Thrombosis Research 131 (2013) 162–165

Contents lists available at SciVerse ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

Intratumoral tissue factor expression and risk of venous thromboembolism in brain tumor patients

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ARTICLE INFO

Article history:
Received 17 April 2012
Received in revised form 21 September 2012
Accepted 25 September 2012
Available online 17 October 2012

Keywords:
Brain tumors
Tissue factor
Immunohistochemistry
Venous thromboembolism

ABSTRACT

Background: Brain tumor patients have an increased risk of venous thromboembolism (VTE). An important role in cancer-related VTE has been suggested for tissue factor (TF), the main initiator of the coagulation cascade. We conducted a prospective cohort study to determine whether expression levels of TF in brain tumors are associated with future VTE.

Patients and Methods: We immunohistochemically determined TF-expression in brain tumor specimens of 96 adult patients (8 low-grade and 82 high-grade gliomas, 6 embryonal tumors) that were included in the Vienna Cancer and Thrombosis Study (CATS). Each patient was prospectively followed until the occurrence of VTE and/or death within a period of two years or loss of follow-up.

Results: Fifteen brain tumor patients (15.6%) developed VTE during follow-up. Seventy-seven brain tumors (80.2%) stained positive for TF. Staining was strong in 13 (13.5%), moderate in 64 (66.7%) and negative in 19 (19.8%) tumors. No statistically significant association between TF-expression (negative, focal, widespread) and the occurrence of VTE was found. The hazard ratio (HR) for VTE was 1.30 (95% confidence interval [CI]: 054 – 3.14, p = 0.567) when patients with negative-, focal- and widespread TF expression were compared and not statistically significant. Also when tumors were categorized into two groups (focal/widespread versus negative TF-expression), the HR for future VTE was not statistically significant (HR: 1.45, 95% CI: 0.44 – 7.37; p = 0.578). An association can still not be definitely excluded, as this study was underpowered.

Conclusions: Our data indicate that TF-expression levels in brain tumors are not strongly associated with future VTE.

Introduction

Venous thromboembolism (VTE) is a frequent complication and leading cause of death in brain tumor patients [1,2]. Up to 34% of brain tumor patients develop VTE during the course of disease. However, not all patients are at the same risk of VTE, and thromboprophylaxis for prevention of VTE is challenging in these patients [3,4].

Tissue factor (TF), a transmembrane glycoprotein, is the main initiator of the blood coagulation cascade in-vivo and has been proposed to play a pivotal role in the development of VTE in brain tumor patients [5]. In recent studies TF-expression in tumor tissue has been reported to predict an increased risk for future VTE in pancreatic- and ovarian cancer patients [6,7]. Whether TF-expression in brain tumors is associated with future VTE has not been investigated yet.

It was the aim of this study to investigate whether TF expression levels in brain tumors are predictive of future VTE.

Patients and Methods

Study Design and Study Population

This study was performed in the framework of the Vienna Cancer and Thrombosis Study (CATS), an ongoing prospective cohort study performed at the Medical University of Vienna. The main objective of CATS is to investigate predictive parameters for the occurrence of VTE in patients with cancer. The detailed study design and methodology along with exclusion and inclusion criteria have been reported previously [8–10]. According to the CATS protocol, all patients undergo a structured interview on their medical history at study inclusion and data on tumor-histology are documented. Each patient is followed

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http://dx.doi.org/10.1016/j.thromres.2012.09.020

until the occurrence of VTE and/or death within a period of two years or loss of follow-up. Information on the course of disease and on the occurrence of VTE is collected for every patient at three-month intervals. If patients do not respond, their family doctors or relatives are contacted. Patients were instructed to contact the Division of Haematology and Haemostaseology (Vienna General Hospital), if they perceive symptoms indicative of VTE (deep vein thrombosis or pulmonary embolism), stroke, myocardial infarction, or peripheral arterial thrombosis. In case of death, data on the time and the cause of death including autopsy protocols are collected. Once a year the Austrian death registry is searched for entries of study participants included in the study. Exclusion criteria for all patients are (a) chemotherapy within the past 3 months prior to study inclusion, (b) surgery or radiotherapy within the past 2 weeks prior to study inclusion, (c) venous or arterial thromboembolism within the past 3 months prior to study inclusion, (d) continuous anticoagulation with vitamin K antagonists or low-molecular-weight-heparins and (e) overt bacterial or viral infection.

Inclusion criteria for our present study were: (a) enrollment into CATS and complete clinical follow-up according to the CATS protocol, an (b) availability of a formalin-fixed and paraffin-embedded (FFPE) brain tumor sample in the bio-bank of the Medial University of Vienna.

The study protocol was approved by the local Ethics Committee, in accordance with the Declaration of Helsinki.

