INTRACEREBRAL HEMORRHAGE AMONG BLOOD DONORS AND THEIR TRANSFUSION RECIPIENTS

Jingcheng Zhao MD PhD[1], Klaus Rostgaard MSc[2,3], Elsa Lauwers PhD[4], Torsten Dahlén MD[1,5], Sisse Rye Ostrowski, MD PhD DMSc[6,7], Christian Erikstrup MD PhD[8,9], Ole Birger Pedersen MD PhD[7,10], Bart De Strooper MD PhD[4,11,12], Robin Lemmens MD PhD[4,11,13], Henrik Hjalgrim MD DrMedSci[2,3,7,14], Gustaf Edgren MD PhD[1,15]

1. Department of Medicine, Solna, Clinical Epidemiology Division, Karolinska Institutet, Stockholm, Sweden.
2. Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark.
3. Danish Cancer Society Research Center, Copenhagen, Denmark.
4. VIB Center for Brain & Disease Research, Leuven, Belgium.
5. Hematology Department, Karolinska University Hospital, Sweden.
6. Department of Clinical Immunology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
7. Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
8. Department of Clinical Immunology, Aarhus University Hospital, Aarhus, Denmark
9. Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
10. Department of Clinical Immunology, Zealand University Hospital, Køge, Denmark
11. Laboratory for the Research of Neurodegenerative Diseases, Department of Neurosciences, Leuven Brain Institute, KU Leuven (University of Leuven), Leuven, Belgium.
12. Dementia Research Institute, University College London, London, UK.
13. Department of Neurology, University Hospitals Leuven, Leuven, Belgium.
14. Department of Hematology, Rigshospitalet, Copenhagen, Denmark
15. Department of Cardiology, Södersjukhuset, Stockholm, Sweden.

Word counts: 422 (abstract), 3835 (manuscript)

Address for correspondence:

Dr Jingcheng Zhao

Clinical Epidemiology Division T2, Department of Medicine, Solna,

Karolinska University Hospital Solna, 171 76 Stockholm, Sweden.

E-mail: jingcheng.zhao@ki.se

Key Points

Question: Is there an association between the occurrence of spontaneous intracerebral hemorrhage among blood donors and the risk of spontaneous intracerebral hemorrhage in patients transfused with their blood?

Findings: In this exploratory retrospective cohort study, which included 759,858 patients in Sweden and 329,512 patients in Denmark, receiving red-cell transfusions from donors who later developed multiple spontaneous intracerebral hemorrhages was significantly associated with an increased risk of developing spontaneous intracerebral hemorrhage compared with receiving a transfusion from donors without subsequent intracerebral hemorrhage (hazard ratios, 2.73 and 2.32 in the Swedish and Danish cohort, respectively).

Meaning: The findings may suggest a transfusion-transmissible agent associated with some types of spontaneous intracerebral hemorrhage, but findings may be susceptible to selection bias and residual confounding and further research is required to understand the potential underlying mechanism.

ABSTRACT

Importance: Recent reports have suggested that cerebral amyloid angiopathy (CAA), a common cause of multiple spontaneous intracerebral hemorrhages (ICH), may be transmissible through parenteral injection of contaminated cadaveric pituitary hormone in humans.

Objective: To determine whether spontaneous ICH in blood donors after blood donation is associated with development of spontaneous ICH in transfusion recipients.

Design, Setting, and Participants: This exploratory retrospective cohort study utilized nationwide blood bank and health register data from Sweden (main cohort) and Denmark (validation cohort) and included all 1,089,370 patients aged 5-80 years recorded to have received a red-cell transfusion from January 1, 1970 (Sweden) or January 1, 1980 (Denmark) until December 31, 2017.

Exposures: Receipt of red-cell transfusions from blood donors who subsequently developed 1) a single spontaneous ICH, 2) multiple spontaneous ICH, or 3) no spontaneous ICH

Main Outcomes and Measures: Spontaneous ICH in transfusion recipients. Ischemic stroke as negative control.

Results: 759,858 patients from Sweden (median [IQR] age, 65 [48 to 73] years; 59% female) and 329,512 patients from Denmark (median [IQR] age, 64 [50 to 73] years; 58% female) were included, with median (IQR) follow up of 5.8 (1.4 to 12.5) years and 6.1 (1.5 to 11.6) years, respectively. Patients transfused with red-cell units from donors who developed multiple spontaneous ICH had a significantly higher risk of a single spontaneous ICH themselves, compared with patients receiving transfusions from donors who did not develop spontaneous ICH, in both the Swedish (unadjusted incidence rate [IR], 3.16 vs 1.12 per 1000 person-years; adjusted hazard ratio [HR], 2.73; 95% confidence interval [CI], 1.72 to 4.35; $p<0.001$) and Danish cohort (unadjusted IR, 2.82 vs 1.09 per 1000 person-years; adjusted HR, 2.32; 95% CI, 1.04 to 5.19; $p=0.04$). No significant difference was found for patients receiving transfusions from donors who developed a single spontaneous ICH in the Swedish (unadjusted IR, 1.35 vs 1.12 per 1000 person-years; adjusted HR, 1.06; 95% CI, 0.84 to 1.36, $p=0.62$) nor Danish cohort (unadjusted IR, 1.36 vs 1.09 per 1000 person-years; adjusted HR, 1.06; 95% CI, 0.70 to 1.60, $p=0.73$), nor for ischemic stroke as a negative control outcome.

