Risk of Venous Thromboembolism Following Hemorrhagic Fever With Renal Syndrome: A Self-controlled Case Series Study

Risk of venous thromboembolism following hemorrhagic fever with renal syndrome: A self-controlled case series study

Anne-Marie Connolly-Andersen[1]*, Heather Whitaker[2], Jonas Klingström[3], Clas Ahlm[1]

1. Unit of Infectious Diseases, Dept. of Clinical Microbiology, Umeå University, Umeå, Sweden
2. Dept. of Mathematics and Statistics, The Open University, Milton Keynes, U.K.
3. Centre for Infectious Medicine, Department of Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

* Corresponding author:
Anne-Marie Connolly-Andersen, PhD
Division of Infectious Diseases, Dept. of Clinical Microbiology, Umeå University
901 85 Umeå, Sweden
Telephone: +46 90 7850921, Fax +46-90-133006
Email: anne-marie.connolly-andersen@umu.se

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Summary: The risk of venous thromboembolism is significantly increased following hemorrhagic fever with renal syndrome and is higher in females compared to males. Our

study is the first to determine the risk of venous thromboembolism following a viral hemorrhagic fever.

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Abstract

Background: Bleeding is associated with viral hemorrhagic fevers; however, thromboembolic complications has received less attention. Hemorrhagic fever with renal syndrome (HFRS) is a mild viral hemorrhagic fever caused by Puumala hantavirus. We previously identified HFRS as a risk factor for myocardial infarction and stroke, but the risk for venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE) is unknown.

Methods: Personal identity numbers from the Swedish HFRS database were cross-linked with the Patient Register to obtain information on all causes for hospitalization during 1964 to 2013. The self-controlled case series method was used to calculate the incidence rate ratio (IRR) for first VTE, DVT and PE during 1998 to 2013.

Results: From 7 244 patients with HFRS, there were 146 with a first VTE of which 74 were DVT and 78 were PE, and 6 patients had both DVT and PE. The overall risk for a VTE was significantly higher during the first two weeks following HFRS onset with IRR 61.8 and 95% confidence interval (CI) 34.7-110.2. The corresponding risk for a DVT was 45.9 (17.2-118.5) and PE 72.7 (34.9-151.2). Sex interacted significantly with the association between HFRS and VTE, with females having a higher risk compared to males.

Conclusion: A significantly increased risk for VTE was found in the time period following HFRS onset. It is important to keep this in mind and monitor HFRS patients, and possibly other viral hemorrhagic fever patients, for early symptoms of VTE.

Introduction

Viral hemorrhagic fever contains members from four viral families: *Bunyaviridae*, *Filoviridae*, *Arenaviridae* and *Flaviviridae*. Pathogenic hantaviruses from the *Bunyaviridae* family cause hantavirus cardiopulmonary syndrome in the Americas and hemorrhagic fever with renal syndrome (HFRS) in Eurasia, and recently hantaviruses have also been reported from Africa [1]. Annually, over 100 000 cases of hantavirus infections are estimated to be diagnosed worldwide [1]. Puumala hantavirus causes HFRS and is a mild viral hemorrhagic fever endemic in Central and Northern Europe [2]. HFRS is a disease characterized by endothelial and platelet activation, coagulation disturbances and renal dysfunction [1, 3-6]. Endothelial dysfunction and hypercoagulability are common during viral hemorrhagic fever, which are risk factors for thromboembolism [2, 7, 8]. Recently, HFRS was identified as a risk factor for the arterial thromboembolic events: acute myocardial infarction (AMI) and stroke [9]. However, no studies have focused on the risk for venous thromboembolism (VTE) following a viral hemorrhagic fever. Therefore, the present study determined the risk of VTE occurrence in a large cohort of patients with HFRS.

