Accepted Manuscript

Submission Date: 2022-01-10
Accepted Date: 2022-03-22
Publication Date: 2022-04-05

Thrombosis and Haemostasis

Do antiangiogenics promote clot instability? Data from the TESEO prospective registry and Caravaggio clinical trial

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DOI: 10.1055/a-1816-8347

Please cite this article as: Carmona-Bayonas A, Verso M, Sánchez Cánovas M et al. Do antiangiogenics promote clot instability? Data from the TESEO prospective registry and Caravaggio clinical trial. Thromb Haemost 2022. doi: 10.1055/a-1816-8347

Conflict of Interest: The authors declare that they have no conflict of interest.

This study was supported by Bristol-Myers Squibb (http://dx.doi.org/10.13039/100002491), Pfizer (http://dx.doi.org/10.13039/100004319), Rovi, Sanofi (http://dx.doi.org/10.13039/100004339)

Abstract:
Background: Venous thromboembolism (VTE) is a common complication in cancer patients. Much of its morbidity stems from the development of fatal pulmonary embolisms (PE). Little is known about the factors involved in clot stability, with angiogenesis possibly being implicated.
Methods: The database is from the TESEO prospective registry that recruits cancer patients with VTE from 41 Spanish hospitals. Independent validation was conducted in a cohort from the Caravaggio trial. The objective is to evaluate the association between exposure to antiangiogenic therapies and the PE/VTE proportion in oncological patients.
Results: 1536 subjects were evaluated; 58.4% (n=894) had a PE and 7% (n=108) received antiangiogenic therapy (bevacizumab in 75%). The PE/VTE proportion among antiangiogenic-treated individuals was 77/108 (71.3%) versus 817/1428 (57.2%) among those receiving other alternative therapies (p=0.004). The effect of the antiangiogenics on the PE/VTE proportion held up across all subgroups except for active smokers or those with chronic obstructive pulmonary disease. Exposure to antiangiogenics was associated with increased PEs, odds ratio (OR) 2.27 (95% CI, 1.42-3.63). In the Caravaggio trial, PE was present in 67% of the individuals treated with antiangiogenics, 50% of those who received chemotherapy without antiangiogenic treatment, and 60% without active therapy (p=0.0016).
Conclusions: Antiangiogenics are associated with increased proportion of PE in oncological patients with VTE. If an effect on clot stability is confirmed, the concept of thrombotic risk in cancer patients should be reconsidered in qualitative terms.

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ABSTRACT

Background: Venous thromboembolism (VTE) is a common complication in cancer patients. Much of its morbidity stems from the development of fatal pulmonary embolisms (PE). Little is known about the factors involved in clot stability, with angiogenesis possibly being implicated.

Methods: The database is from the TESEO prospective registry that recruits cancer patients with VTE from 41 Spanish hospitals. Independent validation was conducted in a cohort from the Caravaggio trial. The objective is to evaluate the association between exposure to antiangiogenic therapies and the PE/VTE proportion in oncological patients.

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Conclusions: Antiangiogenics are associated with increased proportion of PE in oncological patients with VTE. If an effect on clot stability is confirmed, the concept of thrombotic risk in cancer patients should be reconsidered in qualitative terms.

Keywords

antiangiogenic therapy

cancer

clot stability

pulmonary embolism

venous thromboembolism

INTRODUCTION

Deep vein thrombosis (DVT) and pulmonary embolism (PE) are typically considered manifestations of the same physiological process, encompassed under the denomination of venous thromboembolism (VTE) (1). Nevertheless, inasmuch as DVT, in and of itself, is not generally lethal in the short term, PE comprises a life-threatening event when fibrin fragments from the clot occlude enough of the pulmonary arterial territory (2,3). Despite its clinical relevance, little progress has been made in elucidating the mechanisms associated with clot stability (4). Nonetheless, factors impacting the viscoelasticity of the fibrin scaffolding must be studied if we are to understand the qualitative consequences of hypercoagulability states, contributing to risk-adapted management (4,5).

Cancer is among the most common causes of acquired thrombophilia (6). Oncological patients with VTE suffer increased short-term mortality, occasionally due to the direct effect of PE (3,7,8). Cancer-related PE entails a 15-day mortality rate of 10.1% (95% confidence interval [CI], 8.4–12.1) (9). Recent years have witnessed emphasis on the need to predict individual thrombotic risk (10). Even so, thromboprophylaxis in high-risk individuals has failed to improve survival (11), making new proposals essential. One way to move forward would be to investigate the mechanisms that give rise to thrombotic instability, prioritizing thromboprophylaxis when embolization entails high risk.

Angiogenesis, one of the most relevant hallmarks of cancer (12,13) which is regulated by the hemostatic system (12), is one of the processes possibly involved. Thus, the transglutaminase activity of factor XIII promotes clot stability and is pro-angiogenic (14). Antiangiogenics are among the most widely used targeted therapies (15). With these underpinnings, we have examined the TESEO thrombosis registry of the Spanish Society of Medical Oncology (SEOM) to evaluate whether antiangiogenic therapies are associated with PE in the oncological population. An independent validation was conducted in a cohort from the Caravaggio trial.

METHOD

Patients and study design

TESEO is an observational study sponsored by SEOM that prospectively and consecutively recruits patients at 41 Spanish hospitals. Inclusion criteria comprise being ≥18 years of age with a solid tumor and objectively detected VTE (e.g., Doppler ultrasound, computed tomography (CT), angiography scans, scheduled CT to assess tumor response, etc.). Exclusion criteria include superficial thrombophlebitis, the appearance of VTE prior to cancer diagnosis or after completing adjuvant treatment (>1 month in both cases). The study was approved by the Research Ethics Committee of all the Autonomous Communities and participating centers. All participants signed a written informed consent form.