Conclusions and relevance: In an exploratory analysis of patients who received red-cell transfusions, patients transfused with red-cells from donors who later developed multiple spontaneous ICH were at a significantly increased risk of developing spontaneous ICH themselves. This may suggest a transfusion-transmissible agent associated with some types of spontaneous ICH, although the findings may be susceptible to selection bias and residual confounding and further research is needed to investigate if transfusion-transmission of CAA might explain this association.

INTRODUCTION

Cerebral amyloid angiopathy (CAA) is the second most common cause of spontaneous intracerebral hemorrhage (ICH) and is characterized by deposition of misfolded beta-amyloid in arteries in the cerebral cortex and leptomeninges.[1,2] Based on a chart review from 2005 to 2010 in Finland, 20% of ICH cases were estimated to be related to CAA compared with 35% of cases from hypertension.[3-5] Recent evidence suggests that CAA exhibits "prion-like" transmissivity, with reports of transmission through cadaveric pituitary hormone contaminated with amyloid-beta and tau protein,[6-8] dura mater grafts,[9] and possibly neurosurgical instruments.[10] In animal models, CAA has been induced by intravenous injection of amyloid-beta.[11,12] As human-to-human transmission through blood transfusion has been shown for prion illness but has not yet been assessed for CAA,[9,13] a recent international consortium identified the assessment of potential transfusion-transmission of CAA as a top priority.[14]

Under the assumption that at least some observed ICH are due to underlying CAA, we hypothesized that transfusion-transmission of CAA may manifest through an increased risk of spontaneous ICH among transfusion recipients exposed to blood from a donor with spontaneous ICH. To probe this hypothesis, this study examined the association between the occurrence of spontaneous ICH in blood donors and their recipients, using a nationwide cohort in Sweden along with validation in a nationwide cohort in Denmark.

METHODS

Study Design and Data Sources

The study and waiver of patient consent was approved by the Regional Ethics Committee in Stockholm and the Ethics Review Authority in Sweden (reference 2018/167-31, 2019-04656, 2021-04890), Statens Serum Institut QA and Compliance (reference 21/00083), and the Data Protection Agency in the Capital Region in Denmark (reference P-2019-99).

This was an exploratory retrospective, observational study to assess if patient risk for spontaneous ICH was associated with receiving red-cell units from donors who later developed spontaneous ICH, exploiting the fact that future ICH incidence among donors was unknown at the time of donation. As CAA-related ICH is reported to have a 7-fold increased risk of recurrence compared with non-CAA-related ICH,[2] we considered multiple episodes of ICH as a more robust proxy for CAA and separately assessed single and multiple occurrences of ICH.

All electronically available data on blood donors, donations, and transfusions were extracted from blood banks in Sweden and Denmark and linked with nationwide health registers using unique personal identifiers (i.e., the SCANDAT3 database).[15] Data from blood banks is available from the late 1960s in Sweden and the early 1980s in Denmark, with essentially nationwide coverage from the mid-1990s and late 1990s in Sweden and Denmark, respectively.[16] Diagnoses of ICH, aneurysms, arteriovenous malformations, trauma, and ischemic stroke were ascertained using National Patient Registers, which contain data on all inpatient care in Denmark and Sweden. Primary cerebral tumors were identified using the Swedish and Danish Cancer Registers. Reporting of diagnoses, procedures, blood donations, and transfusions to the data sources used in this study is mandated by law, which helps ensure their completeness. Data was kept and analyzed separately in Sweden and Denmark.

Participants

Using nationwide data from blood banks and national registers, we constructed a historical cohort of transfused patients and blood donors from 1970 to 2017 in Sweden (main cohort) and from 1980 to 2017 in Denmark (validation cohort). The study cohorts comprised all patients transfused with a red-cell unit between ages 5 and 80 years in Sweden and Denmark without a previously recorded blood transfusion, a prior diagnosis of spontaneous ICH, or a cerebral tumor.