Methods

Subject and data sources

HFRS is a notifiable disease in Sweden; therefore, the Public Health Agency of Sweden registers all HFRS diagnosed individuals by their personal identity number (PIN). Every Swedish citizen is issued a PIN at birth by the Swedish Tax Agency. The diagnosis of HFRS is confirmed by detection of specific IgM and/or IgG seroconversion using an indirect immunofluorescence method or ELISA in accredited laboratories, as previously described [9]. The data reported into the HFRS database includes dates of HFRS onset, sample date, diagnosis and reporting as previously reported by us [9]. Data on venous thromboembolism (VTE) and date of HFRS onset were obtained by crosslinking the HFRS database with the inpatient register (IPR), Cause of Death Register and Outpatient Register using the PIN for each patient. All data were anonymized by the Swedish National Board of Health and Welfare. The first VTE (including deep vein thrombosis (DVT) and pulmonary embolism (PE)) was identified between 1964 through 2013 using ICD7 through ICD10 (see Supplementary Table 1 for ICD codes). It is only possible to calculate the risk of a VTE following HFRS onset after 1997 (where the disease became notifiable). But since the self-controlled case series method requires a full exposure risk history (364 days from HFRS) prior to initiation of the study period only individuals with a first VTE during 1998 to 2013 were included. Ethical approval was granted by the Regional Ethical Board of Stockholm, Sweden.

Study design and statistical analysis

The incidence rate ratio (IRR) and 95% confidence interval (CI) of venous and/or arterial thromboembolism during risk periods following HFRS onset compared with control periods was determined using the self-controlled case series method. The risk period was divided into

weeks following HFRS disease onset: 1-2, 3-4, 5-8, 9-12, 13-26 and 27-52 weeks (or for follow-up studies: 1-4, 5-8, 9-12 and 13-52 weeks). Exposure to the virus is unlikely in the immediate time period following VTE due to hospitalization and recovery. In addition, HFRS diagnosis may be delayed [9]. We therefore excluded the 26 weeks prior to HFRS disease onset to allow for these factors. The control period spanned the period 1st of January 1998 until 31st December 2013 or date of death, excluding the 26-week pre-exposure buffer period and the risk period (see Figure 1). Patients with VTE as the cause of death at the time of event were excluded to meet the assumptions of the self-controlled case series method [10].

All models adjusted for age using the age groups: 0-49; 50-59; 60-69; 70-79 and > 80 years of age at thromboembolism event, since the incidence of VTE increases with age [11, 12]. A likelihood ratio test was applied to examine the interactions between sex or seasonality and VTE risk following HFRS onset. Seasons were divided into colder months: October through March and warmer months: April through September. Median ages were compared using non-parametric Mann-Whitney test. Gender distribution was compared using χ^2 test.

Sensitivity analyses were run excluding individuals with other known risk factors for VTE: cancer, surgery or hospitalization for any reason 6 weeks prior to the event and the age-adjusted IRR for VTE following HFRS was calculated separately [11, 12]. The cancer diagnoses codes were as follows: ICD-7 codes 140-239; ICD-8 codes 140-239; ICD-9 codes 140-239 and ICD-10 codes C00-D48. Two separate analyses were performed where individuals diagnosed with cancer were excluded. The first analysis excluded individuals with diagnosis of cancer at any time point of their life regardless when the VTE occurred. The second analysis excluded only individuals with a cancer diagnosis within ± 12 months of VTE event.

We analyzed whether the length of hospitalization differed between the control period (including buffer period) and the risk period, thereby predisposing to VTE, using the non-

parametric Mann-Whitney U-test. Since anti-coagulation treatment indicates other risk factors and affects the coagulation system, individuals on anti-coagulation therapy prior to event were excluded in a follow-up analysis and the IRR for VTE following HFRS calculated. These patients were identified using the ICD-10 code Z921 (long-term usage of anti-coagulants in personal history); anatomical therapeutic chemical (ATC) code: B01AA03 (warfarin) or B01AB04 (fragmin); atrial fibrillation or heart valve prosthesis of non-biological origin (ICD-7: 433.12 and 433.13; ICD-8: 427.92 and 427.90; ICD-9: 427D, 427A and V43D; and ICD-10: I48 or Z952).

Statistical analyses of date were performed using SPSS (version 24) or Stata (version 12.0 SE). All *P*-values are 2-sided and $P < 0.05$ is considered statistically significant.