The data were validated in patients from the randomized Caravaggio trial, the rationale and design of which have been previously published (16). Essentially, it is a non-inferiority phase III study that recruited subjects with cancer and incidental or symptomatic VTE. This population was randomized to receive apixaban or dalteparin. All the participants with active cancer at the time of VTE, except for those with hematological diseases, were selected for the validation. The scientific committee and independent statistical team analyzed the results separately.

Objectives and variables

The objective is to examine the association between the use of antiangiogenic therapies and PE/VTE proportion. PE was defined in the registry as an intraluminal contrast-filling defect measuring ≥2 mm visualized on two CT sections (CT pulmonary angiography or conventional contrast enhanced CT scans). In the event of isolated subsegmental PEs, investigators were required to verify the information with a thoracic radiologist. Diagnosis by Doppler ultrasound followed the usual criteria (e.g., non-compressibility, intraluminal thrombus, flow abnormality, etc.) according to the practice of each center. Diagnostic criteria in the Caravaggio trial were comparable (16).

Antiangiogenic therapy was considered to comprise any drug (antibody or tyrosine kinase inhibitor) targeting any molecule pertaining to the family of vascular endothelial growth factor (VEGF), its receptors, or other analogous molecules involved in angiogenesis (15). In both cohorts (TESEO and Caravaggio), antineoplastic treatment was defined as the therapy the patient was receiving at the time of thrombosis or had completed in the 30 days prior.

To appraise the association, model-building was carried out by means of subject-matter knowledge regarding causal mechanisms or sources of bias (17). The candidate predictors for the multivariable model were initially chosen after a review of the literature and conversation with the executive committee of the TESEO registry, made up of medical oncologists with expertise in thrombosis and cancer. Thus, variables that could theoretically affect the appearance or diagnosis of PE were selected as confounding factors or mediators: suspected VTE, prior VTE, associated chemotherapy, tumor type (colorectal vs others), tumor stage TMN (IV vs others), Eastern Cooperative Oncology Group Performance Status (ECOG-PS), and age. Other variables evaluated were the concurrent presence of DVT and PE characteristics (e.g., site, extension, association with symptoms).

Sensitivity analyses were also performed to examine how different factors influenced the conclusions. The point of these analyses is for the results to be more tenable if the magnitude of benefit did not change on the basis of the stratification factors (e.g., the association holds when only the CT-detected incidental events are considered). The selection criterion for these factors was based on the assumption that the PE detection

patters would be more homogeneous within each category, thereby probing the possibility of detection biases. To this end, bivariate analyses were used, stratified by the presence of active cancer, tumor stage, tumor type, type of antiangiogenic, cancer treatment, type of diagnosis (suspected vs not suspected), diagnostic method (CTPA, scheduled or unplanned CT), presence of recurring events, active smoking, comorbidities, and the use of antiplatelets agents. Other endpoints consisted of rate of venous rethrombosis and major/clinically relevant bleeding, as per the International Society on Thrombosis and Haemostasis (ISTH). Rethrombosis was defined as the appearance of a second thrombotic event following proper management of the index VTE or progression of the previous episode despite appropriate anticoagulant therapy.

Statistical methods

The Aalen–Johansen estimator was used to obtain the cumulative incidence function for rethrombosis and bleeding, in the presence of death as a competing event. Standard descriptive statistics were used, including absolute and relative frequencies, and differences in proportions. We provided 95% CIs when appropriate and considered a significance level of $P < 0.05$ in all statistical tests. Two-tailed P values were calculated. Comparisons between proportions were conducted by bivariate $\chi 2$-tests. Inference was accompanied by sensitivity analyses contemplating other factors that could impact results (see above). The association between PE and antiangiogenic therapy was further assessed by means of multivariable binary logistic regression, specified with the previously mentioned covariates. The Aalen–Johansen estimator was applied to obtain the cumulative incidence function for rethrombosis and bleeding, in the presence of death as a competing event. These descriptive analyses were executed using R version 4.01 (18).

RESULTS AND DISCUSSION

Patient characteristics

The TESEO registry database contains 1536 subjects with VTE diagnosed between July 2018 and December 2020. Of them, 58.2% (n=894) had a PE, whereas 41.8% (n=642) had other VTEs. Other concurrent thromboses were present in 174/894 (19.4%) of the patients with PE. At the time of VTE, 7% of the individuals (n=108) were receiving antiangiogenic therapy. Baseline characteristics are displayed in **Table 1**. PE were most often diagnosed by conventional computed tomography scan (either scheduled or unplanned) performed for reasons other than suspicion of pulmonary embolism in 59.6% (n=533), followed by computed tomography pulmonary angiography in 35.2% (n=315) (see diagnostic methods in **Annex Table 1**). The most common antiangiogenics were antibodies (bevacizumab in 81, ramucirumab in 2), VEGF trap (aflibercept in 10), or tyrosine kinase inhibitors (TKIs) (cabozantinib in 4; sunitinib in 3; sorafenib in 2; axitinib, pazopanib, regorafenib, and vandetanib in one case each, and an unspecified TKI in another 2) (**Table 2**).

The validation cohort from the Caravaggio trial comprises 1034 cases. Of them, 56% (n=579) were diagnosed with PE, whereas 44% (n=455) had a DVT. The remaining baseline characteristics can be found in **Table 3**. At the time of recruitment, 86/1034 (8.3%) of the Caravaggio study population were receiving antiangiogenic therapy (see **Table 2**).