Transfusion Exposure

In the main analyses, exposure was restricted to transfusions administered in the first 180-days ("180-day exposure assessment window") following the first registered transfusion and was classified hierarchically in this order: receipt of at least one red-cell unit from a donor who eventually developed multiple spontaneous ICHs, receipt of at least one red-cell unit from a donor who eventually developed a single spontaneous ICH, or neither of the above. Patients transfused with red-cells from donors with a history of ICH prior to blood donation were excluded. A fixed-length exposure assessment window was used to ensure unambiguous exposure classification and to mitigate possible reverse causality, as in previous studies on transfusion transmission using this type of register data.[17-19] The length of the exposure assessment window was a compromise between having a sufficiently long period to capture relevant transfusion exposure but not too long as to exclude possibly relevant outcome events. The choice of 180 days was informed by (1) the fact that the majority of patients in our study population were transfused only within this 180-day period, and (2) the belief that outcome events in the first 180 days were unlikely to be related to development of transfusion-related CAA given that the development of CAA is thought to require longer incubation periods.

Outcomes

The main outcomes were development of single and multiple spontaneous ICH among transfusion recipients. We also assessed ischemic stroke as a negative control since it shares risk factors for spontaneous ICH but is not associated with CAA.[20] Diagnosis of spontaneous ICH and ischemic stroke was ascertained using the 8th, 9th, and 10th revisions of the International Classification of Diseases (ICD), using the admission date as the diagnosis date (eMethods). Primary cerebral tumor diagnoses were ascertained from National Cancer Registers using the 7th, 9th, and 10th revision of the International Classification of Diseases. ICH with an ICD code for trauma or vascular malformation within 30 days or primary cerebral tumor within 180 days were excluded. Multiple spontaneous ICH or ischemic stroke was defined as having at least two inpatient episodes at least 30 days apart with a diagnosis of spontaneous ICH or ischemic stroke, and the diagnosis date was approximated with the admission date for the second inpatient episode.

Statistical Analyses

In the main analyses, follow-up started 180 days after a patient's first transfusion and ended on the date of diagnosis of spontaneous ICH (first or second, in separate models), death, emigration, or end of follow-up (December 31, 2017). We also censored patients 180 days after an additional transfusion, to avoid exposure misclassification, or upon diagnosis of a cerebral tumor. Patients were excluded if they, during the exposure assessment window, received a platelet or plasma transfusion, received an autologous red-cell unit, or a red-cell unit from a non-identified donor, a donor with a diagnosis of spontaneous ICH prior to donation, parent, or child.

Cause-specific hazard ratios were estimated using a stratified Cox proportional hazards model, with strata constituted by hospital where the first transfusion was administered, further adjusting for the index calendar year, age, sex (as a categorical term), age and sex interaction, number of transfusions, indication for transfusion (categorized as 9 hierarchical groups defined using a previously derived algorithm[21,22]), and ABO-RhD blood group. All numerical covariates

were treated as restricted cubic splines with 5 equally spaced knots. Adjusting for the index calendar year also accounts for the potential available follow-up of blood donors, which is right-censored at the end of study. Corresponding E-values were computed using the 'E-Value Calculator' by Mathur et al.[23,24] The proportional hazards assumption was confirmed to hold by varying the length of follow-up from 5 to 40 years.[25]

Standardized cause-specific cumulative incidence functions, with corresponding cumulative incidence differences, were estimated in the main Swedish cohort using a flexible parametric survival model with the baseline hazard modeled as a restricted cubic spline with 5 knots. Competing risks by death, primary cerebral tumor, and additional blood transfusions were accounted for by separately modeling for these outcomes (eFigure 1). For computational efficiency, we limited the number of non-exposed patients per stratum to 3, matching on the number of transfusions, region, and date of entry (± 1 year). Patients with more than 100 red-cell transfusions were excluded to reduce the number of patients without matching controls. Estimates were adjusted for the same confounders as for the cause-specific hazard ratios, except for hospital that was replaced by region.

All data processing and statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc) including the *%stratify* macro[26], and Stata 15 (StataCorp). Standardized cumulative incidence was estimated using the *stmp2* and *standsurv* packages for Stata.[27,28] Statistical significance was set to p=0.05, tests were two-tailed and were not adjusted for multiple testing. Analyses were performed on data as-recorded without imputation or exclusion of missing data. A directed acyclic graph and a statistical analysis plan are provided in eMethods.

Secondary and Sensitivity analyses

In secondary analyses, we assessed the exposure time-varyingly throughout follow-up. In this way, rather than censoring patients upon receipt of an additional transfusion, patients could instead change exposure classification if they later received transfusions from a donor who

subsequently developed a single spontaneous ICH or multiple spontaneous ICH. Analogous to the main analyses, the start of follow-up commenced 180 days after the first recorded red-cell transfusion and the association of transfusions with the outcome were assessed with a 180-day delay. Given that potential transmission of beta-amyloid or other potential agents causing CAA would likely not manifest for several years or decades after exposure, we imposed a 5-year delay to the start of follow-up to assess if there were any sustained long-term associations for both the main and time-varying exposure analyses. Details are available in eMethods.

