Original article

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Factor V Leiden and G20210A prothrombin mutation and the risk of subclavian vein thrombosis in patients with breast cancer and a central venous catheter

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Background: To analyze the influence of the prothrombotic gene mutation factor V G1691A (factor V Leiden) and prothrombin G20210A on the risk of a first episode of catheter-related deep venous thrombosis (DVT) in a group of patients with breast cancer treated with chemotherapy.

Patients and methods: Between January 1999 and February 2001, the occurrence of a first symptomatic DVT was investigated in a cohort of 300 consecutive patients with locally advanced or metastatic breast cancer treated at a single institution with fluorouracil-based chemotherapy, administered continuously through a totally implanted access port. A nested case–control study included 25 women (cases) with catheter-related DVT and 50 controls without DVT matched with cases for age, identical chemotherapy, stage of disease and prognostic features. The G1691A factor V and G20210A prothrombin mutation genotypes were analyzed.

Results: Five cases [20%; 95% confidence interval (CI) 9% to 39%)] and two controls (4%; 95% CI 1% to 14%) were heterozygous carriers of G1691A factor V ($P = 0.04$). The age-adjusted odds ratio for catheter-related DVT was 6.1 (95% CI 1.1–34.3). Only one patient (case) had the G20210A prothrombin gene mutation. Time from start of chemotherapy infusion to DVT was not significantly different between patients with (median 31 days) and without (median 43 days) G1691A factor V mutation ($P = 0.6$).

Conclusions: Factor V Leiden carriers with locally advanced or metastatic breast cancer have an increased risk of developing catheter-related DVT during chemotherapy.

Key words: breast cancer, factor V Leiden, vein thrombosis

Introduction

In patients with cancer, the increasing use of dose-intensive and continuous infusion chemotherapy based upon fluorouracil requires reliable, long-term central venous catheters (CVC) for both blood sampling and drug administration. Another reason for the implant of durable CVC is the increasing application of supportive care measures, such as intravenous antiemetics, analgesics, antibiotics and parenteral nutrition [1]. One of the major complications of CVC is thrombosis of the subclavian vein and, in approximately one-third of deep venous thrombosis (DVT) cases, pulmonary embolism [2–4]. In cancer patients, activation of the coagulation pathway favors venous thromboembolism (VTE), which is the second leading cause of death in these patients [5–7]. Even though the association between cancer and hypercoagulability is well established, the pathogenesis of VTE in these patients has not been entirely elucidated. While coagulation activation is encountered in up to 90% of cancer patients, only 4–15% of them develop DVT or pulmonary embolism [8]. Chemotherapy increases the risk of VTE by damaging the vascular endothelium and decreasing plasma levels of naturally occurring coagulation inhibitors [9]. The highest incidence of VTE occurs during advanced disease, being influenced by larger cancer cell burden, immobilization for pathologic bone fractures, immobilization due to tumor cachexia and mechanical compression of the veins by tumor mass [10].

Even though the usefulness of coagulation markers in predicting thrombosis during chemotherapy and/or hormonal treatment in patients with cancer is not established, it is well known that some genetically based abnormalities of coagulation are generally associated with an increased risk for VTE [11]. Inherited resistance to activated protein C is a prothrombotic condition resulting from a gain-of-function mutation of coagulation factor V, commonly referred to as factor V Leiden. A single guanine (G) to adenine (A) missense mutation in the factor V gene at nucleotide 1691 substitutes G for A, resulting in a mutant protein resistant to the anticoagulant action of activated protein C [12]. This mutation is the

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most common inherited risk factor for VTE, with a prevalence of ~5% in the general Caucasian population and 20–50% among patients with VTE. The estimated risk of DVT in heterozygous carriers of the mutation is five- to 10-fold higher, whereas for homozygous carriers is 80- to 100-fold higher, than in non-carriers [11]. Another prothrombotic gain-of-function mutation has been identified in the 3′ untranslated region of the prothrombin gene (the substitution of A for G at position 20 210). The mutant allele is present in ~2% of the general population and increases the risk of DVT by three- to five-fold [11]. Since the etiology of VTE is multifactorial [13], and considering that factor V Leiden and the G20210A prothrombin mutation are the most common inherited risk factors for VTE, we hypothesized a role for these mutations in causing catheter-related DVT in patients with advanced cancer treated with chemotherapy. With this background, this case–control study was carried out in patients with locally advanced or metastatic breast cancer who developed DVT during continuous infusion of fluorouracil-based chemotherapy through an implanted CVC.

