Increased Risk of Acute Myocardial Infarction and Stroke During Hemorrhagic Fever with Renal Syndrome: A Self-Controlled Case Series Study

Running title: Connolly-Andersen et al.; HFRS increases risk for AMI and stroke

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Abstract

**Background**—We recently observed that cardiovascular causes of death were common in patients with hemorrhagic fever with renal syndrome (HFRS), which is caused by hantaviruses. But it is not known whether HFRS is a risk factor for acute cardiovascular events: acute myocardial infarction (AMI) and stroke.

**Methods and Results**—Personal identification numbers from the Swedish HFRS patient database (1997 to 2012; n = 6643) were cross-linked with the National Patient Register from 1987-2011. Using the self-controlled case series method, the incidence rate ratio (IRR) of AMI/stroke in the 21 days following HFRS were calculated against two different control periods either excluding (analysis 1) or including (analysis 2) fatal AMI/stroke events. The IRRs (95% confidence intervals) for analysis 1 and 2 for all AMI events were 5.53 (2.6-11.8) and 6.02 (2.95-12.3); and for first AMI events 3.53 (1.25-9.96) and 4.64 (1.83-11.77). The IRRs for analysis 1 and 2 for all stroke events were 12.93 (5.62-29.74) and 15.16 (7.21-31.87); and for first stroke events 14.54 (5.87-36.04) and 17.09 (7.49-38.96). The majority of stroke events occurred in the first week following HFRS. Seasonal effects were not observed, and apart from one study, neither sex nor age interacted with the associations observed in this study.

**Conclusions**—There is a significantly increased risk for AMI and stroke in the immediate time period following HFRS. Therefore HFRS patients should be carefully monitored during the acute phase of disease to ensure early recognition of symptoms of impending AMI or stroke.

**Key words:** acute myocardial infarction, epidemiology, cerebrovascular disease/stroke, Hemorrhagic fever with renal syndrome, Hantavirus
**Introduction**

Globally, cardiovascular diseases (CVD) are the leading cause of death and a major source of disability. A majority of these are caused by ischemic heart disease, such as acute myocardial infarction (AMI), stroke, and other cerebrovascular diseases. Several factors, including infectious diseases, have been associated with increased risk for CVD. The host inflammatory response to infection often results in release of pro-inflammatory cytokines and activation of platelets, leukocytes and endothelial cells that can activate pro-coagulant pathways and can inhibit anticoagulant pathways. This can tip the balance of hemostasis towards a pro-thrombotic state, laying the foundation for acute cardiovascular events such as AMI and stroke.

Viral hemorrhagic fevers (VHF) are characterized by fever, hemorrhages and shock. Abnormal coagulation and vascular dysfunction are common observations during VHF, which could be risk factors for CVD. Four viral families contain members that cause VHF: Bunyaviridae, Flaviviridae, Filoviridae and Arenaviridae. Pathogenic hantaviruses in the Bunyaviridae family cause hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. HFRS caused by Puumala virus (PUUV) is considered a mild VHF characterized by thrombocytopenia, enhanced coagulation and fibrinolysis, and disseminated intravascular coagulopathy (DIC).

In a highly endemic area for PUUV in Northern Sweden, we observed a tendency of HFRS-patients to develop acute cardiovascular events. Further, we have recently showed that CVD is a common cause of death during acute HFRS in Sweden. Together, this suggests a possible association between acute HFRS and CVD, and we therefore decided to investigate more in detail if HFRS is a risk factor for CVD. To test this, we compared the incidence of AMI and stroke in the immediate time periods before and following HFRS disease onset, using the
self-controlled case series method (SCCS).\textsuperscript{12}

Methods

Participants and databases

HFRS is a notifiable disease in Sweden since 1997. To diagnose HFRS, a blood sample is obtained from the patient and analyzed by one of the three HFRS diagnostic laboratories in Sweden. Samples are analyzed for seroconversion, either by an indirect immunofluorescence method or by ELISA.\textsuperscript{13} The attending medical doctor and diagnostic laboratory then separately report the HFRS diagnosis using the patient’s personal identification number (PIN) to the Swedish Institute for Communicable Disease Control (SICDC). This report is entered into the national HFRS database administered by the SICDC. All patients diagnosed with HFRS in Sweden from 1\textsuperscript{st} of January, 1997 until 31\textsuperscript{st} of December, 2012 were included in the present study.

The National Patient Register is a nationwide database administered by the Swedish National Board of Health and Welfare (NBHW), consisting of among others the Inpatient Register (IPR) and the Outpatient Register (OPR).\textsuperscript{14} The IPR contains information on somatic hospital discharges nationwide and has complete national coverage since 1987. The OPR contains information on hospital-based outpatient care diagnoses.\textsuperscript{14}

The PINs from the HFRS database were cross-linked with the National Patient Register to retrieve information regarding potential AMI/stroke events. At the time of our request to the NBHW the IPR only had registered data up until 2011. All data were anonymized by the NBHW before analyses were performed. This study received ethical approval from the Regional Ethical Review Board, Stockholm, Sweden.
AMI and stroke events and determination of HFRS index date

Individuals with international classification of disease (ICD; World Health Organization) main and/or contributing diagnosis codes for AMI/stroke were selected from the HFRS-database cross-linked IPR. The ICD-9 diagnosis codes 410 and 411 and ICD-10 diagnosis codes I21-22 were used for AMI. The diagnoses codes for stroke were: ICD-9 codes 430-434, 436, and ICD-10 codes I60-64. Stroke was further sub-classified into hemorrhagic stroke (ICD-10 codes I60-I62) and ischemic stroke (ICD-10 codes I63-I64). The date of an AMI or stroke event is the date of hospitalization found in the IPR for the patient. A first AMI/stroke event is defined as the first time the patient is hospitalized due to AMI or stroke in the IPR in the time period 1987 to 2011.