Multiple sensitivity analyses were conducted in the main Swedish cohort. First, we assessed exposure and outcome using only spontaneous ICH registered as the primary diagnosis in the National Patient Register, in case follow-up care was inadvertently coded as new ICH episodes. Second, as a negative control, we assessed ischemic stroke as outcome in transfused patients instead of spontaneous ICH. Third, we assessed ischemic stroke in both donors as the exposure and in transfused patients as the outcome as an additional negative control. Fourth, we removed censoring for additional transfusions. Fifth, based on the co-occurrence of CAA and Alzheimer's disease,[1,2] we assessed patients transfused with red-cells from donors who were later diagnosed with a single episode of ICH as well as any type of dementia during follow-up (using the National Patient Registers, see eMethods for ICD codes).

RESULTS

Cohort creation

A total of 759,858 patients were included in the analysis in Sweden and 329,512 patients were included in Denmark. Baseline cohort characteristics are displayed in Table 1. The proportion of female patients was 59% and 58%, and the median age was 65 years (interquartile range [IQR], 48-73) and 64 years (IQR, 50-73) in Sweden and Denmark, respectively. Additionally, baseline characteristics for the matched cohort used to estimate cumulative incidence in the Swedish cohort are presented in eTable 1; the remaining patients without matching controls (n=192, 3%) were excluded for this analysis. Descriptive statistics on blood donors are presented in eTable 2 and descriptive statistics of exposure and outcomes are available in eTable 3. In total, 8598 (1.1%) and 3695 (1.1%) patients were exposed to a blood unit from a donor who later developed a single spontaneous ICH, and 862 (0.1%) and 448 (0.1%) to a donor who later developed multiple spontaneous ICH, in Sweden and Denmark, respectively. Descriptive statistics stratified by exposure during the 180-day exposure assessment window are presented in Table 2. In the main Swedish cohort, the median (IQR) follow-up in years for patients exposed to blood donors who did not develop spontaneous ICH, developed a single spontaneous ICH, and developed multiple spontaneous ICH was 6.1 (2.0-12.5), 7.1 (1.8-16.2), and 8.2 (1.8-19.4) respectively; follow-up for blood donors was 12.7 (6.1-20.0), 20.0 (14.2-24.8), and 22.2 (15.8-26.7), respectively.

Main analyses

Cause-specific hazard ratios for single spontaneous ICH outcomes in patients are displayed in Figure 1A and 1B. Descriptive statistics for transfused patients, including frequency and timing of ICH, stratified by donor spontaneous ICH status are presented in Table 2. Compared to receipt of transfusion from donors who did not develop spontaneous ICH, receipt of red-cell transfusions from donors who subsequently developed multiple spontaneous ICH was significantly associated with developing a spontaneous ICH in the main Swedish cohort (18 vs

5185 events; unadjusted incidence rate [IR], 3.16 vs 1.12 per 1000 person-years; HR, 2.73; 95% CI, 1.72 to 4.35), corresponding to E-values of 4.9 and 2.8 for the point estimate and lower confidence interval for the adjusted hazard ratio, respectively. The same association was significant in the validation Danish cohort (6 vs 1901 events; unadjusted IR, 2.82 vs 1.09 per 1000 person-years; HR, 2.32; 95% CI, 1.04 to 5.19). In the majority of cases, donors developed spontaneous ICH more than 10 years after their blood was transfused (Table 2). The corresponding adjusted cumulative incidence difference associated with developing spontaneous ICH was 2.3% (95% CI, 0.6 to 4.0%) in the main Swedish cohort after 30 years (Figure 2). No significant association was seen among recipients of transfusions from donors who later developed only a single spontaneous ICH, neither in Sweden (67 vs 5185 events; unadjusted IR, 1.35 vs 1.12 per 1000 person-years; HR, 1.06; 95% CI, 0.84 to 1.36) nor in Denmark (23 vs 1901 events; unadjusted IR, 1.36 vs 1.09 per 1000 person-years; HR, 1.06; 95% CI, 0.70 to 1.60). With a five-year latency, hazard ratios for spontaneous ICH in recipients of red-cell units from donors with multiple spontaneous ICH donors remained significant (9 vs 2190 events; unadjusted IR, 2.28 vs 0.82 per 1000 person-years; HR, 2.84; 95% CI, 1.47 to 5.49) in the Swedish cohort, but there was only 1 exposed case in the Danish cohort. There were only 2 events of multiple ICH among patients who received red-cells from donors who developed multiple ICH in Sweden, and none in Denmark (eTable 4).