Results

From 1997 where HFRS became a notifiable disease, there were a total of 7 244 patients registered in The HFRS database up to 2014. From these patients, that were diagnosed with HFRS from 1997, 6 435 (88.8%) were hospitalized for any cause during 1964-2013 (IPR). 2 663 (36.8%) were hospitalized due to HFRS (IPR) either as the main or contributing cause of hospitalization. 483 (6.7%) had deceased during 1997-2013 (Cause of Death register). During 1998 to 2013 there were 146 HFRS patients with a first VTE (2%). Of these, 34 patients (23%) had their first VTE during our specified risk period (0-52 weeks following HFRS onset).

The baseline demographics and the number of individuals with known risk factors for VTE are shown in Table 1. Generally, the individuals in the study groups were older compared to the HFRS cohort. In addition, the sex distribution differed significantly between the PE study group compared to the HFRS cohort (Table 1).

Venous thromboembolism following HFRS onset

The IRR for a first VTE was significantly increased in the first half year (apart from 9-12 weeks) following HFRS (Table 2) compared to the control period. VTE was further divided into DVT and PE, where the IRR for DVT was significantly increased the first year following HFRS. The IRR for PE was significantly higher in the first month only following HFRS. The IRR for PE in the first two weeks following HFRS was higher than that for DVT. Sex had a significant modifying effect on the association between HFRS and VTE (Supplementary Table 2). The IRR for VTE for females was higher compared to males in the four weeks following HFRS onset. Similarly, the risks for DVT and PE were higher in females compared to males in the same time period. Season did not have a confounding effect on VTE (Supplementary Table 2). Other risk factors for VTE include cancer diagnosis, surgery and

immobilization [11, 12]. However, the association between HFRS and VTE was still significant even after excluding individuals who had had a cancer diagnosis at 1) any time point during the observation period or 2) within ± 12 months of HFRS (Supplementary Table3). The association between HFRS and VTE was still significant even after excluding individuals who had undergone surgery or were hospitalized for any reason 6 weeks prior to HFRS, respectively (Supplementary Table 3). In addition, anti-coagulation treatment could attenuate the association between HFRS and VTE. Yet, the association was similar even after these individuals were excluded (Supplementary Table 3). Another risk factor for VTE is immobilization. If patients are hospitalized for a longer time during HFRS (risk period) compared to hospitalizations during the control period, this could predispose to VTE [11, 12]. We did not find a significant difference in the length of hospitalization in the control period vs. the risk period (see Supplementary Table 4).

Discussion

This study of all Swedish HFRS patients from 1997 to 2014 determines an increased risk for venous thromboembolism following a viral hemorrhagic fever. We previously identified HFRS as a risk factor for acute myocardial infarction and stroke [9].

We found a statistically significant modifying effect of sex on the association between HFRS and VTE with females having a higher IRR for a first VTE compared to males. Furthermore, though the IRRs for females were higher, the length of time with increased risk for VTEs following HFRS onset was longer in males. Only one female under 59 years of age had VTE during the risk period indicating that reproductive risk factors are not an explanation for our results. The cytokine profile differs between males and females during acute HFRS, whether this translates into an increased risk for VTE is unknown [13]. Other risk factors for VTE include cancer, surgery and immobilization (hospitalization) [11, 14] and a protective factor is anti-coagulants, though when we performed sensitivity analyses including these factors, they did not modify our results.

Previous studies have associated infections with increased risk for VTE [15-17] . In a study with a similar methodology to ours, the risk for a DVT and PE was increased approximately two-fold in the first two weeks following a urinary tract infection and similarly for DVT following a systemic respiratory tract infection.[15]. Notably, we observe in our study of HFRS patients a much higher risk for DVT (≈ 46-fold) and PE (≈ 73-fold) in the same period, indicating the potency of the interaction between hantaviral infection, the endothelium and the coagulation system. Our results are further supported by autopsies of patients who died due to Puumala hantavirus infection, who had thromboses in pulmonary vessels [18]. That HFRS patients have coagulopathy is indicated by almost one-third of HFRS patients fulfilling

criteria for disseminated intravascular coagulation [5], increased platelet activation during HFRS [4], deregulated fibrinolysis [6] and the identification of HFRS as a risk factor for the two specific cardiovascular events AMI and stroke [9]. Endothelial cells are a key player in VHF pathogenesis and clinical outcome [19], and hantaviruses target endothelial cells during infection resulting in increased coagulation and platelet adhesion [20]. Furthermore, the increased risk for arterial and venous thromboembolism during HFRS, as shown in our previous and current study, indicates that endothelial cells are affected during hantaviral infection regardless of their location [9]. Thereby there are several mechanisms during hantavirus infection that could explain the increased risk for thromboembolism that we show in this cohort based study.