The self-controlled case series (SCCS) method is highly sensitive to the accuracy of dates. Therefore, we first selected individuals who had had an AMI/stroke within ± 2 years of the date of report to the SICDC. Then each individual was re-assigned a new date, henceforth called the HFRS index date, based on availability of information in the HFRS database, IPR and OPR. The preferred date was the date of disease onset, if entered into the HFRS database. This date was then followed in preference by the date of hospitalization/visit to an outpatient clinic with the ICD-10 diagnosis code for HFRS A98.5 in the IPR and the OPR, respectively. If HFRS was reported to the SICDC whilst the patient was hospitalized, the date of hospitalization was then used. Lastly, the dates of blood sampling, diagnosis and report were utilized from the HFRS database in order of preference.

Statistical analyses

We used the SCCS method, a conditional Poisson regression method, to calculate relative incidence rate ratios (IRR) for AMI and stroke. This method is based on intra-person comparisons of incidence rates (IR) of the outcome of interest (AMI or stroke event) following
exposure (HFRS) in an observation period subdivided into risk and control periods.\textsuperscript{12, 15} Hence, the method is conditional on participants having an AMI or stroke event within the observation period relative to the HFRS index date (figure 1). To avoid immortal time bias\textsuperscript{16} which can occur when a control period is included prior to exposure (here HFRS), we performed two separate analyses. In analysis 1 the observation period was ± 365 days from the HFRS index date, and + 365 days from the HFRS index date for analysis 2. Individuals that died within 365 days of their AMI or stroke event were excluded from analysis 1 but not in analysis 2. The incubation period for HFRS is normally three weeks,\textsuperscript{8} but the exact date of infection is normally not possible to determine for infected individuals. Since we do not know the effect of hantavirus-infection during the incubation period of HFRS on AMI and stroke incidence rates, we therefore included a separate risk period (“buffer”) ranging from 30 days to 1 day prior to the HRFS index date in analysis 1 (figure 1). The risk periods for both analyses were defined as: day 1 to 21; and day 22 to 90 after the HFRS index date. Furthermore, both analyses had the days 91 to 365 following HFRS index date as a control period. Analysis 1 also had days 365 to 31 prior to the HFRS index date as a control period (figure 1).

A seasonal pattern has been observed for HFRS and AMI in Sweden; both are more common during the colder months of the year.\textsuperscript{17, 18} Therefore we performed separate analyses where we adjusted for the potential confounder seasonality by dividing the year into two parts; colder months (October through March) and warmer months (April through September).

We analyzed whether the median age and sex differed between the original HFRS cohort and the study groups for AMI/stroke events using the non-parametric tests Mann-Whitney U-test for age differences and the $\chi^2$ test for sex distribution.

To investigate whether sex and age act as effect modifiers on the potential association
between HFRS and AMI/stroke, we included interactions between sex and exposure, and age and exposure in the analysis. Likelihood ratio tests were thereafter carried out to test the hypotheses that no interaction terms should be included in the model.\textsuperscript{12}

In Sweden, the median age for AMI and stroke onset is 70 and 75 years, respectively.\textsuperscript{19, 20} Therefore, individuals were divided into the age groups ≤ 70 years and > 70 years of age in the AMI study groups. The individuals in the stroke study groups were divided into the age groups ≤ 75 years of age and > 75 years of age.

Data were analyzed using SPSS (version 20) and Stata (version 12.0 SE).

Results

Totally 6643 individuals were diagnosed with HFRS in Sweden during 1997 to 2012 with a median age of 52 years (interquartile range (IQR) 39-62 years) and a 1.48:1 male to female ratio (table 1). The distribution of the various dates used for determining the HFRS index date is shown in supplementary table 1 for the HFRS cohort and for the AMI/stroke study groups in supplementary table 2.

Acute myocardial infarction

In total, 320 of the HFRS-diagnosed individuals had been hospitalized due to AMI at one or more time points during 1987 until 2011 (figure 2). The study group for analysis 1 consisted of 51 individuals with 55 AMI events within ± 365 days of HFRS index date, of which there were 41 first (75\%) and 14 recurrent AMI events (25\%), respectively (figure 2, table 1). The study group for analysis 2 consisted of 37 individuals with 40 AMI events within + 365 days of HFRS, of which 27 (68\%) were first and 13 (32\%) were recurrent AMI events (figure 2, table 1). The median age for individuals with AMI events was significantly higher than for the original HFRS
cohort. There was a higher proportion of males to females in analysis 1 compared to the original HFRS cohort, but not for analysis 2 (Table 1).

In analysis 1, the IRR in the first three weeks following HFRS was 5.53 (95% CI 2.6-11.8) for all AMI events and 3.53 (95% CI 1.25-9.96) for first AMI events (Table 2). The IRR did not differ significantly from the control periods in risk period 2 (22-90 days following HFRS onset). In analysis 2, the IRR in the first three weeks following HFRS was 6.02 (95% CI 2.95-12.3) for all AMI events and 4.64 (95% CI 1.83-11.77) for first AMI events (Table 2). As in analysis 1, the IRR for the risk period 22 to 90 days following HFRS did not differ significantly from the control periods. A histogram of AMI events in relation to HFRS is displayed in supplementary figure 1.