Secondary analyses

Results from the secondary time-varying exposure models are presented in Table 3. Similar to the main analyses, receiving red-cell transfusions from donors who subsequently developed multiple spontaneous ICH, compared with donors who did not develop spontaneous ICH, was significantly associated with developing a single spontaneous ICH in the main Swedish cohort (21 vs 7686 events; unadjusted IR, 2.60 vs 1.28 per 1000 person-years; HR, 1.95; 95% CI, 1.26-3.03). In Denmark, the corresponding hazard ratio was similar but with wider confidence intervals and was not significant (10 vs 3080 events; unadjusted IR, 2.96 vs 1.27 per 1000

person-years; HR, 1.74 ; 95% CI, 0.72 to 4.19). With a 5-year delay, the hazard ratios were 1.74 (95% CI, 0.96 to 3.15) and 2.00 (95% CI, 0.64 to 6.23), in the Swedish and Danish cohort respectively. Comparing patients transfused with red-cells from donors who later developed a single spontaneous ICH to patients transfused with red-cells from donors who did not develop spontaneous ICH, there was no significant difference in risk of a single spontaneous ICH among recipients in the Swedish cohort (114 vs 7686 events; unadjusted IR, 1.55 vs 1.28 per 1000 person-years; HR, 1.01; 95% CI, 0.84 to 1.23) or in the Danish cohort (36 vs 3080 events; unadjusted IR, 1.29 vs 1.27 per 1000 person-years; HR, 0.75; 95% CI, 0.48 to 1.16). Similar to the main analyses, multiple spontaneous ICH among transfused patients were rare.

Sensitivity Analysis

Sensitivity analyses for the main Swedish cohort are shown in Figure 1C. The associations observed in the main analyses among recipients of transfusions from donors with multiple spontaneous ICH were significant when removing censoring for additional transfusions after the 180-day exposure assessment window (HR, 2.35; 95% CI, 1.48 to 3.75). Furthermore, the hazard ratio was increased by restricting the spontaneous ICH definition to only registrations where ICH was coded as the main diagnosis (HR, 3.21; 95% CI, 1.89 to 5.44). No significant associations were seen when using ischemic stroke as the outcome (HR, 1.12, 95% CI, 0.79 - 1.58) or when using ischemic stroke both in blood donors as the exposure and in transfusion recipients as the outcome, among recipients of transfusions from donors who had a single stroke (HR, 0.97; 95% CI, 0.92 to 1.03) and multiple strokes (HR, 0.88; 95% CI, 0.76 to 1.02). Receiving transfusions from donors who subsequently developed both a single spontaneous ICH and dementia was also significantly associated with developing a single spontaneous ICH (HR, 2.44; 95% 1.52 to 3.94).

DISCUSSION

In this exploratory retrospective cohort study, there was a significantly increased risk of spontaneous ICH among patients who received red-cell transfusions from donors who themselves developed multiple spontaneous ICH after donating blood, but not from donors who developed a single spontaneous ICH. Findings from the main analyses using a nationwide Swedish cohort were validated in a nationwide Danish cohort and were robust to several of the sensitivity analyses.

Among the sensitivity analyses, there was a significantly increased risk of spontaneous ICH among patients who were transfused with red-cells from donors who subsequently developed a single spontaneous ICH and dementia. Using ischemic stroke as a negative control, there were no significant associations for ischemic stroke when it was used as either an outcome in patients transfused from donors who developed spontaneous ICH or as both an exposure and outcome in donors and transfused patients, respectively**.** In secondary analyses allowing time-varying exposure, hazard ratios were similar for the main Swedish cohort, however, numerically higher but non-significant in the Danish cohort and in cohorts with delayed follow-up, although these cohorts had few cases with wide confidence intervals around the hazard ratio estimates.

The observed increased risk of spontaneous ICH associated with receiving a red-cell transfusion from a donor who later developed multiple spontaneous ICH, corresponding to a 30-year cumulative incidence difference of 2.3%, is a novel finding. We are not aware of blood donor factors that have been reported to be associated with an increased risk of spontaneous ICH in transfusion recipients. The cause-specific hazard ratios in the main analyses of the main Swedish cohort correspond to E-values of 4.9 and 2.8 for the point estimate and lower confidence interval, respectively, which indicates additional unmeasured confounding associated with both donors developing multiple spontaneous ICH and recipients developing a spontaneous ICH by a risk ratio of at least 4.9-fold or 2.8-fold each would be required to explain away the observed association. At the time of transfusion, future spontaneous ICH in donors is unknown and factors that affect blood allocation are measured and controlled for. We are not

aware of additional significant confounders, especially confounders that would apply to spontaneous ICH but not ischemic stroke. It therefore seems unlikely for the results to be explained entirely by residual confounding.