Patients and methods

Patients

Between January 1999 and February 2001, we investigated a cohort of 300 consecutive women with locally advanced or metastatic breast cancer treated at a single institution with chemotherapy administered continuously through a totally implantable CVC. Among them, 182 were identified by retrospective scrutiny of the medical charts and 118 were prospectively enrolled and followed up. The study protocol was approved by the Ethical Scientific Committee of the European Institute of Oncology. Written informed consent was obtained from all patients. A nested case–control study was carried out, taking as cases women with an objectively documented first episode of catheter-related subclavian vein thrombosis (CRT). For each case we enrolled as controls two women without DVT, matched with cases for age (±5 years), identical chemotherapy, stage of disease and prognostic features (i.e. extent and site of metastases, pT size, pN degree of involvement, grade, Ki67 expression, vascular invasion, steroid hormone receptor expression and HER2/*neu* overexpression in the tumor). Patients who received previous chemotherapy and/or hormonal therapy, those with previous episodes of VTE, and those with congenital deficiencies of antithrombin, protein C and protein S, as well as antiphospholipid antibodies, were not eligible for the study. No patient received anticoagulant or antiplatelet aggregation prophylaxis. The diagnosis of catheter-related DVT was made by color Doppler ultrasonography of the internal jugular and subclavian veins when indicated by clinical signs such as the appearance of arm/facial venous engorgement, swelling, redness and/or pain. When clinical signs persisted and ultrasound examination was negative, the latter was repeated after 5–7 days. When symptoms suggestive of pulmonary embolism developed, radionuclide lung scanning was performed. In patients with a documented thrombus, the catheter was removed and heparin was given intravenously for at least 5 days, followed by oral anticoagulant therapy with warfarin for a period of at least 3 months.

Insertion and maintenance of Port-A-Cath®

A single type of port constructed of titanium and silicone rubber (Dome Port™; Bard Inc., Salt Lake City, UT, USA), attached to 9.6-F silastic open-ended catheter tubing, was implanted in the operating room under fluoroscopic control by the same experienced staff of surgeons using maximal sterile-barrier precautions. A standard introductory percutaneous subclavian approach was used, consisting of the direct puncture of a subclavian vein, introduction of a guide wire, radiological confirmation of correct wire position, dilatation of the introductory route, creation of a subcutaneous tunnel and introduction of the catheter with the tip positioned in the superior vena cava. The patients received local anesthesia with no additional intravenous sedation; a single dose (2 g) of cefazoline sodium was given intravenously 15 min before implantation. Postoperative chest radiography was performed routinely to detect inadvertent pneumothorax and to confirm correct catheter placement. To prevent clot formation and catheter blockage, implanted ports were flushed with 20 ml of normal saline and then filled with sterile, heparinized saline after each infusion of medication or blood withdrawal (5 ml of a solution containing 50 IU/ml). If the port remained unused for a long time, the heparin lock was changed once every 21 or 28 days. This maintenance program was carried out on an outpatient basis by a experienced nurses.