No statistically significant confounding effects were observed for seasonality on the correlation between HFRS and AMI, although a borderline effect was observed for season in analysis 1 and 2 of first AMI events (supplementary table 3). The season-adjusted IRR in analysis 1 and 2 for first AMI events in the time period 1-21 days following HFRS was 2.96 (95% CI 1.03 – 8.47) and 3.8 (95% CI 1.46-9.9), respectively. Apart from the study group for all AMI events in analysis 2, no modifying effects of age at HFRS > 70 and sex were found on the association between HFRS and AMI (supplementary table 4 and 5). The model containing the interaction term HFRS x age was borderline significant for analysis 2, which meant that the IRRs for AMI were likely different for individuals above and below 70 years of age (supplementary table 4). We therefore performed a follow up analysis where the IRRs for analysis 2 of all AMI events were re-calculated separately for the two age groups. For individuals above 70 years of age (n = 14; events = 16) the IRR for an AMI in the first three weeks following HFRS was 15.33 (95% CI 5.15-45.62), for individuals below 70 years of age (n = 23; events = 24) the IRR for an
AMI in the first three weeks following HFRS was 2.92 (95% CI 0.99-8.63).

**Stroke**

Of all HFRS-diagnosed, in total 258 individuals were hospitalized due to stroke at one or more time-points during 1987 until 2011 (figure 2). The study group for analysis 1 consisted of 26 individuals who had 28 stroke events ± 365 days of HFRS; of these 22 (79%) were a first stroke event and 6 (21%) were recurrent stroke events (table 1). Of the 28 stroke events, 22 (78.6%) were ischemic and 6 (21.4%) were hemorrhagic. For the first stroke events, 18 (81.8%) were ischemic and 4 (18.2%) were hemorrhagic.

The study group for analysis 2 consisted of 30 individuals who had 31 stroke events within + 365 days of HFRS (table 1); 25 (81%) were a first, and 6 (19%) were recurrent, stroke events, respectively. Of the 31 stroke events, 22 (71%) were ischemic and 9 (29%) were hemorrhagic. For the first stroke events, 18 (72%) were ischemic and 7 (28%) were hemorrhagic.

The median age for those diagnosed with stroke was significantly higher than the median age of the original HFRS cohort. However, the sex distribution for all study groups with stroke did not differ significantly from the HFRS cohort (table 1).

In analysis 1 the IRR for the first 3 weeks following HFRS was 12.93 (95% CI 5.62-29.74) for all stroke events and 14.54 (95% CI 5.87-36.04) for first stroke events (table 3). In analysis 2 the IRR for the first 3 weeks following HFRS was 15.16 (95% CI 7.21-31.87) for all stroke events and 17.09 (95% CI 7.49-38.96) for first stroke events (table 3). The IRRs for the second risk period (after 3 weeks following HFRS onset) did not significantly differ from the control periods in either analysis (table 3). There were no stroke events in the third week, we therefore calculated the IRRs for the first and for the second week following HFRS.

Interestingly, in analysis 1 the IRRs in the first week following HFRS were 33.94 (95% CI
14.18-81.28) and 37.41 (95% CI 14.38-97.35) for all and first stroke events, respectively. There was no significant difference from the control periods for the IRRs in the second week. Furthermore, the IRRs for analysis 2 were 36.4 (95% CI 16.6-79.78) and 41.08 (95% CI 17.75-95.09) for all and first stroke events in the first week following HFRS, respectively. The IRRs for all and first stroke events in the second week following HFRS were 9.1 (95% CI 2.6-31.96) and 12.32 (95% CI 3.48-43.66), respectively. A histogram of stroke events in relation to time after HFRS is shown in supplementary figure 2.

There were no significant seasonal effects on stroke events (supplementary table 3). No effects could be observed on the association between HFRS and stroke by either age at HFRS > 75 and sex in analysis 1 and 2 (supplementary table 4 and 5).

Discussion

Our results show that HFRS is linked to a transient but significant increased risk for AMI and stroke, suggesting a role for hantavirus-infection in triggering these events. This finding was demonstrated by two separate analyses taking into account the impact of fatal AMI/stroke events using the SCCS method. The SCCS method is sensitive to timing and dates;\textsuperscript{12} therefore it is crucial the HFRS index dates are close to disease onset. The least accurate date; date of report to the SICDC, was only used in approximately 5% of the events for the AMI study groups and this was not even an issue in the stroke study groups. Thereby the majority of dates fall within the first risk period enabling a good temporal distinction between AMI/stroke events occurring in the risk and control periods.

A critical assumption for the SCCS method is that the occurrence of an event (AMI or stroke) does not alter the likelihood of exposure (HFRS).\textsuperscript{21} This is termed the immortal time bias.
and could potentially inflate the IRR for AMI and stroke.\textsuperscript{16} In essence, we retrospectively select for patients that have been diagnosed with HFRS and AMI/stroke. Individuals who develop fatal AMI/stroke events before a theoretical PUUV-infection will obviously not figure in our cohort. Therefore the IR of AMI/stroke in the year prior to HFRS in our study could be underestimated. This issue we circumvent by omitting individuals who die within 365 days of their AMI/stroke events in analysis 1. Hence we avoid introducing the immortal time bias in our studies.