Association between PE and antiangiogenic therapy in the TESEO cohort

PE was suffered by 77/108 (71.2%, 95% CI, 62.1-78.9%) of the individuals treated with an antiangiogenic versus 817/1428 (57.2%, 95% CI, 54.6-59.7%) subjects who were receiving other therapies (difference in proportions, 14.0%, 95% CI, 4.1-22.5%) ($\chi 2=8.186$, degrees of freedom (d.f.) =1, p=0.004) (**Annex Figure 1**). In order to make a preliminary evaluation of possible detection biases, the difference in proportions of subjects with or without an antiangiogenic was calculated in different subgroups. The effect of the antiangiogenic on the proportion of PE was maintained in all cases except in active smokers or patients with chronic obstructive pulmonary disease (COPD) (**Figure 1**). In particular, the association held in the subgroup of metastatic cancer and was independent of whether the antiangiogenic was bevacizumab and of diagnostic method (CTPA, scheduled or unplanned CT). Likewise,

despite the fact that most of the truly asymptomatic events, as well as symptomatic events for reasons other than the VTE, occurred most often after PE (see **Annex Table 2**), the association between PE diagnosis and the use of antiangiogenics is independent of the presence or absence of these symptoms (**Figure 1**). We then fitted a multivariable binary logistic regression (**Figure 2, Annex Table 3**). In this model, exposure to antiangiogenics was associated with a higher proportion of PE with an odds ratio (OR) of 2.27 (95% CI, 1.42-3.63). While other confounding factors were significant, the model was not causally specified to make inferences in that regard (**Annex Table 3**). The detailed breakdown of thrombosis locations does not support the notion that the association between PE and antiangiogenics is dependent on the more proximal location of lower limb thrombosis in subjects who have received these therapies, given that the proportion of DVT at the femoral-iliac level is comparable in both groups (see **Annex Table 4**). A sensitivity analysis revealed that the association persisted when the antineoplastic treatment was categorized as 'non-antiangiogenic', 'treatment with antiangiogenic', and 'no therapy' with PE in 56.0% (95% CI, 52.8-59.1) (521/930), 71.2% (95% CI, 62.1-78.9) (77/108) and 59.4% (95% CI, 55.0-63.6%) (296/498), respectively ($\chi 2=9.742$, d.f.=2, p=0.007).

Characteristics of VTE and prognosis

In patients with PE, the data do not contradict the hypothesis that the rate of multiple (47.9% vs 41.6%, $\chi 2=1.12$, d.f.=1, p-value=0.289) or central PEs (58.4% vs 67.5%, $\chi 2=2.43$, d.f.=1, p-value=0.118) is similar in subjects without vs with antiangiogenics, respectively. The 12-month cumulative incidence of venous rethrombosis was 6.2% (95% CI, 4.8-7.8) and 5.2% (95% CI, 1.6-11.8), for subjects with or without antiangiogenics, respectively (Fine-Gray test, p-value=0.852). The cumulative incidence of clinically relevant or major bleeding was 6.7% (95% CI, 5.4-8.3) vs 4.3% (95% CI, 1.4-10.0) with or without antiangiogenic therapy, respectively (Fine-Gray test, p-value=0.425). Finally, median overall survival (OS) in patients with stage IV tumors with any VTE was 18.6 months (95% CI, 10.5-NA) vs 9.2 months (95%

CI, 8.1-10.7) in subjects with or without antiangiogenics, respectively (log-rank test, p-value=0.02).

Validation in the Caravaggio trial

At the time of randomization, 56/86 of the individuals treated with antiangiogenic therapy (65.1%), 218/438 who received chemotherapy without any antiangiogenic (49.7%), and 305/510 of the participants without active treatment (59.8%) had PE ($\chi 2$=12.791, d.f.= 2, p-value=0.0016).

DISCUSSION

Antiangiogenic drugs have been used as antitumor therapy for more than 20 years, but their association with venous thrombotic risk remains unclear. An initial meta-analysis found that individuals treated with bevacizumab suffered more VTE with a relative risk (RR) of 1.33 (95% CI, 1.13-1.56) with respect to subjects treated with other therapies (19). In a second meta-analysis, Hurwitz et al found no differences in incidence of all-grade VTEs for bevacizumab versus controls (20). Extending to the rest of antiangiogenics, Abdel-Qadir et al found insufficient evidence to contradict the null hypothesis (similar thrombotic risk), although the margins of error were compatible with substantially increased odds, hence, the 'absence of effect' interpretation could require additional data (e.g., for DVT, OR 1.20, 95% CI, 0.86-1.66) (21). One notable limitation was that most randomized controlled trials (RCTs) did not report the type of thromboembolism, preventing the PE/VTE proportion from being estimated. In a third meta-analysis, Liu et al found PE to be uncommon in RCTs of antiangiogenics (~1.7%) (22), impeding the ability to capture how the PE/VTE proportion varied based on these treatments. However, thromboembolism is not a common side effect in clinical trials, patients are not generally asked specifically about them, and half of all cases are asymptomatic. Therefore, it is likely that VTE are underdiagnosed (23).

Our study differs from those analyses as it does examine the relative frequency of PEs across subjects who have had any VTE and, consequently, makes it possible to probe into

the qualitative characteristics of the events in a broad, prospective cohort. Mainly, we have found that exposure to antiangiogenics was associated with a marked increase in the proportion of PE over DVT. This was consistent across all subgroups, except for active smokers and subjects with COPD. These results were confirmed in the Caravaggio clinical trial.

