

Online methods section

Study design and population

The Vienna Cancer and Thrombosis Study (CATS) is an ongoing, prospective and observational cohort study initiated in year 2003 at the Medical University of Vienna. The study is conducted in accordance with the Declaration of Helsinki after approval by the institutional ethics committee. The aim of CATS is to investigate factors which are associated with the occurrence of symptomatic venous thromboembolism (VTE) in patients with cancer. All patients with solid tumors included in the CATS between October 2003 and December 2009 were eligible for this study.

Detailed information about the methodology of CATS and the exact inclusion and exclusion criteria have been reported previously (1-3). The inclusion criteria have been as follows: (i) patients with newly diagnosed cancer of the breast, lung, stomach, colon, pancreas, prostate, and other sites (mainly gynecologic system and sarcoma); or progression of disease after complete or partial remission; (ii) histological confirmation of diagnosis; (iii) age 18 years or older; (iv) willingness to participate; and (v) written informed consent. Exclusion criteria have been (i) the presence of overt bacterial or viral infection; (ii) continuous anticoagulation with vitamin K antagonists or low-molecular weight heparins; and (iii) venous or arterial thromboembolism within the last three months. For patients with disease progression, additional exclusion criteria have been (iv) chemotherapy within the last 3 months and (v) surgery or radiotherapy within the last 2 weeks before study inclusion. All study participants underwent tumor staging or, in case of disease progression after remission, re-staging prior to study inclusion. The study participants were followed prospectively until the end of follow-up (2 years), occurrence of VTE, death, loss of follow-up, or withdrawal of consent. At study inclusion, every patient underwent a

structured interview, patients' medical history was recorded, and blood samples were drawn.

Outcome measure and diagnosis of venous thromboembolism

The main outcome measure was the occurrence of symptomatic VTE within two years after study inclusion. No routine screening for VTE was performed. All patients were briefed about VTE and asked to immediately report to our clinic, if they recognized any VTE symptoms. Additionally, we contacted every patient approximately every 3 to 4 months to perform a follow-up. When a patient presented with symptoms of VTE, objective methods were used to confirm the diagnosis of VTE. Duplex sonography or venography were applied for diagnosis of deep vein thrombosis (DVT), and computed tomography or ventilation/perfusion lung scan were applied for diagnosis of pulmonary embolism (PE). An independent adjudication committee consisting of experts in the fields of angiology, radiology, and nuclear medicine evaluated and approved all VTE events. These experts were informed about patients' medical history, but were unaware of tumor stage and laboratory results. Incidentally detected VTE (e.g. PE detected in a routine computerized tomography) was counted as an event, when the adjudication committee decided that the event was of clinical significance.

Classification of local, regional and distant stage

Based on the TNM Classification specified by the Union for International Cancer Control (UICC) (4) we defined 3 tumor stages: local, regional, and distant. Patients with absence of lymph node metastases and absence of distant metastases were classified as local stage (TxN0M0). Regional stage was defined by a positive lymph node status but concurrent absence of distant metastases (TxN1-3M0). The

presence of distant metastases led to the classification of distant stage, irrespective of the lymph node status (TxNxM1). Lymph node metastases and distant metastases were detected and verified by imaging techniques (e.g. computerized tomography), cancer biopsy, or cancer surgery.

Blood sampling and laboratory analyses

At study inclusion, venous blood samples were drawn from each patient. Detailed information about the procedure of blood sampling and applied laboratory methods for measurement of D-dimer, prothrombin fragment 1 and 2 (F1 + F2), clotting factor VIII (FVIII), platelets, and soluble P-selectin (sP-selectin) is given in previous publications (1-3, 5).

Statistical analyses

Characteristics of patients were described by median and interquartile-range (IQR) because of non-normally distributed continuous variables, and by frequencies and percentages for categorical variables. Of main interest were differences between patients with different tumor stages: local, regional, and distant. The Kruskal-Wallis test was applied to compare the distribution of (non-normally distributed) biomarkers, such as D-dimer, F1+F2, FVIII, platelets, sP-selectin, leukocytes, and hemoglobin, between patients with these 3 tumor stages. In case of a significant overall test result, a Mann-Whitney test for patients with local versus regional and local versus distant stage was applied and corrected with a Bonferroni correction for multiple testing. The median of the follow-up distribution was estimated by the Kaplan-Meier method with reverse meaning of the status indicator (6). Kaplan-Meier analysis was used to visualize the association between stage and risk of VTE. Univariate and multivariable Cox-regression analyses were used for calculating the risk of VTE from study

inclusion until last follow-up, patient's death, or maximal duration of follow-up of two years. The first multivariable Cox-regression model (model 1) comprised regional stage (versus local stage), distant stage (versus local stage), surgery, radiotherapy, chemotherapy, age, and newly diagnosed cancer (versus progression of disease). The hazard ratio (HR) of the continuous age variable is given per each 10-year increase. We assumed that surgery, chemotherapy, and radiotherapy would entail a modified risk for VTE not only limited to the exact time-point of the procedure, but also for a certain period afterwards. Therefore, 3 time-dependent binary variables were included in the statistical model that indicated times of possible influence on the VTE risk by surgery (from the day of surgery plus 6 consecutive weeks), chemotherapy (from the first day of a treatment cycle until the last day plus 4 weeks), or radiotherapy (from the first day of treatment until the last day plus 4 weeks). A second Cox-regression model (model 2) additionally considered selected laboratory biomarkers (D-dimer, leukocytes, platelets, and hemoglobin) and clinicopathological factors from model 1 (tumor stage (local, regional, and distant) and newly diagnosed cancer (versus progression of disease)). Selection of covariates was orientated according to the availability of risk parameters in clinical practice.

For reasons of comparability with other studies and for enabling an easy practicability in clinical routine, laboratory biomarkers were dichotomized: Biomarkers also used in the Khorana VTE prediction score were dichotomized according to cut-off levels used in this score (7) (leukocytes $> 11 \times 10^9/L$, platelets $\geq 350 \times 10^9/L$, hemoglobin $< 100g/L$) and D-dimer was dichotomized according to the 75th percentile of the CATS study population (cut-off: $1440\mu g/L$) as already described by Ay and colleagues (2009) (2). Multivariable Cox-regression models were tested for all pair-wise interactions and interactions with $\log(\text{time})$ by means of candidate variables and a $P < 0.01$ was considered to indicate a significant interaction. However, no significant

interaction was found. For hypothesis tests we considered $P < 0.05$ as statistically significant and all tests were two-sided. Statistical computations were performed with SAS System V9.2 (2008, SAS Institute, Cary, NC) and SPSS 17 (SPSS, Inc. 2001, Chicago, IL, www.spss.com).

References

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