds ratios) were obtained: Distant metastasis of 1.338 (P=0.479), ECOG (scores 2, 3 and 4) of 3,039 (P=0.009), sex of 2.557 (P=0.023) and D-dimer of 5.072 (P<0.001). The second logistic regression (regression 2) had, as variables, distant metastasis, ECOG (scores 2, 3 and 4), sex and P-selectin, which revealed odds ratios for distant metastasis of 2.488 (P=0.040), ECOG (scores 2, 3 and 4) of 4.150 (P=0.003), sex of 2.709 (P=0.029), and P-selectin of 3.385 (P=0.007) (Table V).

The data reported above were used to prepare 4 predictive scoring models, ranging from 0 to 1, of which 1 indicated the presence of TEE and 0 indicated 'no event'. The score predictor 1 was built from regression 1 (with D-dimer) based on the sum of the approximate values of the odds ratio obtained in the regression. For this model, the sum is able to range from 0 to 1 point. In the group with 0 (zero) points, the probability of having TEE was 5.7%; for 3 points, 23.5%; for 5 points,

Table IV. Association between laboratory characteristics and thromboembolic events.

	Positive			Negative			
Variable	n	Mean	SD	n	Mean	SD	P-value
Hemoglobin (g/dl)	58	10.29	1.92	112	11.05	2.07	0.007
Hematocrit (%)	58	31.38	5.22	112	33.51	5.80	0.007
Platelets (k/mm^3)	58	220.14	97.61	112	258.83	302.99	0.645
Creatinine (mg/dl)	59	1.19	1.40	100	1.01	1.12	0.136
FBN (mg/dl)	52	411.46	149.96	94	435.81	143.01	0.240
TNF-α (pg/ml)	48	14.76	6.48	88	17.85	19.57	0.215
AFXa (%)	52	102.94	25.72	94	106.81	30.35	0.439
hsCRP (mg/l)	49	68.42	73.39	89	43.92	61.70	0.056
IL-6 (pg/ml)	44	24.83	27.02	76	21.72	48.40	0.032
Dimer-D (ng/ml)	45	4,615.38	6,460.54	91	977.52	2,145.50	<0.001
P-selectin (ng/ml)	44	33.60	23.35	73	20.40	6.92	<0.001

SD, standard deviation; FBN, fibrinogen, TNF-α, tumoral necrosis factor-α; AFXa, activated factor Xa; hsCRP, ultrasensitive C-reactive protein; IL-6, interleukin-6.

Table V. Logistic regression of thromboembolic events with the variables: Distant metastasis, ECOG (2,3 and 4), sex, and D-dimer (regression 1).

							CI, 95%	
	Variable	B	SD	Wald	df	P-value	OR	IL	SL
D-dimer	Metastasis	0.291	0.411	0.501	1	0.479	1.338	0.597	2.996
	ECOG score (2, 3 4)	1.112	0.424	6.882	1	0.009	3.039	1.325	6.973
	Sex	0.939	0.412	5.198	1	0.023	2.557	1.141	5.730
	D-dimer	1.624	0.426	14.519	1	<0.001	5.072	2.200	11.694
	Constant	-2.438	0.460	28.115	1	<0.001	0.087		
P-selectin	Metastasis	0.911	0.444	4.207	1	0.040	2.488	1.041	5.943
	ECOG score (2, 3 and 4)	1.423	0.479	8.825	1	0.003	4.150	1.623	1.613
	Sex	0.997	0.457	4.758	1	0.029	2.709	1.106	6.635
	P-selectin	1.219	0.452	7.280	1	0.007	3.385	1.396	8.208
	Constant	-2.440	0.514	22.573	1	0.000	0.087		

B, regression coefficient; SD, standard deviation; df, degrees of freedom; the Wald test (t-test), OR, odds ratio; CI, confidence interval; LI, inferior limit, LS, superior limit; ECOG, classification according to the Eastern Cooperative Oncology Group.