The hypothesis put forth by our study is compelling because it carries a prediction regarding the role of the VEGF/VEGFR signaling pathway on clot stability and embolic load that can be tested using an animal model. The literature includes some mechanisms that would account for the disparate incidence of DVT and PE in specific situations, generally involving abnormal fibrinolysis or the transglutaminase activity of factor XIII (FXIIIa, or fibrin stabilizing factor) (24). Thus, thrombosis in the context of factor V Leiden have been reported as unlikely to embolize given the increased activity of FXIIIa induced by thrombin (24). In contrast, Shaya et al have demonstrated that direct thrombin inhibitors decrease clot stability in a murine model of thrombosis, raising the associated embolic load (5). Nevertheless, this mechanism would not explain the variation of embolization risk in other thrombophilic defects (25) or other, more general hypercoagulability states, making it necessary to look for other possible explanations. Key to this is that FXIIIa has a pro-angiogenic effect through the crosslink of αvβ3 integrin with VEGFR-2, which entails the ligand-independent activation of VEGFR-2 (14,26). VEGFR-2 phosphorylation also appears to control the pro-angiogenic activity of FXIIIa (26). However, it is not clear how antiangiogenic therapy affects the transglutaminase activity of FXIIIa, thereby affecting clot stability. Further, the interaction between antiangiogenics and the hemostatic system is possibly more complex, involving other elements, such as the endothelium, platelet adhesion, induction of plasminogen activator inhibitor-1, etc. (27,28). Moreover, the relationship between active smoking and antiangiogenic therapy has not yet been resolved, although some exploratory analyses point toward a decreased therapeutic benefit in smokers (29). In any event, active smoking *per se* is associated with resistance to thrombolysis (30), which would offset the destabilizing effect of the antiangiogenic.

If confirmed, this observation could have practical implications such as prioritizing prevention of potentially fatal episodes, mainly those associated with PE. While attributing fatality to the PE can be complex (31), the use of dalteparin lowered the rate of lethal thrombosis from 8% to 0 in the FRAGEM RCT (32), which would possibly translate to improved OS in an adequately powered trial.

Our study has various limitations. We have ruled out possible detection bias of incidental VTE to the best of our ability, although we cannot definitively exclude the possibility of a case being missed. In any case, the data presented here are a subanalysis of two different prospective studies, conducted in different settings, which in total encompass the experience of more than 2570 participants. The fact that the association holds up across multiple subgroups with presumably homogeneous detection patterns (e.g., similar use of CT to reevaluate response to anticancer therapy, etc.) reduces the possibility of bias. Be that as it may, our results have generated a hypothesis that must be confirmed experimentally. As for generalizing the results to all antiangiogenics, it must be remembered that bevacizumab comprised 75%, most often used to treat advanced colorectal cancer.

In conclusion, antiangiogenic therapy was associated with an increased PE/VTE proportion in cancer patients. If these results are confirmed, the description of this new phenomenon should inform experimental studies to elucidate the mechanism that modifies clot stability, which would require the concept of thrombotic risk to be redefined, based on the qualitative impact with implications for thromboprophylaxis in oncological patients.

What is known on this topic:

- Pulmonary embolism (PE) and deep-vein thrombosis (DVT) have been assumed to share a similar pathophysiological substrate.
- The mechanisms associated with clot stability and that prevent embolization have yet to be adequately elucidated.
- No clear molecular links between pro-angiogenic mechanisms and processes promoting clot stability are currently known.

- Inasmuch as the incidence of PE in clinical trials of anti-angiogenic drugs has been low, it has not been possible to establish any causal association.

What does this paper add?

- Antiangiogenics appear to promote clot instability, fostering the development of pulmonary embolisms in both the prospective TESEO registry and the Caravaggio RCT.
- The effect of antiangiogenics on clot stability was maintained in all subgroups except in active smokers
- This should inform experimental studies to elucidate the mechanism that modifies clot stability

Funding source

This study was supported by unrestricted grants from Sanofi, Leo Pharma Rovi, and BMS-Pfizer. The sponsors of this research have not participated in data collection, analysis, or interpretation, in writing the report, or in the decision to submit the article for publication.

Ethical statement

This study was performed in accordance with the ethical standards of the Declaration of Helsinki and its subsequent amendments. This observational, non-interventional trial was approved by the Research Ethics Committees of all centers, and by the Spanish Agency of Medicines and Medical Devices (AEMPS).

Informed consent statement Signed informed consent was obtained from all patients.

Consent for publication

Informed consent and approval by the competent national authorities includes permission for publication and dissemination of the data.

Conflict of interest

All authors declare that they have no conflict of interest regarding the scope of this work. This is an academic study.

Acknowledgements

The authors would like to thank the investigators of the TESEO registry, the Thrombosis Working Group of the Spanish Society of Medical Oncology (SEOM), Priscilla Chase Duran for editing the manuscript and Natalia Cateriano, Miguel Vaquero, IRICOM S.A. for supporting the registry website. Gustavo Reporte has helped in the interpretation of the mathematics of the model.

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Figure 1. Differences between proportions of pulmonary embolism with or without antiangiogenic. Positive differences indicate more embolisms in subjects receiving an antiangiogenic. Newcombe's method was used to calculate the confidence interval for the difference between proportions. Abbreviations: TNM, Tumor, node, metastases; VTE, venous thromboembolism; CT, computed tomography; DVT, deep vein thrombosis; COPD, chronic obstructive pulmonary disease; PE, pulmonary embolism.

Figure 2. Binary logistic regression. The dichotomous response variable is the detection of pulmonary embolism. Abbreviations: ECOG PS, Eastern Cooperative Group Performance Status; VTE, venous thromboembolism; TNM, Tumor, node, metastases.