One advantage of SCCS is that multiplicative effects of fixed confounders factor out; therefore the baseline risk for cardiovascular events can be considered to be removed when analyzing shorter periods of time.\textsuperscript{12} However, it is possible that fixed effects can still act as effect modifiers.\textsuperscript{12} For instance although seroprevalence for PUUV is equally represented in both sexes, the disease HFRS is over-represented in middle-aged men.\textsuperscript{17, 22} Thereby sex and age group might be factors that determine who is more likely to be diagnosed with HFRS. In our study, we investigated whether there was a significant difference in the IRRs for AMI or stroke following HFRS between males vs. females (sex) and age groups. Therefore we included a separate test to identify whether an interaction term should be included in our model for age group and HFRS or sex and HFRS, respectively. No significant modifying effects were found for sex and HFRS or age and HFRS on the association between HFRS and AMI/stroke, apart from analysis 2 of all AMI events where age had a significant interaction. When calculated separately, the age group above 70 years had a 15-fold higher risk of developing AMI in the first three weeks following HFRS. It is possible that higher age might increase the risk for AMI following HFRS. This is also in line with previous observations where mortality following HFRS increased with age.\textsuperscript{23}

Further, we compared the age and sex distribution between the participants of the study groups to the original complete HFRS cohort, and found that those that had had AMI and stroke
were significantly older. In general, there was a trend towards a higher proportion of males in the study groups compared to the original HFRS cohort, although it was only statistically significant in AMI analysis 1.

In Sweden, an increased AMI incidence has been reported for the colder months compared to the warmer months, and a seasonal trend could perhaps be present in analysis of first AMI events. The season-adjusted IRR for analysis of first AMI events in the first three weeks following HFRS was still significantly increased. There were no seasonal effects in the other study groups.

Our study was based on data obtained from the National Patient Register. It has been reported that the validity for diagnosis codes relating to AMI and stroke was 100 and 98.6%, respectively, indicating a very good quality of the data.

Previous studies have linked acute infections with an increased risk for AMI and stroke. One of these studies was based on the United Kingdom General Practice Research Database using SCCS, the same method as used here. In that study, the IRR for a first AMI event in the first 3 days following disease onset of systemic respiratory and urine tract infections was increased 5 – and 3-fold, respectively. This is similar to the findings in our study where the IRR for first AMI events is increased approximately 4-fold in the first three weeks following HFRS compared to the control periods. Further, Smeeth et al., show that the risk for a first stroke event was significantly increased approximately 3-fold the first week following both upper respiratory and urine tract infections. It is noteworthy that our IRRs are so high for a first stroke event following HFRS; approximately 40-fold higher than the control periods, indicating that HFRS is a strong risk factor for developing acute cerebrovascular events. To our knowledge, no prior studies have uncovered an association between an acute infection and stroke of the magnitude
observed here.

The possibility that HFRS is a trigger for acute cardiovascular events is in line with previous studies focusing on hantaviral infection and pathogenesis. HFRS-patients have abnormal coagulation with elevated thrombosis and fibrinolysis, and disseminated intravascular coagulopathy. Additionally, hantavirus infection seems to have a negative impact on heart functions; cardiac disturbances have been observed in over half of HFRS-patients. Furthermore, the central nervous system was also shown to be affected by hantavirus-infection with reports of cerebral hemorrhage, and presence of PUUV RNA and increased levels of the monocyte/macrophage activation marker neopterin in cerebrospinal fluid. HFRS also cause intravascular damage, and dysfunctional platelets. Endothelial cells are infected with hantaviruses with subsequent activation. Consequently, hantavirus-infection induces an enhanced state of inflammation and coagulation, which might contribute to the here reported increased risk for AMI/stroke during acute HFRS. The observed enhanced coagulopathy in patients in prior studies, could also explain why the majority of stroke cases in our study were ischemic, rather than hemorrhagic as could possibly be expected from a hantaviral hemorrhagic fever.

There are case reports suggesting an association between Dengue and stroke, but to our knowledge this issue has not been studied in a cohort of Dengue virus-infected patients. Furthermore, Dengue was also shown to cause cardiac disease in a recent study. We do not know whether our results could apply to other VHF, even though coagulation disorders are more frequent. It remains to be investigated if VHF in general is associated with increased risk of AMI/stroke.

We identified an increased risk for AMI and stroke in the first weeks following HFRS,
but a limitation of our study was the small number of events and the low precision of the estimated IRR. In order to determine the IRR more precisely, more cases would have to be included. However, the HFRS database contains information on all diagnosed HFRS cases in Sweden since 1997 (n=6643); which we have included in our study. Therefore it would be impossible to increase the size of our cohort further. As for other infectious diseases there is a considerable proportion of unrecognized HFRS cases, whether our findings also apply to these is unknown. Since fewer females are diagnosed with HFRS, there is a risk of selection bias with regards to sex. Finally, the majority of exposure dates were based on the date of disease onset. It is possible that the date of disease onset was inaccurate in some cases due to patient recall bias and error in entering data, although probably this would introduce an error of a few days, and the risk period is measured in weeks.

We conclude that HFRS is associated with a transient but significantly increased risk for the acute cardiovascular events AMI and stroke, suggesting that hantavirus can trigger severe cardiovascular events in infected patients.

**Acknowledgments:** We gratefully acknowledge Prof. Paddy Farrington of The Open University for his valuable input and advice regarding the SCCS method.