39.3%; for 6 points, 50.0%; and for 8 points and 11 points, 56.7% and 78.6%, respectively. The prediction score 2 was constructed using the sum of the approximate odds ratio of regression 2 (with P-selectin). This score ranges from 0 to 12 points: 0 points shows a 13.6% probability of presenting TEE; for 2 points, 25.0%; for 3 points, 11.8%; for 4 points, 0.00%; for 5 points, 38.5%; for 6 points, 40.0%; for 7 points, 55.6%; for 8 points, 60.0%; for 9 points, 88.9%; for 10 points 100.0% and for 12 points, 83.3% (Table VI).

In order to facilitate an understanding of what the clinical findings signify, the predicted score 1 (with ECOG 1, 2 and 3, sex and D-dimer) was classified into four levels of risk: Low risk (0-3 points), moderate risk (5-6 points), high risk (8 points) and very high risk (9 points), with the following likelihood of a TEE: 14.5, 40.6, 56.7 and 78.6%, respectively (Table VII). As in the previous model, the predictive score 4 was also prepared with the intention of classifying the likelihood of having a TEE, with reference to the predictive score 2 with P-selectin. The tracks were divided as follows: 0-4 points, classified as low risk (14.0%); 5-6 points, classified as moderate risk (38.9%); 7-8 points, classified as high risk (57.1%); and 9 or more points, classified as 88.2% (Table VII).

Discussion

This study corroborated, in part, data that previously reported the TEE as an important phenomenon to be judiciously evaluated for patients with cancer subjected (or not) to treatment, as

Table VI. Score predictors of a thromboembolic event, based on logistic regression.

Variable	Score	Negative (%)	Positive (%)	Total
D-dimer	0	33 (94.3)	2 (5.7)	35
	3	26 (76.5)	8 (23.5)	34
	5	17 (60.7)	11 (39.3)	28
	6	2 (50.0)	2 (50.0)	4
	8	13 (43.3)	17 (56.7)	30
	11	3 (21.4)	11 (78.6)	14
P-selectin	0	19 (86.4)	3 (13.6)	22
	2	6 (75.0)	2 (25.0)	8
	3	15 (88.2)	2 (11.8)	17
	4	3 (100.0)	0 (0.0)	3
	5	16 (61.5)	10 (38.5)	26
	6	6 (60.0)	4 (40.0)	10
	7	4 (44.4)	5 (55.6)	9
	8	2 (40.0)	3 (60.0)	5
	9	1 (11.1)	8 (88.9)	9
	10	0 (0.0)	2 (100.0)	2
	12	1 (16.7)	5 (83.3)	6

Table VII. Predictive score for TEE risk.

Variable	Score	Risk classification	Negative (%)	Positive (%)	Total
D-dimer					
	0 to 3	Low risk	59 (85.5)	10 (14.5)	69
	5 to 6	Moderate risk	19 (59.4)	13 (40.6)	32
	8	High risk	13 (43.3)	17 (56.7)	30
	9	Very high risk	3 (21.4)	11 (78.6)	14
P-selectin	0 to 4	Low risk	43 (86.0)	7 (14.0)	50
	5 to 6	Moderate risk	22 (61.1)	14 (38.9)	36
	7 to 8	High risk	6 (42.9)	8 (57.1)	14
	≥9	Very high risk	2 (11.8)	15 (88.2)	17

we have identified a total of 36.03% of confirmed TEEs. Such events are particularly significant considering that, among 297 patients recruited in a period of 231 days, the occurrence of thromboembolism was confirmed for 107 of them, with an average of a TEE confirmed every 2.15 days. These figures confirm the importance of this issue, and the urgency to improve strategies of prevention and early diagnosis.

The average age of the patients was 56.58 years, and therefore no relevant difference between groups with or without a TEE was observed. Nevertheless, there was a statistically significant difference between the sex. In men, the positive group represented 48.62%, whereas for the negative group, it was 34.64%. However, these data are contestable, since the TEE diagnostic confirmation occurred in 51.37% of the women and 48.62% of the men (P=0.028), which corroborated data published previously in the literature (4,5,9,14).