Genetic studies

Blood was collected from the antecubital vein without venous stasis using a Vacutainer PrecisionGlide™ needle 0.8 × 38 mm into four 3.15 ml Vacutainer® tubes containing 0.129 M sodium citrate. The first 5 ml were discarded. Citrated platelet-poor plasma was prepared using two centrifugation steps: 5 min at 2150 *g* at room temperature and 10 min at 11 000 *g* at 4°C. The blood samples were processed within 30 min of collection. The plasma samples were stored at –70°C in aliquots of 500 µl until analysis. Analyses for G1691A factor V and G20210A prothrombin were performed in all patients. Genomic DNA was extracted from white blood cells using the NucleoSpin Blood L kit (Macherey-Nagel, Oensingen, Switzerland), according to the manufacturer's instructions. Analyses were performed on blinded samples. The physician was not aware of the mutation status. In addition, technicians performing assays were unaware of the patient's status with respect to CRT and the ultrasound technicians were blinded to the genetic results.

Genotypes were determined by PCR and hybridization with allele-specific oligonucleotides. The allele specific oligonucleotide for factor V A1691 was 3′-TGGACAGGCAAGGAATAC-5′, and for factor V G1691 3′-GGACAGGCGAGGAATAC-5′. The prothrombin fragment of 230 bp was cleaved by *Hin*dIII in case the G→A mutation was present, and yielded two smaller fragments of 190 and 40 bp. Dots were visualized on radiograph films (DuPont, Brussels, Belgium) after overnight radiation. Some samples were retested by restriction fragment-length polymorphism.

Statistical analysis

Fisher's exact test was used to compare genotypes in cases and controls. The different distribution of individual characteristics in cases and controls was tested using the Fisher's exact test for categorical variables and the non-parametric Mann–Whitney *U*-test for continuous variables. Unconditional logistic regression analysis was used to estimate the age-adjusted odds ratio as a measure of thrombosis risk in carriers of factor V Leiden or prothrombin mutation compared with non-carriers. All tests were two-sided and significance referred to $P \leq 0.05$.

Results

The main characteristics of the study population are reported in Table 1. From the cohort of 300 eligible women with locally advanced or metastatic breast cancer, 25 (8.3%) who developed CRT and 50 controls were included in this nested case–control study. No case of pulmonary embolism was diagnosed. Age distribution, menopausal status, previous surgery and type of chemotherapy were similar in cases and controls. The number of cycles was greater in controls (median six) than in cases (median three) ($P < 0.001$). Five cases and two controls were heterozygous carriers of G1691A factor V, giving a prevalence of 20% [95% confidence

Table 1. Individual characteristics of the study and control groups

	Cases (n = 25)	Controls (n = 50)	P value
Age (years)[a]	51 (46–55)	50 (43–54)	0.4
Menopausal status [n (%)]			
Premenopausal	11 (44)	25 (50)	0.8
Postmenopausal	14 (56)	25 (50)	
Tumor stage [n (%)]			
Localized (locally advanced)	15 (60)	29 (59)	>0.9
Metastatic	10 (40)	21 (41)	
Number of cycles[a]	3 (2–5)	6 (4–6)	<0.001
Surgery [n (%)]			
Yes	24 (96)	48 (98)	>0.9
No	1 (4)	1 (2)	

[a]Median and interquartile range (25th and 75th percentile).

interval (CI) 9% to 39%] and 4% (95% CI 1% to 14%), respectively (P = 0.04). The age-adjusted odds ratio for catheter-related DVT was 6.1 (95% CI 1.1–34.3). The median time elapsed from start of chemotherapy to thrombosis was not significantly different between those with (31 days) and without (43 days) the mutation (P = 0.6). Only one case and no control carried the G20210A mutation in the prothrombin gene (P = 0.30). The age-adjusted odds ratio for the presence of either mutation was 7.6 (95% CI 1.4–41.0).

Discussion

To our knowledge, this is the first study evaluating the role of the prothrombotic, gain-of-function mutations of the factor V and prothrombin gene as risk factors for CVC-related DVT in a homogeneously treated group of patients with breast cancer. The study aimed to identify features that would help to predict thrombotic complications. It shows that in patients who develop catheter-related DVT while receiving continuous infusion 5-fluorouracil-based chemotherapy for advanced breast cancer, the prevalence of factor V Leiden is five times higher than in those without thrombosis, giving a six-fold greater relative risk.