Concerning other viral hemorrhagic fevers, case reports for Dengue virus indicate a similar tendency for venous thromboembolism that we observed in our study [21]. Also, thrombosis is relevant for the outcome in viral hemorrhagic fever, which was indicated in a study where increased von Willebrand factor levels in patients with hemorrhagic manifestations following infection with Sudan ebolavirus led to speculation that these patients have excessive thrombosis [22]. In addition, case reports of two other ebolavirus infected patients indicated a hypercoagulable phase following infection that warranted treatment with low-molecular-weight heparin [23]. However, the risk for thromboembolism following these viral hemorrhagic fevers has yet to be determined and quantified.

A strength of our study is that it comprises all individuals in Sweden with verified HFRS since 1997 until 2014. In addition, we have data from the IPR since 1964, with complete coverage from 1987 [24]. We could therefore identify the first venous thromboembolic event with high precision from 1987 up until 2013.

The self-controlled case series method requires precision of the exposure date (HFRS disease onset), since the method is sensitive to timing and dates [10]. For nearly 80% of our patients, we have the actual date of HFRS disease onset, which is another strength of our study. The least accurate date, the date of report to the Public Health Agency of Sweden, constitutes only 0.7% of the dates. However, in order to circumvent potential imprecision of HFRS disease onset, we included a 6-month buffer prior to the HFRS date. This ensures that a patient who receives a delayed HFRS diagnosis or registration date with venous thromboembolism during that delay period, will not affect the actual IRR for a thromboembolism.

A limitation of this study is that we do not have access to information regarding whether the individuals are on systemic hormone replacement therapy, use oral contraceptives or are pregnant since these are risk factors for VTE [11, 14].

In Sweden, the IPR has been extensively validated concerning the codes for AMI and stroke diagnoses but not for VTE [24]. There is a potential risk for a less accuracy and over- or underestimation of VTE events, which is a limitation of our study. However, that risk should equally apply to both the control period and the risk period. Thereby the probability of it affecting the IRR following HFRS is likely low.

An additional limitation is that although we did not find a significant difference in the length of hospitalization between the control periods compared to the risk period, the association between HFRS and VTE could still be mediated in part by immobility. Further *in vivo* studies are required to ascertain the link between HFRS and VTE.

We show a significant but transient association between venous thromboembolism and HFRS. Previous studies show a reduction in venous thromboembolism in patients with infectious disease that were treated with thromboprophylaxis [25]. The Wells score for DVT and PE do

not include infectious diseases as a risk factor. A scoring system that takes infectious disease, among others, into account showed a reduction in VTE following identification of high-risk groups with subsequent thromboprophylaxis treatment [26].

Our study raises the question whether some patients with HFRS, or other viral hemorrhagic fevers, could potentially benefit from similar thromboprophylaxis in the short-term period. Further clinical studies are warranted to support such recommendations.

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Figure legends.

Figure 1. Study design for analyses of the association between hemorrhagic fever with renal syndrome (HFRS) and venous thromboembolism using the self-controlled case series method. HFRS patients are included from 1st of January 1997 until 30th June 2014. The control period spans the period 1st of January 1998 until 31st December 2013 or date of death, excluding the 26-week pre-exposure buffer period and the risk period. The risk period spans 365 days following HFRS disease onset, and is further stratified into 1-2, 3-4, 5-8, 9-12, 13-26 and 27-52 weeks following HFRS onset (or for follow-up studies: 1-4, 5-8, 9-12 and 13-52 weeks following HFRS onset).