No difference was identified between the groups associated with systemic arterial hypertension, diabetes mellitus or smoking. Regarding the group positive for TEE, the percentage with a hypertensive condition was 40.43%, consistent with the values identified in the general population, ranging from 22.3 to 43.9% and averaging 32.5%; however, in the population aged 60-69 years, these values could increase up 50%, and >70 years, may reach 75% (39-41). The frequency of diabetes was 14.89%, higher than the average observed in the Brazilian population (5%). However, analyzing higher age groups, for example, between 70 and 79 years, the percentage can rise up to 17.5% (42). This is a relevant fact in this population, since the association between diabetes and abnormal vascular events has been firmly established. Active smoking was identified in 12.46% of the patients, slightly below the national average, which is 16.1% (43). It is known that smoking is an independent risk factor for TEEs (16,44); however, the difference identified (8.26% in the positive group and 14.98% in the negative group) could be accounted for by chance, since the P-value was not significant (P=0.234).

The literature provides robust data concerning the association between decreased mobility and occurrence of TEEs (2,17,45). A large number of patients presented ECOG scores of 0 or 1 (55.22%). A total of 55.96% of the patients had restriction (ECOG 2, 3 and 4) in the positive group, with the smallest percentage being identified in the negative group (38.3%). Similar reasoning can be applied to mobility, since many patients had restrictions on their locomotion (35.14% in the positive group and 76.59% in the negative group), confirming once again that there is a correlation between restriction on movement and the occurrence of TEE.

Although obesity is a risk factor for TEE (18), 22.71% of the patients had obesity grades I, II or III (the BMI was classified as follows: <18, underweight; 18-24.9, normal weight; 25-29.9, obesity grade I; 30-34.9, obesity grade II; ≥30, obesity grade III); the BMI values were 14.02% for the positive group, and 27.26% for the negative group. Therefore, the obesity rate was lower in the TEE positive group. It is possible that this association has been influenced by other factors, as patients with metastases, clearly weakened, or at an advanced stage of the cancer disease are at higher risk for TEEs; however, they are also underweight, due to the state of patient consumption.

The occurrence of TEEs in the cancer population may reach 25%. In the current study, 18 (16.82%) of the 107 patients who were receiving anticoagulant therapy were confirmed to have TEEs due to previous TEE treatment.

The laboratory tests revealed the association between hemoglobin and hematocrit with TEEs, possibly due to the correlation of a decreased in the levels of hemoglobin with advanced cancer and, consequently, a higher risk of progressing to TEEs. IL-6, D-dimer and P-selectin levels were statistically different between the positive and negative groups, which revealed a potential use of these markers as ancillary tests for TEEs in patients with cancer.

The results from the present analysis disclosed several interesting laboratory characteristics that partly differed from those reported in the literature. Among them, the most intriguing was the value identified for P-selectin. While retaining the ability to differentiate between the groups with or without TEEs, the average values that were identified were much lower than had been expected. Also, the ROC curve indicated cut-off values for the D-dimer evaluation that were higher than those reported in the literature (633 ng/ml compared with 500 ng /ml, respectively).

In conclusion, despite being associated with more advanced tumors and more features of aggressive behavior (46), in terms of elevated serum levels of FBN, no association with TEEs was identified. Similar findings were observed regarding other parameters generally associated with TEEs (47), such as AFXa. The levels of hsCRP were revealed to be much higher than the values of reference, which was as expected (35), although these were not of value in potentially being of use for the prediction of TEEs. However, the cut-off value of P-selectin for patients with cancer was different from that reported in the literature in the general population, and the model using D-dimer and P-selectin has been revealed in the present study to be potentially suitable for use in a panel of tests to predict TEEs, either independently or in a prediction score.

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