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**Conflict of Interest Disclosures:** None.
References:


Table 1. Characteristics of hemorrhagic fever with renal syndrome (HFRS) cohort and the study groups

<table>
<thead>
<tr>
<th></th>
<th>Individuals</th>
<th>Age</th>
<th>p-value†</th>
<th>Female (%)</th>
<th>Male (%)</th>
<th>p-value‡</th>
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<tbody>
<tr>
<td><strong>HFRS cohort</strong></td>
<td>6643</td>
<td>52</td>
<td>(39-62)</td>
<td>-</td>
<td></td>
<td></td>
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<tr>
<td><strong>AMI study groups</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analysis 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All events</td>
<td>51</td>
<td>64</td>
<td>(57-72)</td>
<td>&lt; 0.001</td>
<td>11 (21.6)</td>
<td>40 (78.4)</td>
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<td>First events</td>
<td>41</td>
<td>62</td>
<td>(55-73)</td>
<td>&lt; 0.001</td>
<td>10 (24.4)</td>
<td>31 (75.6)</td>
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<td>Analysis 2</td>
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</tr>
<tr>
<td>All events</td>
<td>37</td>
<td>65</td>
<td>(27.5-72)</td>
<td>&lt; 0.001</td>
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<td>27 (73)</td>
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<tr>
<td>First events</td>
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<td>(57-71)</td>
<td>&lt; 0.001</td>
<td>8 (29.6)</td>
<td>19 (70.4)</td>
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<td><strong>Stroke study groups</strong></td>
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<tr>
<td>All events</td>
<td>26</td>
<td>69</td>
<td>(58-78)</td>
<td>&lt; 0.001</td>
<td>8 (30.8)</td>
<td>18 (69.2)</td>
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<tr>
<td>First events</td>
<td>22</td>
<td>69</td>
<td>(57.8-78)</td>
<td>&lt; 0.001</td>
<td>6 (27.3)</td>
<td>16 (72.7)</td>
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<tr>
<td>Analysis 2</td>
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<tr>
<td>All events</td>
<td>30</td>
<td>73</td>
<td>(57.8-80)</td>
<td>&lt; 0.001</td>
<td>20 (33.3)</td>
<td>10 (66.7)</td>
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<tr>
<td>First events</td>
<td>25</td>
<td>73</td>
<td>(57-80)</td>
<td>&lt; 0.001</td>
<td>7 (28)</td>
<td>18 (72)</td>
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</table>

*Age in years is shown as the median with interquartile range in brackets. Information regarding the age of six individuals was missing from the HFRS cohort.
†Statistical analysis of the difference in median age between the HFRS cohort versus the study groups (Mann-Whitney U-test)
‡Statistical analysis analyzing the observed versus the expected proportion of females and males in the study groups compared to the HFRS cohort (χ² test)
Table 2. Incidence rate ratios (IRR) of acute myocardial infarctions in individuals diagnosed with hemorrhagic fever with renal syndrome (HFRS), Sweden

<table>
<thead>
<tr>
<th></th>
<th>Analysis 1</th>
<th>Analysis 2</th>
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<tr>
<td></td>
<td>No. of events</td>
<td>IRR (95% CI)</td>
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<tr>
<td><strong>All AMI events</strong></td>
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<tr>
<td>1-21</td>
<td>8</td>
<td>5.53 (2.6 – 11.8)</td>
</tr>
<tr>
<td>22-90</td>
<td>5</td>
<td>1.05 (0.42 – 2.66)</td>
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<tr>
<td>Control period</td>
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<tr>
<td>Total</td>
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<td>40</td>
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<tr>
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<tr>
<td>1-21</td>
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<td>3.53 (1.25 – 9.96)</td>
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<tr>
<td>22-90</td>
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<td>1.07 (0.38 – 3.03)</td>
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<td>Control period</td>
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<tr>
<td>Total</td>
<td>41</td>
<td>27</td>
</tr>
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</table>

$^\dagger$Observation period: ± 365 days from HFRS; all events within observation period included. The control period consists of the days +91 to +365 following HFRS.

$^\dagger$Observation period: ± 365 days from HFRS; individuals with fatal events within +365 days of AMI event are excluded. The two control periods consists of: - 365 to – 31 days preceding and +91 to +365 days following HFRS.

$^\dagger$The z-test was performed to test the hypothesis that there was no difference in the incidence of AMI events between the specified risk period and the control period.
Table 3. Incidence rate ratios (IRR) of stroke in individuals diagnosed with hemorrhagic fever with renal syndrome, Sweden

<table>
<thead>
<tr>
<th>Days</th>
<th>No. of events</th>
<th>Analysis 1</th>
<th>Analysis 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IRR (95%CI)</td>
<td>p-value$^|$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p-value$^|$</td>
</tr>
<tr>
<td>All stroke events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-21</td>
<td>8</td>
<td>12.93 (5.62–29.74)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>1-7</td>
<td>7</td>
<td>33.94 (14.18–81.28)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>8-14</td>
<td>1</td>
<td>4.84 (0.65–36.32)</td>
<td>0.13</td>
</tr>
<tr>
<td>15-21</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22-90</td>
<td>2</td>
<td>0.98 (0.23–4.24)</td>
<td>0.98</td>
</tr>
<tr>
<td>Control period</td>
<td>18</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>First stroke events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-21</td>
<td>7</td>
<td>14.54 (5.87–36.04)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>1-7</td>
<td>6</td>
<td>37.41 (14.38–97.35)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>8-14</td>
<td>1</td>
<td>6.23 (0.82–47.41)</td>
<td>0.077</td>
</tr>
<tr>
<td>15-21</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22-90</td>
<td>1</td>
<td>0.63 (0.08–4.81)</td>
<td>0.66</td>
</tr>
<tr>
<td>Control period</td>
<td>14</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

$^*$Observation period: ± 365 days from HFRS; individuals with fatal events within +365 days of the stroke event are excluded. The two control periods consists of: -365 to – 31 days preceding and +91 to +365 days following HFRS.