Table 1. Baseline characteristics broken down by use of antiangiogenic (TESEO study)

	Overall	**No antiangiogenic, N (%)**	**Antiangiogenic N (%)**
Age, median	66 (20-92)	66 (20-92)	66 (28-86)

Sex, male	807 (52.6)	745 (52.2)	62 (57.4)
ECOG-PS			
0	349 (22.7)	328 (23.0)	21 (19.4)
1	801 (52.1)	737 (51.6)	64 (59.3)
2	297 (19.3)	280 (19.6)	17 (15.7)
3	83 (5.4)	77 (5.4)	6 (5.6)
4	6 (0.4)	6 (0.4)	0
Most common tumors			
Colorectum	307 (20)	238 (16.7)	69 (63.9)
Lung - Non-small cell	297 (19.3)	291 (20.4)	6 (5.6)
Breast	160 (10.4)	158 (11.1)	2 (1.9)
Pancreas	146 (9.5)	146 (10.2)	0
Stomach	77 (5.0)	77 (5.3)	2 (1.9)
Ovarian	62 (4.0)	59 (4.1)	3 (2.8)
Bladder	56 (3.6)	56 (3.9)	0
Endometrial	32 (2.1)	30 (2.1)	2 (1.9)
Bile duct / gallbladder	42 (2.7)	42 (2.9)	0
Esophagus	32 (2.1)	32 (2.2)	0
Brain	39 (2.5)	31 (2.2)	8 (7.4)
Prostate	34 (2.2)	34 (2.4)	0
Kidney	25 (1.6)	17 (1.2)	8 (7.4)
Liver	17 (1.1)	14 (1.0)	3 (2.8)
Other	210 (13.6)	203 (14.2)	5 (4.6)
Histology, adenocarcinoma	1075 (70%)	988 (69.2)	87 (80.6)
TNM stage IV	1091 (71.0)	992 (69.5)	99 (91.7)
Active tumor	1262 (82.2)	1161 (81.3)	101 (93.5)
Use of chemotherapy	889 (57.9)	805 (56.4)	84 (77.8)
VTE, type of detection			
Suspected	747 (48.6)	701 (49.1)	46 (42.6)
Unsuspected	771 (50.2)	709 (49.6)	62 (57.4)
Unknown	18 (1.2)	18 (1.3)	0
Type of VTE			
DVT	642 (41.8)	611 (42.8)	31 (28.7)
PE + without DVT	720 (46.9)	656 (45.9)	64 (59.3)
PE + DVT	174 (11.3)	161 (11.3)	13 (12.0)
Severity, NCI-CTC			
Grade 1	-	-	-
Grade 2	682 (44.9)	647 (45.9)	35 (32.4)
Grade 3	780 (51.4)	709 (50.3)	71 (65.7)
Grade 4	47 (3.1)	45 (3.2)	2 (1.9)
Grade 5	9 (0.6)	9 (0.6)	0
Unknown	18 (1.1)	18 (1.2)	0
Total	1536 (100%)	1428 (100%)	108 (100%)

Abbreviations: ECOG-PS, Eastern Cooperative Group Performance Status; TNM, Tumor, node, metastases; VTE, venous thromboembolism; DVT, deep vein thrombosis; PE, pulmonary embolism; NCI-CTC, National Cancer Institute Common Toxicity Criteria.

Table 2. Antiangiogenic drugs. Notes: N=Number of patients taking antiangiogenic therapy at randomization

	TESEO registry, N (%)	**Caravaggio trial, N (%)**
Aflibercept (recombinant fusion protein)	10 (9.2)	4 (4.7)
Bevacizumab (antiangiogenic monoclonal antibody)	81 (75.0)	30 (34.9)
Ramucirumab (antiangiogenic monoclonal antibody)	2 (1.8)	2 (2.3)
Axitinib (tyrosine kinase inhibitor)	1 (0.9)	0
Cabozantinib (tyrosine kinase inhibitor)	4 (3.7)	1 (1.2)
Imatinib (tyrosine kinase inhibitor)	0	1 (1.2)
Lenvatinib (tyrosine kinase inhibitor)	0	0
Pazopanib (tyrosine kinase inhibitor)	1 (0.9)	3 (3.5)
Sorafenib (tyrosine kinase inhibitor)	2 (1.8)	0
Sunitinib (tyrosine kinase inhibitor)	3 (2.7)	2 (2.3)
Regorafenib (tyrosine kinase inhibitor)	1 (0.9)	2 (2.3)
Vandetanib (tyrosine kinase inhibitor)	1 (0.9)	0
Other, not specified	2 (1.8)	84 (97.7)
Total	108	86

Patients were included in only one treatment group. Percentages were calculated on total number of patients taking antiangiogenic therapies at randomization. Subjects with hematological cancer and history of cancer were excluded from analysis.