Immunohistochemistry

For anti-TF immunostaining, sections were cut at a thickness of 3–5 μm from routinely FFPE tumor tissue blocks. Slides underwent heat induced epitope retrieval at 95 °C for 20 minutes in Flex TRS low solution (Dako, Glostrup, Denmark). Sections were incubated with a monoclonal IgG1 mouse anti-tissue factor antibody (No. 4509, American Diagostica, Inc., Greenwich, CT, USA) at a dilution of 1:200 for 60 minutes at room temperature. Detection of immunostaining was performed using the ChemMate® kit (Dako) and diaminobenzidine (DAB) was used as chromogen. For specificity controls, the primary antibody was omitted or substituted by isotype-matched antibodies, which resulted in negative staining reactions. As a positive control we used a glioblastoma sample with ample TF expression.

All sections were reviewed by two observers experienced in histopathological evaluation of primary brain tumours (J.A.H. and M.P.) together on a multi-headed microscope. In each individual case both observers evaluated the same immunostained slide together and immunohistological grading was performed in agreement. The observers were blinded to clinical and pathologic information. The intensity of TF staining was semiquantitatively classified into 3 degrees as follows: (−), negative; (+), focal expression (≤80% positive tumor cells) and widespread expression (>80% positive tumor cells) based on the estimated proportion of the entire tumor cell population staining positively for TF.

Statistical Analysis

Continuous variables were described by the median and the 25th-75th percentile. Categorical variables were described by the absolute numbers and percentages. The Kruskal-Wallis test was used to compare immunohistochemical expression of TF and levels of circulating TF between groups of patients. The median follow-up time was calculated with the reverse Kaplan-Meier method. Survival probabilities were estimated by the Kaplan-Meier method.

Univariate Cox regression analysis was used to evaluate the effect of immunohistochemical expression of TF on the development of VTE. Evaluating the potential predictive value of immunohistochemical expression of TF on the development of VTE, the observation endpoint was fatal or non-fatal VTE. Data were censored at death, end of observational period after 2 years or loss to follow-up. Analyzing the outcome VTE, the prognostic variable immunohistochemical expression of TF was considered only in a univariate Cox regression model due to the rather small number of events (i.e. VTE).

Results

Characteristics of Study Population

Ninety-six brain tumor patients diagnosed with a brain tumor between December 2003 and March 2010 were included in this study. Ninety patients (93.8%) had gliomas including 8 patients (8.3%) with low-grade astrocytomas, 15 patients (15.6%) with anaplastic astrocytomas, 4 patients (4.1%) with anaplastic oligodendrogliomas, 7 patients (7.3%) with anaplastic oligoastrocytomas and 56 patients (58.3%) with glioblastomas. Six patients (6.3%) had embryonal neoplasms including 1 medulloblastoma and 5 primitive neuroectodermal tumors (PNET). Information on basic patient characteristics, tumor histology and cancer treatment are given in Table 1.

Immunohistochemical Analysis and Description of Tissue Factor Expression

TF-expression was widespread in 13- (13.5%), focal in 64- (66.7%) and negative in 19 (19.8%) brain tumor specimens. The highest levels of TF expression were found in glioblastomas followed by low-grade astrocytomas, anaplastic oligodendrogliomas, anaplastic astrocytomas, anaplastic oligoastrocytomas and embryonal tumors (Kruskal-Wallis Test: $p<0.001$). Detailed information on the staining intensity in different brain tumor histologies is given in Table 2. TF expression patterns in different tumor specimens are shown in Fig. 1A - D. Tissue factor-expression occurred primarily in neoplastic astrocytes (Fig. 1A – C) but also in reactive astrocytes that infiltrated or surrounded brain tumors (Fig. 1D). Particularly in glioblastomas TF-expression was accentuated in perinecrotic areas and around microvascular proliferates (Fig. 1A). On the cellular level TF was most strongly expressed on cell surfaces and less in the cytoplasm (Fig. 1B – D). Reactive non-malignant astrocytes showed high TF-expression up to the smallest extensions of their cellular processes (Fig. 1D).

Tissue Factor Expression and Thromboembolic Events

Fifteen brain tumor patients (15.6%) developed a VTE event during follow-up. VTE event rates according to different brain tumor histologies are given in Table 2. Venous thromboembolic event characteristics are given in Table 3. In univariate Cox-regression analysis no

Table 1
Baseline Characteristics of brain tumor patients (n = 96).

Age at study entry, mean ± standard deviation, yrs	51 ± 15
Gender	
Female, n (%)	31 (32.3)
Male, n (%)	65 (67.7)
Brain tumor histology n (%)	
Gliomas:	
Low-grade astrocytoma	8 (8.3)
Anaplastic astrocytoma	15 (15.6)
Glioblastoma	56 (58.3)
Anaplastic Oligoastrocytoma	7 (7.3)
Anaplastic Oligodendroglioma	4 (4.2)
Embryonal tumors:	
Medulloblastoma	1 (1)
Primitive Neuroectodermal tumor	5 (5.2)
Brain cancer treatment during observation period, n (%)	
Chemotherapy	76 (79.1)
Radiotherapy	66 (68.8)
Surgery	12 (12.5)