$^\dagger$Observation period: + 365 days from HFRS; all stroke events within observation period are included. The control period consists of the days +91 to +365 days following HFRS.

$^\|$ The z-test was performed to test the hypothesis that there was no difference in the incidence of stroke events between the specified risk period and the control period.
Figure Legends:

**Figure 1.** Analyses of HFRS and AMI/stroke using the self-controlled case series method from the years 1997 to 2010. The control periods span over the year prior to and following the HFRS index date. Therefore, in analysis 1, the range of years in control period 1 is from 1996 to 2010, and in control period 2 the range of years is from 1997 to 2011. In analysis 2, there is only one control period, which includes the range of years from 1997 to 2011. Outline of the observation period for analysis 1 and 2 of the association between hemorrhagic fever with renal syndrome and the two acute cardiovascular events acute myocardial infarction and stroke.

**Figure 2.** Study profile. The PINs from HFRS patients registered in the HFRS database were cross-linked with the Swedish National Patient Register to identify AMI/stroke events (Inpatient Register) in the year preceding and following HFRS.
AMI/stroke dates:
-365 days

HFRS index dates:
1997 - 2010

AMI/stroke dates:
+365 days

Control period 1
(analysis 1)

Day1
Control period 1 (analysis 2)
Control period 2 (analysis 1)

Analysis 1

-365 days
-30
1
2
90
365 days

Control period
Buffer
Risk periods
Control period

Analysis 2

1
21
90
365 days

Risk periods
Control period

Figure 1
HFRS database
n = 6643

Inpatient Register
n = 5663

Cases with AMI
n = 320

AMI ± 2 years of HFRS report to SICDC
n = 97

AMI ± 365 days of HFRS index date
n = 57
Events = 61

Excluded:
- 6 fatal cases

Analysis 1
± 365 days of HFRS
All AMI events:
n = 51
Events = 55
First AMI events:
n = 41
Events = 41

Analysis 2
+ 365 days of HFRS
All AMI events:
n = 37
Events = 40
First AMI events:
n = 27
Events = 27

Cases with stroke
n = 258

Stroke ± 2 years of HFRS report to SICDC
n = 72

Stroke ± 365 days of HFRS index date
n = 35
Events = 37

Excluded:
- 9 fatal cases

Analysis 1
± 365 days of HFRS
All stroke events:
n = 26
Events = 28
First stroke events:
n = 22
Events = 22

Analysis 2
+ 365 days of HFRS
All stroke events:
n = 30
Events = 31
First stroke events:
n = 25
Events = 25

Analysis 2
+ 365 days of HFRS
All AMI events:
n = 51
Events = 55
First AMI events:
n = 41
Events = 41
Increased Risk of Acute Myocardial Infarction and Stroke During Hemorrhagic Fever with Renal Syndrome: A Self-Controlled Case Series Study
Anne-Marie Connolly-Andersen, Edvin Hammargren, Heather Whitaker, Mats Eliasson, Lars Holmgren, Jonas Klingström and Clas Ahlm

Circulation, published online January 7, 2014;

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**SUPPLEMENTAL MATERIAL**

Supplementary table 1. The types of dates registered in the HFRS database for the HFRS cohort 1997 to 2012

<table>
<thead>
<tr>
<th>Type of date</th>
<th>Frequency (%)</th>
<th>Difference in days between date of report to the SICDC and other dates, median (IQR)</th>
<th>Difference in days between date of HFRS disease onset and other dates, median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of Disease onset</td>
<td>5313 (80)</td>
<td>15 (11-22)</td>
<td>-</td>
</tr>
<tr>
<td>Sample date</td>
<td>5959 (89.7)</td>
<td>8 (5-13)</td>
<td>6 (4-8)</td>
</tr>
<tr>
<td>Diagnosis date</td>
<td>5584 (84.1)</td>
<td>5 (1-10)</td>
<td>9 (6-12)</td>
</tr>
<tr>
<td>Date of report to the SICDC</td>
<td>6643 (100)</td>
<td>-</td>
<td>15 (11-22)</td>
</tr>
<tr>
<td>Total</td>
<td>6643 (100)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*When the HFRS patient is reported to the SICDC, the date of disease onset, sample date or diagnosis date may also be included. However, these dates are not available in all cases therefore the date of report to SICDC was initially used to select individuals for the study groups.*
Supplementary table 2. The source of origin for the revised HFRS dates

<table>
<thead>
<tr>
<th>Source for HFRS date</th>
<th>Acute myocardial infarction</th>
<th>Stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Analysis 1†</td>
<td>Analysis 2‡</td>
</tr>
<tr>
<td></td>
<td>All AMI events</td>
<td>First AMI events</td>
</tr>
<tr>
<td>Date of Disease onset§</td>
<td>34 (66.7)</td>
<td>26 (63.4)</td>
</tr>
<tr>
<td>Date of Hospitalization#</td>
<td>7 (13.7)</td>
<td>6 (14.6)</td>
</tr>
<tr>
<td>Sample date§</td>
<td>8 (15.7)</td>
<td>7 (17.1)</td>
</tr>
<tr>
<td>Diagnosis date§</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Date of report to the SICDC§</td>
<td>2 (3.9)</td>
<td>2 (4.9)</td>
</tr>
<tr>
<td>Total</td>
<td>51 (100)</td>
<td>41 (100)</td>
</tr>
</tbody>
</table>

The frequency of HFRS patients that had an AMI or stroke within the observation period for the specified analysis is shown and the number in brackets refers to the percentage within the study group.