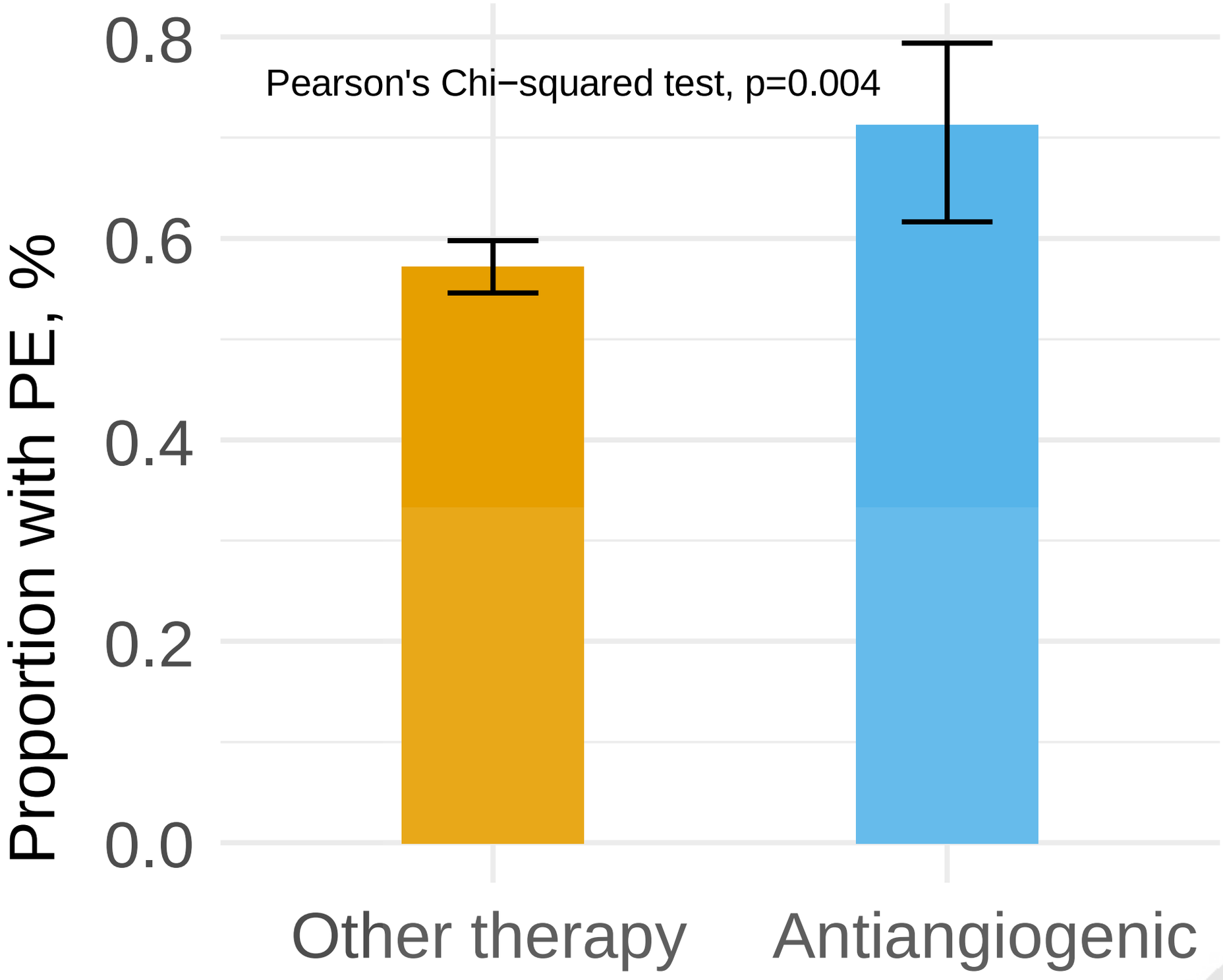
Table 3. Outcomes & characteristics in the validation cohort (Caravaggio trial)

	Overall	**At least one antiangiogenic therapy at randomization, N (%)**	**At least one therapy other than antiangiogenic at randomization, N (%)**	**No therapy at randomization, N (%)**
Age, median	69	64	69	69
Sex, male	505 (48.8)	42 (48.8)	207 (47.3)	256 (50.2)
Most common tumors	229 (22.1)	26 (30.2)	107 (24.4)	96 (18.8)
Colorectum	195 (18.9)	30 (34.9)	57 (13.0)	108 (21.2)
Lung	134 (13.0)	7 (8.1)	55 (12.6)	72 (14.1)
Genitourinary	149 (14.4)	9 (10.5)	88 (20.1)	52 (10.2)
Breast		0	43 (9.8)	43 (8.4)
Pancreatic or Hepatobiliary		5 (5.8)	44 (10.0)	65 (12.7)
Gynecological		5 (5.8)	22 (5.0)	27 (5.3)
		0	10 (2.3)	10 (2.0)