This study was motivated by the need to evaluate possible transfusion-transmission of agents causing CAA. Although the study does not directly assess CAA, several findings are interesting in this context. First, there was a significant association for developing spontaneous ICH among patients transfused with red-cells from donors who developed multiple but not single spontaneous ICH. We expect CAA to be more prevalent among donors who develop multiple spontaneous ICH as CAA-related ICH has been reported to have a 7-fold increase for recurrent ICH compared with CAA-unrelated ICH.[2] Second, a significantly increased risk was found for patients transfused with red-cells from donors who developed a single spontaneous ICH and dementia, which could be interpreted in the context of CAA frequently co-occurring with Alzheimer's dementia.[29] Third, there were not any significant associations for ischemic stroke, which is strongly associated with other etiologies of spontaneous ICH, including hypertension, but is not associated with CAA.[1,20] Fourth, it is possible that the potential true association for CAA-related ICH is underestimated as our study also includes non-CAA-related ICH among both donors and recipients.

In this study, there was an increased cumulative incidence of ICH within a decade of exposure, while other studies have reported that longer time is needed for amyloid-beta to manifest into clinical disease.[30] A recent systematic review of 23 cases of iatrogenic CAA reported a mean latency of 34 years (range, 25 to 46 years) among patients potentially exposed to amyloid-beta through dura mater grafts or other neurosurgical procedures during early childhood (mean age, 3.3 years; range 0.1 to 20 years).[31] The difference in exposure age may affect possible incubation periods, since the median age at study entry in this study was 64 to 65 years. In line with recent experimental evidence, incubation periods may be shorter if amyloid-beta is inoculated in elderly patients with pre-existing subclinical amyloid-beta pathology.[32] This is speculative, as this study does not conclusively determine transmission of agents causing

CAA and includes non-CAA related ICH. Future studies should also assess neuroradiology to validate the diagnosis of CAA and biomarkers for amyloid-beta pathology.

This study has several strengths. Strong residual confounding is unlikely given that future development of ICH in donors is unknown at the time of donation, and measured confounders should account for most relevant confounders. The large binational cohort with up to almost 5 decades of follow-up allowed observation of rare events with possibly long incubation periods. It also allowed the exposure to be tiered, using both single and multiple spontaneous ICH as proxies, where the latter is rarer but may have a higher positive predictive value for CAA.[2] Our results were robust to several key assumptions shown in the sensitivity analyses, and we were able to use ischemic stroke as a negative control.

Limitations

This study has several limitations. First, the analyses did not assess CAA directly and instead used single and multiple spontaneous ICH as proxies for CAA. While this finds support in the literature,[2,33] we did not have access to neuropathology or neuroradiology to directly assess CAA as an exposure or outcome in our cohort. A specific diagnosis code for CAA exists in the tenth revision of the International Classification of Diseases, but it was rarely used. Since spontaneous ICH is an infrequent and a late-stage manifestation of CAA,[33-35] many donors who do not develop ICH are expected to have CAA, which in turn may have attenuated the observed associations. Second, we defined spontaneous ICH as the absence of diagnoses related to trauma or vascular malformations, however, we did not have data to validate the sensitivity of diagnosis codes for trauma or vascular malformations. Our definition of multiple spontaneous ICH may misclassify spontaneous ICH that occurred less than 30 days apart or at the same time. Third, despite using all computerized transfusion records in two countries over several decades, both the exposure and the outcome were rare which led to small numbers of events. The study was especially underpowered to assess multiple spontaneous ICH as the outcome, and we were unable to consider transfusions of other types of blood products. Fourth, based on current

386 understanding that incubation periods for iatrogenic CAA is multiple decades[31], we assumed
387 that the incubation period was at least 180 days and did not assess events within the first 180
388 days. Fifth, it is possible that the difference in follow-up across exposure groups may have led to
389 uncontrolled selection bias; however, because we controlled for calendar year of transfusion we
390 do not believe this materially affected our findings.
391
392 *Conclusions*
393 In an exploratory analysis of patients who received red-cell transfusions, patients transfused
394 with red-cells from donors who later developed multiple spontaneous ICH were at a
395 significantly increased risk of developing spontaneous ICH themselves. This may suggest a
396 transfusion-transmissible agent associated with some types of spontaneous ICH, although the
397 findings may be susceptible to selection bias and residual confounding and further research is
398 needed to investigate if transfusion-transmission of CAA might explain this association.

ACKNOWLEDGEMENTS

Data Sharing Statement: See Supplemental Online Content.