†Analysis 1 includes individuals with an AMI or stroke within ± 365 days of the revised HFRS date. If individual died within 365 days of their AMI or stroke event, they are excluded in order to avoid immortal time bias.

‡Analysis 2 includes individuals with an AMI or stroke within +365 days of the revised HFRS date. The individuals who died within 365 days of their AMI or stroke event are also included in this analysis.

§The date of disease onset, sample date, HFRS diagnosis date and date of report to SICDC are all obtained from the HFRS database from the SICDC.

#The date of hospitalization is obtained from the IPR. This date is used if the date of disease onset is not available. If the patient is hospitalized due to HFRS (ICD-10 diagnosis code: A985) or if the sample date or diagnosis date is in the same period of hospitalization.
Supplementary table 3: Analysis of the potential association between HFRS (main risk factor) and AMI/stroke in the first risk period (1-21 days following HFRS) or season (potential confounder) and AMI/stroke.

<table>
<thead>
<tr>
<th>Events</th>
<th>Risk factor</th>
<th>Effect</th>
<th>IRR</th>
<th>95% CI</th>
<th>p-value‡</th>
<th>IRR</th>
<th>95% CI</th>
<th>p-value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>HFRS‡</td>
<td>Main</td>
<td>4.9</td>
<td>2.27-10.63</td>
<td>&lt; 0.001</td>
<td>5.49</td>
<td>2.64-11.41</td>
</tr>
<tr>
<td></td>
<td>Season</td>
<td>Confounder</td>
<td>1.52</td>
<td>0.87-2.67</td>
<td>0.14</td>
<td>1.45</td>
<td>0.75-2.83</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>First</td>
<td>HFRS‡</td>
<td>Main</td>
<td>2.96</td>
<td>1.03-8.47</td>
<td>0.043</td>
<td>3.8</td>
<td>1.46-9.9</td>
</tr>
<tr>
<td></td>
<td>Season</td>
<td>Confounder</td>
<td>1.93</td>
<td>0.998-3.73</td>
<td>0.051</td>
<td>2.24</td>
<td>0.96-5.27</td>
<td>0.063</td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>HFRS‡</td>
<td>Main</td>
<td>12.14</td>
<td>5.2-28.35</td>
<td>&lt; 0.001</td>
<td>13.75</td>
<td>6.32-29.94</td>
</tr>
<tr>
<td></td>
<td>Season</td>
<td>Confounder</td>
<td>1.34</td>
<td>0.61-2.96</td>
<td>0.46</td>
<td>1.38</td>
<td>0.59-3.21</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>First</td>
<td>HFRS‡</td>
<td>Main</td>
<td>12.88</td>
<td>5.1-32.5</td>
<td>&lt; 0.001</td>
<td>13.96</td>
<td>5.91-32.95</td>
</tr>
<tr>
<td></td>
<td>Season</td>
<td>Confounder</td>
<td>1.87</td>
<td>0.74-4.74</td>
<td>0.18</td>
<td>2.08</td>
<td>0.76-5.69</td>
<td>0.15</td>
</tr>
</tbody>
</table>

*Observation period: ± 365 days from HFRS; individuals with fatal events within +365 days of the AMI/stroke event are excluded. The two control periods consists of: -365 to – 31 days preceding and +91 to +365 days following HFRS.
†Observation period: + 365 days from HFRS; all AMI/stroke events within observation period are included. The control period consists of the days +91 to +365 days following HFRS.
‡ The z-test was performed to test the null hypothesis:
H₀ (HFRS): There is no difference in the incidence rate of AMI/stroke during the risk period vs. the control period
H₀ (season): There is no difference in the incidence rate of AMI/stroke between summer and winter months
§ The season-adjusted IRRs for AMI and stroke are shown for HFRS in this table
Supplementary table 4: Analysis including interactions of age (potential effect-modifier) on the association between HFRS (main risk factor) and AMI/stroke in the first risk period (1-21 days following HFRS).