The type of treatment used (including 5-fluorouracil continuous infusion) is highly effective in controlling disease and its related symptoms, with a rather low burden of subjective side effects. In a previous study on 182 breast cancer patients treated with this type of chemotherapy [14], we observed a 7.7% incidence of VTE, similarly distributed among patients with early/locally advanced disease or overt metastases. Thrombosis associated with a CVC is a serious complication in these patients, and is associated with significant morbidity (pain, edema) and some risk of pulmonary embolism. The impact of thrombosis on continuation of treatment was clear, because the number of chemotherapy courses in cases (median three) was lower than in controls (median six) (P <0.001), owing to catheter removal and discontinuation of therapy in all patients with catheter-related DVT.

Studies similar to ours were previously carried out mostly in patients with hematological malignancies. Fijnheer et al. [15], who investigated the occurrence of thrombosis associated with CVCs in 277 consecutive patients receiving allogenic bone marrow transplantation, found that of 13 patients heterozygous for factor V Leiden, seven (54%) had a subclavian vein thrombosis, while only 9% of the patients without factor V Leiden had this complication (relative risk 7.7; 95% CI 3.3–17.9) On the other hand, Sifontes et al. [16] found no increased risk of thrombosis related to factor V Leiden in children with various types of cancer, even though the only child heterozygous for the factor V Leiden mutation had thrombosis in association with an indwelling venous catheter. Rees et al. [17] found no association between thrombosis and factor V Leiden in a small cohort of patients with acute promyelocytic leukaemia. Finally, no association between DVT and the presence of inherited prothrombotic markers was found in 60 children with a CVC and acute lymphoblastic leukemia treated with the prothrombotic agent L-asparaginase [18].

This study has some limitations. First, the confidence interval of the relative risk of thrombosis is wide, owing to the small number of patients included in the analysis. Secondly, patients were not routinely screened for CRT; therefore, there is the possibility that some of the controls might have had CRT and were in fact cases. Finally, a selection bias for living patients able to sign the informed consent might have occurred, since part of our study population was retrospectively identified through medical charts. However, given the relatively low rate of embolization of CVC-related vein thromboses, the probability of having missed fatal cases is low. In addition, all breast cancer patients who received chemotherapy between January 1999 and February 2001 and developed a symptomatic CRT during treatment have been evaluated in this study.

The consensus recommendation for the prevention of VTE in patients with long-term indwelling CVC includes the prophylactic use of low-dose warfarin (1 mg a day) or low molecular weight heparins [19]. Results of two randomized trials of these treatments on the prevention of thromboembolic complications in patients with cancer and CVC showed some benefit, but failed to modify clinical practice [20, 21]. The results of more recent studies show that the incidence of CRT has decreased substantially over the past decade, probably due to improved quality and handling of catheters. Hence, the benefit of primary prophylactic anticoagulation remains questionable [22, 23]. Moreover, a recent retrospective study revealed a high incidence of excessive anticoagulation (expressed by INR elevation) when minidose warfarin was given along with an intermittent dose of fluorouracil-based infusion chemotherapy [24]. The identification of a subgroup of patients with cancer at high risk for DVT would be a reasonable strategy to avoid medical devices that enhance the risk of thrombosis, or, more likely, to consider the implementation of primary antithrombotic prophylaxis. Our study suggests that factor V Leiden carriers with advanced or metastatic breast cancer are at higher risk of developing catheter-related DVT during chemotherapy. We do not advise genetic testing for all patients with breast cancer receiving continuous infusion chemotherapy through a totally implantable CVC, because the number of patients to be tested to avoid few

episodes of VTE is likely to confer little cost-effectiveness to this strategy. However, it is reasonable to offer testing to patients with a positive history of VTE (even when not cancer-related). In patients so identified, there could be two options: the implementation of antithrombotic prophylaxis or the choice of alternative cytotoxic treatments not requiring continuous infusion and the implantation of CVC. A validation of our findings in a prospective study is warranted.

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