We would like to acknowledge all the blood banks and transfusion clinics in Sweden and Denmark and the Danish Transfusion database who have contributed data to this study.

Dr Zhao and Mr Rostgaard had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of Interest Disclosures:

Dr Zhao reports grants from Karolinska Institutet and grants from Vetenskapsrådet to the research group (Edgren) during the conduct of the study. Dr Edgren reports grants from Swedish Research Council (Vetenskapsrådet, 2017-01954) and grants from Region Stockholm (20180499) during the conduct of the study. Dr Hjalgrim reports grants from Helsefonden (21-B-0432). Dr Pedersen reports grants from Danish Regions during the conduct of the study; grants from Danish Independent Research Council Research in IBD on same cohort and grants from Zealand Region Denmark Research on same cohort outside the submitted work. Dr de Strooper has had consulting engagements for Eisai, Abbvie, consulting and a small number of shares in Muna TX as founding director and is the founding director of Agustine TX outside the submitted work.

Role of the Funder/Sponsor: The study sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

REFERENCES

1. Biffi A, Greenberg SM. Cerebral amyloid angiopathy: a systematic review. J Clin Neurol 2011;7:1-9.

2. Charidimou A, Imaizumi T, Moulin S, et al. Brain hemorrhage recurrence, small vessel disease type, and cerebral microbleeds: A meta-analysis. Neurology 2017;89:820-9.

3. Meretoja A, Strbian D, Putaala J, et al. SMASH-U: a proposal for etiologic classification of intracerebral hemorrhage. Stroke 2012;43:2592-7.

4. Jia Y, Li G, Song G, et al. SMASH-U aetiological classification: A predictor of long-term functional outcome after intracerebral haemorrhage. Eur J Neurol 2022;29:178-87.

5. Yeh SJ, Tang SC, Tsai LK, Jeng JS. Pathogenetical subtypes of recurrent intracerebral hemorrhage: designations by SMASH-U classification system. Stroke 2014;45:2636-42.

6. Jaunmuktane Z, Mead S, Ellis M, et al. Evidence for human transmission of amyloid-β pathology and cerebral amyloid angiopathy. Nature 2015;525:247-50.

7. Purro SA, Farrow MA, Linehan J, et al. Transmission of amyloid-β protein pathology from cadaveric pituitary growth hormone. Nature 2018;564:415-9.

8. Ritchie DL, Adlard P, Peden AH, et al. Amyloid-β accumulation in the CNS in human growth hormone recipients in the UK. Acta Neuropathol 2017;134:221-40.

9. Cali I, Cohen ML, Haik S, et al. Iatrogenic Creutzfeldt-Jakob disease with Amyloid-β pathology: an international study. Acta Neuropathol Commun 2018;6:5.

10. Jaunmuktane Z, Quaegebeur A, Taipa R, et al. Evidence of amyloid-β cerebral amyloid angiopathy transmission through neurosurgery. Acta Neuropathol 2018;135:671-9.

11. Morales R, Duran-Aniotz C, Bravo-Alegria J, et al. Infusion of blood from mice displaying cerebral amyloidosis accelerates amyloid pathology in animal models of Alzheimer's disease. Acta Neuropathol Commun 2020;8:213.

12. Burwinkel M, Lutzenberger M, Heppner FL, Schulz-Schaeffer W, Baier M. Intravenous injection of beta-amyloid seeds promotes cerebral amyloid angiopathy (CAA). Acta Neuropathol Commun 2018;6:23.

13. Brown P, Brandel JP, Sato T, et al. Iatrogenic Creutzfeldt-Jakob disease, final assessment. Emerg Infect Dis 2012;18:901-7.

14. Lauwers E, Lalli G, Brandner S, et al. Potential human transmission of amyloid β pathology: surveillance and risks. Lancet Neurol 2020;19:872-8.

15. Zhao J, Rostgaard K, Hjalgrim H, Edgren G. The Swedish Scandinavian donations and transfusions database (SCANDAT3-S) - 50 years of donor and recipient follow-up. Transfusion 2020;60:3019-27.

16. Edgren G, Rostgaard K, Vasan SK, et al. The new Scandinavian Donations and Transfusions database (SCANDAT2): a blood safety resource with added versatility. Transfusion 2015;55:1600-6.

17. Edgren G, Hjalgrim H, Rostgaard K, et al. Transmission of Neurodegenerative Disorders Through Blood Transfusion: A Cohort Study. Annals of internal medicine 2016;165:316-24.

18. Edgren G, Hjalgrim H, Rostgaard K, et al. Searching for unknown transfusion-transmitted hepatitis viruses: a binational cohort study of 1.5 million transfused patients. J Intern Med 2018;284:92-103.