<table>
<thead>
<tr>
<th>Events</th>
<th>Risk factor</th>
<th>Effect</th>
<th>IRR</th>
<th>95 % CI</th>
<th>p-value £</th>
<th>IRR</th>
<th>95 % CI</th>
<th>p-value £</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Analysis 1*</td>
<td>Analysis 2†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMI</td>
<td>All</td>
<td>HFRS</td>
<td>Main</td>
<td>4.16</td>
<td>1.46-11.85</td>
<td>2.92</td>
<td>0.99-8.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>Interaction</td>
<td>2</td>
<td>0.43-9.21</td>
<td>0.6</td>
<td>5.25</td>
<td>1.13-24.42</td>
<td>0.058</td>
</tr>
<tr>
<td>First</td>
<td>HFRS</td>
<td>Main</td>
<td></td>
<td>3.79</td>
<td>1.13-12.64</td>
<td>2.82</td>
<td>0.81-9.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>Interaction</td>
<td>0.77</td>
<td>0.07-8.3</td>
<td>0.88</td>
<td>4.67</td>
<td>0.61-35.49</td>
<td>0.21</td>
</tr>
<tr>
<td>Stroke</td>
<td>All</td>
<td>HFRS</td>
<td>Main</td>
<td>14.55</td>
<td>5.46-38.77</td>
<td>13.14</td>
<td>4.93-35.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>Interaction</td>
<td>0.67</td>
<td>0.1-4.36</td>
<td>0.66</td>
<td>1.4</td>
<td>0.31-6.33</td>
<td>0.86</td>
</tr>
<tr>
<td>First</td>
<td>HFRS</td>
<td>Main</td>
<td></td>
<td>16.17</td>
<td>5.42-48.23</td>
<td>15.33</td>
<td>5.15-45.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>Interaction</td>
<td>0.72</td>
<td>0.1-5.17</td>
<td>0.83</td>
<td>1.29</td>
<td>0.24-6.83</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Observation period: ± 365 days from HFRS; individuals with fatal events within +365 days of the AMI/stroke event are excluded. The two control periods consists of: -365 to – 31 days preceding and +91 to +365 days following HFRS.

Observation period: + 365 days from HFRS; all AMI/stroke events within observation period are included. The control period consists of the days +91 to +365 days following HFRS.

The likelihood ratio test was performed to test the null hypothesis $H_0$: There is no difference between the model containing the interaction term (age x HFRS) and the model without the interaction term.

The individuals were divided into two groups based on their age at HFRS:
AMI: 1) < 70 years of age 2) ≥ 70 years of age
Stroke: 1) < 75 years of age 2) ≥ 75 years of age
Supplementary table 5: Analysis including interactions of sex (potential effect-modifier) on the association between HFRS (main risk factor) and AMI/stroke in the first risk period (1-21 days following HFRS).

<table>
<thead>
<tr>
<th>Events</th>
<th>Risk factor</th>
<th>Effect</th>
<th>Analysis 1</th>
<th>Analysis 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IRR</td>
<td>95 % CI</td>
<td>p-value‡</td>
</tr>
<tr>
<td>AMI</td>
<td></td>
<td>IRR</td>
<td>95 % CI</td>
<td>p-value‡</td>
</tr>
<tr>
<td>All</td>
<td>HFRS</td>
<td>Main</td>
<td>6.17</td>
<td>2.73-13.95</td>
</tr>
<tr>
<td>Sex&quot;</td>
<td>Interaction</td>
<td>0.52</td>
<td>0.06-4.83</td>
<td>0.95</td>
</tr>
<tr>
<td>First</td>
<td>HFRS</td>
<td>Main</td>
<td>3.49</td>
<td>1.05-11.56</td>
</tr>
<tr>
<td>Sex&quot;</td>
<td>Interaction</td>
<td>1.04</td>
<td>0.09-11.47</td>
<td>1</td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
<td>IRR</td>
<td>95 % CI</td>
<td>p-value‡</td>
</tr>
<tr>
<td>All</td>
<td>HFRS</td>
<td>Main</td>
<td>14.55</td>
<td>5.46-38.77</td>
</tr>
<tr>
<td>Sex&quot;</td>
<td>Interaction</td>
<td>0.67</td>
<td>0.1-4.36</td>
<td>0.66</td>
</tr>
<tr>
<td>First</td>
<td>HFRS</td>
<td>Main</td>
<td>14.55</td>
<td>4.97-42.56</td>
</tr>
<tr>
<td>Sex&quot;</td>
<td>Interaction</td>
<td>1</td>
<td>0.13-7.45</td>
<td>0.88</td>
</tr>
</tbody>
</table>

*Observation period: ± 365 days from HFRS; individuals with fatal events within +365 days of the AMI/stroke event are excluded. The two control periods consist of: -365 to – 31 days preceding and +91 to +365 days following HFRS.
†Observation period: + 365 days from HFRS; all AMI/stroke events within observation period are included. The control period consists of the days +91 to +365 days following HFRS.
‡ The likelihood ratio test was performed to test the null hypothesis H₀: There is no difference between the model containing the interaction term (sex x HFRS) and the model without the interaction term.
The individuals were divided into the two categories: Male or female.
Supplementary figure 1. The temporal association between AMI events and HFRS. Each dot is a single AMI event in the designated time period. An individual could have more than one AMI event within the specified observation period, apart from when the first events only are shown. The three months following hemorrhagic fever with renal syndrome (risk period) is displayed in a higher resolution as events per week in the small histogram. The histogram for analysis 1 for all (A) and first (B) AMI events, respectively is displayed, and the histogram for analysis 2 for all (C) and first (D) AMI events, respectively, is displayed.

Supplementary figure 2. The temporal association between stroke events and HFRS. Each dot is a single stroke event, and an individual may have had more than one stroke event within the specified observation period, apart from when the first stroke events only are shown. The three months following hemorrhagic fever with renal syndrome (risk period) is displayed in a higher resolution with events per week in the smaller histogram. The histogram for analysis 1 for all (A) and first (B) stroke events are shown, and the histogram for analysis 2 for all (C) and first (D) stroke events are displayed.