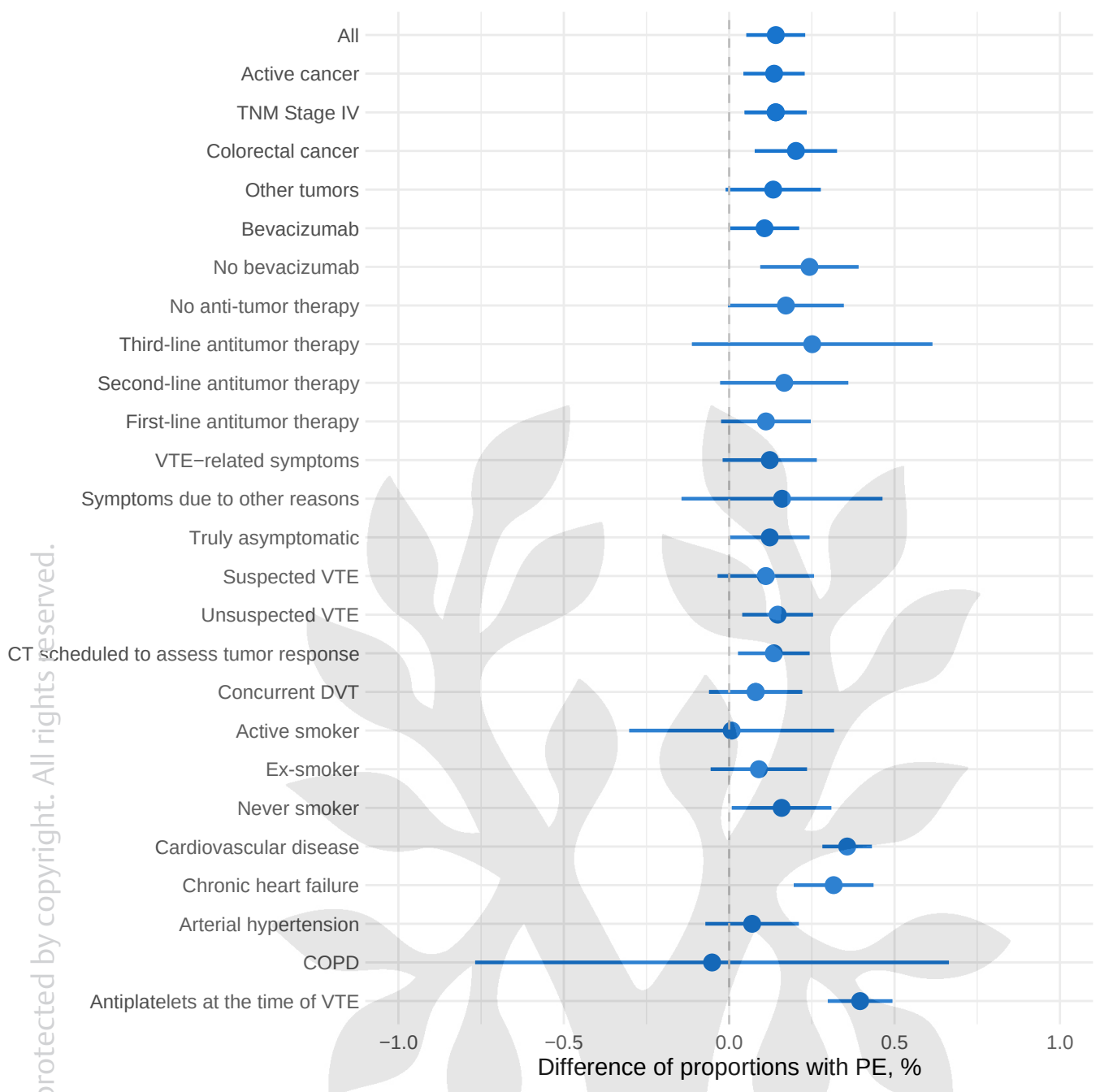
Upper GI	86 (8.3)	0	5 (1.1)	8 (1.6)
Head and Neck	114 (11.0)	1 (1.2)	2 (0.5)	7 (1.4)
Bone / Soft Tissue	54 (5.2)	3 (3.5)	5 (1.1)	22 (4.3)
Skin - Melanoma	20 (1.9)			
Other	13 (1.3)			
	10 (1.0)			
	30 (2.9)			
TNM stage IV (metastatic)	503 (48.6)	61 (70.9)	220 (50.2)	222 (43.5)
Active tumor	1034 (100)	86 (100)	438 (100)	510 (100)
VTE, type of detection				
Symptomatic	819 (79.2)	63 (73.3)	352 (80.4)	404 (79.2)
Unsuspected	215 (20.8)	23 (26.7)	86 (19.6)	106 (20.8)
Type of VTE				
DVT	455 (44.0)	30 (34.9)	220 (50.2)	205 (40.2)
PE + without DVT	493 (47.7)	53 (61.6)	184 (42.0)	256 (50.2)
PE + DVT	86 (8.3)	3 (3.5)	34 (7.8)	49 (9.6)
Total	1034	86	438	510

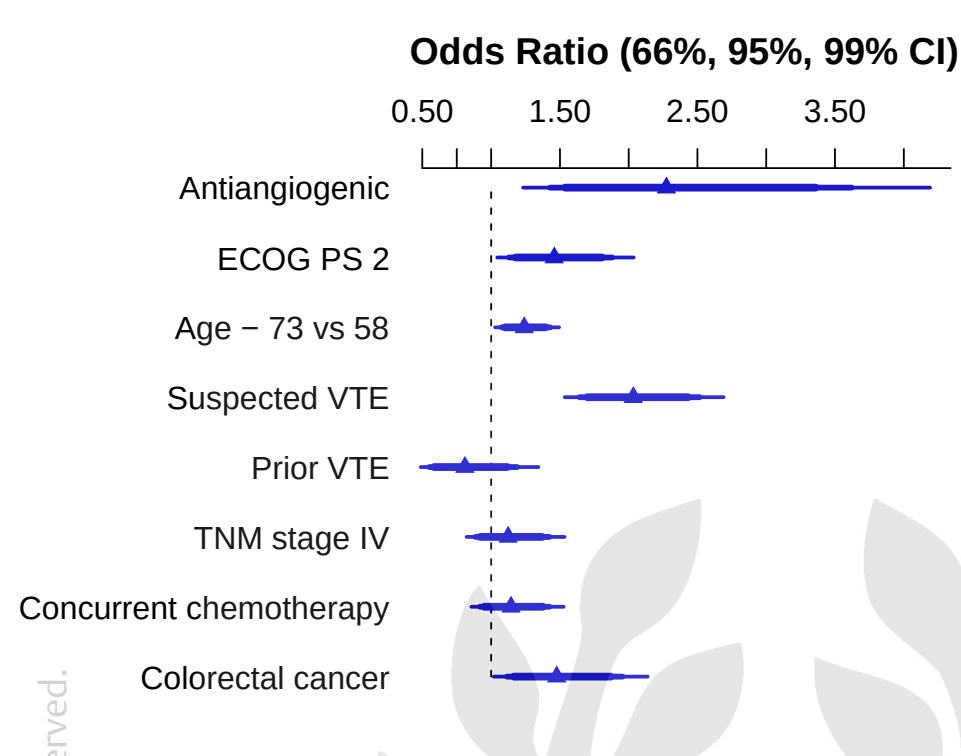
Notes: Percentages were calculated on total number of patients in each group. Subjects with hematological cancer and history of cancer were excluded from analysis. Abbreviations: TNM, Tumor, node, metastases; VTE, venous thromboembolism; DVT, deep vein thrombosis; PE, pulmonary embolism.

Annex Figure 1. Proportion of patients with pulmonary embolism.



Abbreviations: PE, pulmonary embolism.




Odds Ratio (66%, 95%, 99% CI)
0.50
1.50
2.50
3.50
Antiangiogenic
ECOG PS 2
Age − 73 vs 58
Suspected VTE
Prior VTE
TNM stage IV
Concurrent chemotherapy
Colorectal cancer

	PE, n (%)	DVT, n (%)
CTPA	315 (35.2)	23 (3.6)
Scheduled CT	472 (52.8)	208 (32.4)
Unplanned CT	61 (6.8)	43 (6.7)
Scintigraphy	10 (1.1)	0
Doppler ultrasound	4 (0.4)	341 (43.1)
MRI	0	5 (0.8)
Venography	0	2 (0.3)
Unknown	32 (3.6)	20 (3.1)
Total	894 (100)	642 (100)

Annex Table 1. Diagnostic methods

Abbreviations: CTPA=Computed Tomography Pulmonary Angiography; Scheduled CT= Scheduled computed tomography scan performed for reasons other than pulmonary embolism suspicion, usually cancer monitoring; Unplanned CT= Unplanned conventional computed tomography scan performed for reasons other than pulmonary embolism suspicion. Scintigraphy= high probability ventilation/perfusion pulmonary scintigraphy according to the PIOPED criteria; MRI= Magnetic resonance imaging

Annex Table 2. Symptoms according to the type of thrombotic event.

	Overall, N (%)	VTE-related symptoms, N (%)	Symptoms due to other reasons, N (%)	Truly asymptomatic, N (%)
PE	720 (46.9)	240 (35.5)	210 (50.2)	270 (61.2)
PE + DVT	174 (11.3)	106 (15.7)	38 (9.1)	30 (6.8)
Isolated DVT	593 (38.6)	321 (47.4)	141 (33.7)	131 (29.7)
Unknown	49 (3.2)	10 (1.5)	29 (6.9)	10 (2.3)
Total	1536 (100.0)	677 (100)	418 (100)	441 (100)

VTE-related symptoms: Presence of new symptoms and/or abnormality in vital signs potentially attributable to pulmonary embolism; Symptoms due to other reasons: Presence of symptoms presumably related to the tumor, co-morbidity, or antineoplastic therapy

Annex Table 3. Logistic regression model for PE.

	Coefficient	S.E.	OR (95% CI)	Wald	P-value
Intercept	-0.3290	0.3244	-	-1.01	0.3105
Antiangiogenic	**0.8162**	**0.2361**	**2.2 (1.4-3.6)**	**3.46**	**0.0005**
ECOG PS 2	0.3823	0.1279	1.4 (1.1-1.8)	2.99	0.0028
Age	0.0141	0.0047	1.0 (1.0-1.0)	2.99	0.0028
Suspected VTE	-0.6382	0.1073	1.8 (1.5-2.3)	-5.95	<0.0001
Prior VTE	0.1366	0.1916	0.8 (0.5-1.2)	0.71	0.4757
TNM stage IV	-0.0747	0.1192	1.11 (0.8-1.36)	-0.63	0.5306
Concurrent chemotherapy	0.0562	0.1104	1.1 (0.8-1.3)	0.51	0.6109
Colorectal cancer	-0.3582	0.1419	1.4 (1.1-1.8)	-2.52	0.0116

Abbreviations: OR = 0dds ratio, CI = confidence interval, S.E. = standard error, PE= pulmonary embolism, ECOG PS = Eastern Cooperative Group Performance Status, VTE = venous thromboembolic event, TNM= tumor-node-metastases.

C=0.624, R2=0.061, likelihood ratio test $\chi 2=71.4$, d.f.=9, p<0.0001

Annex Table 4. Location of VTE depending on the use of antiangiogenics.

	Overall, N (%)	No antiangiogenic, N (%)	Antiangiogenic, N (%)
Head & neck	89 (5.8)	84 (5.9)	5 (4.6)
Head & neck + PE	6 (0.4)	5 (0.4)	1 (0.9)
Upper extremities	58 (3.8)	56 (3.9)	2 (1.9)
Upper extremities + PE	4 (0.3)	4 (0.3)	0
Catheter-related	89 (5.8)	84 (5.9)	5 (4.6)
Catheter-related + PE	8 (0.5)	7 (0.5)	1 (0.9)
Splanchnic	112 (7.3)	106 (7.4)	6 (5.6)
Splanchnic + PE	12 (0.8)	12 (0.8)	0
Inferior cava vein	28 (1.8)	27 (1.9)	1 (0.9)
Inferior cava vein + PE	8 (0.5)	7 (0.5)	1 (0.9)
Iliac	34 (2.2)	32 (2.2)	2 (1.9)
Iliac +PE	17 (1.1)	15 (1.1)	2 (1.9)
Femoral	139 (9.0)	130 (9.1)	9 (8.3)
Femoral + PE	74 (4.8)	67 (4.7)	7 (6.5)
Calf vein	73 (4.8)	71 (5.0)	2 (1.9)
Calf vein + PE	35 (2.3)	33 (2.3)	2 (1.9)
Lower extremity, NOS	18 (1.2)	18 (1.3)	0
Lower extremity, NOS + PE	15 (1.0)	15 (1.1)	0
Unknown	49 (3.2)	48 (3.4)	1 (0.9)
PE without DVT	720 (46.9)	656 (45.9)	64 (59.3)
Total	1536 (100.0)	108 (100.0)	108 (100.0)

Abbreviation: PE= pulmonary embolism, DVT= deep vein thrombosis, NOS= not otherwise specified.
Note: inferior cava vein includes: inferior vena cava, renal & suprahepatic vein; calf vein includes: anterior tibial/ posterior tibial/ fibular veins.