19. Dahl V, Majeed A, Wikman A, Norda R, Edgren G. Transmission of viral hepatitis through blood transfusion in Sweden, 1968 to 2012. Euro Surveill 2020;25.

20. Reijmer YD, van Veluw SJ, Greenberg SM. Ischemic brain injury in cerebral amyloid angiopathy. J Cereb Blood Flow Metab 2016;36:40-54.
21. Shanwell A, Andersson TM, Rostgaard K, et al. Post-transfusion mortality among recipients of ABO-compatible but non-identical plasma. Vox Sang 2009;96:316-23.
22. Auvinen MK, Zhao J, Lassén E, et al. Patterns of blood use in Sweden from 2008 to 2017: A nationwide cohort study. Transfusion 2020;60:2529-36.
23. Mathur MB, Ding P, Riddell CA, VanderWeele TJ. Web Site and R Package for Computing E-values. Epidemiology 2018;29:e45-e7.
24. VanderWeele TJ, Ding P. Sensitivity Analysis in Observational Research: Introducing the E-Value. Annals of internal medicine 2017;167:268-74.
25. Hernán MA. The hazards of hazard ratios. Epidemiology 2010;21:13-5.
26. Rostgaard K. Methods for stratification of person-time and events - a prerequisite for Poisson regression and SIR estimation. Epidemiologic perspectives & innovations : EP+I 2008;5:7.
27. Lambert P. STPM2: Stata module to estimate flexible parametric survival models. Statistical Software Components: Boston College Department of Economics; 2010.
28. Lambert PC. Marginal estimates through regression standardization in competing risks and relative survival models. Stata Users Group2020.
29. Greenberg SM, Bacskai BJ, Hernandez-Guillamon M, Pruzin J, Sperling R, van Veluw SJ. Cerebral amyloid angiopathy and Alzheimer disease - one peptide, two pathways. Nat Rev Neurol 2020;16:30-42.
30. Jaunmuktane Z, Brandner S. Invited Review: The role of prion-like mechanisms in neurodegenerative diseases. Neuropathol Appl Neurobiol 2020;46:522-45.

31. Banerjee G, Samra K, Adams ME, et al. Iatrogenic cerebral amyloid angiopathy: an emerging clinical phenomenon. Journal of Neurology, Neurosurgery & Psychiatry 2022;93:693-700.

32. Friesen M, Meyer-Luehmann M. Aβ Seeding as a Tool to Study Cerebral Amyloidosis and Associated Pathology. Front Mol Neurosci 2019;12:233.

33. Jäkel L, De Kort AM, Klijn CJM, Schreuder F, Verbeek MM. Prevalence of cerebral amyloid angiopathy: A systematic review and meta-analysis. Alzheimers Dement 2021.

34. Jäkel L, Kuiperij HB, Gerding LP, et al. Disturbed balance in the expression of MMP9 and TIMP3 in cerebral amyloid angiopathy-related intracerebral haemorrhage. Acta Neuropathol Commun 2020;8:99.

35. McCarron MO, Nicoll JA, Ironside JW, Love S, Alberts MJ, Bone I. Cerebral amyloid angiopathy-related hemorrhage. Interaction of APOE epsilon2 with putative clinical risk factors. Stroke 1999;30:1643-6.

511 **Table 1.** Characteristics of study population.

	Sweden (Main)	**Denmark (Validation)**
Patients, N	759 858	329 512
Male, N (%)	308 738 (41%)	139 488 (42%)
Female, N (%)	451 120 (59%)	190 024 (58%)
Age at first transfusion, N(%)		
5-17 y	14 602 (2%)	4883 (1%)
18-39 y	122 849 (16%)	43 758 (13%)
40-64 y	242 368 (32%)	117 925 (36%)
65-80 y	380 039 (50%)	162 946 (49%)
Median (IQR)	65 (48-73)	64 (50-73)
Decade at first transfusion, N (%)		
1970	10 798 (1%)	0 (0%)
1980	76 167 (10%)	13 277 (4%)
1990	224 493 (30%)	88 623 (27%)
2000	262 278 (35%)	182 508 (55%)
2010	186 122 (24%)	45 104 (14%)
Year at first transfusion, Median (IQR)	2002 (1995-2009)	2003 (1998-2007)
Follow-up in years[a], N (%)		
<5 y	352 221 (46%)	144 726 (44%)
5-9 y	157 270 (21%)	81 476 (25%)
10-19 y	182 014 (24%)	85 821 (26%)
20+ y	68 353 (9%)	17 489 (5%)
Median (IQR)	5.8 (1.4-12.5)	6.1 (1.5-11.6)
Total, person years	6 096 106	2 459 076
Uncensored after the 180-day exposure assessment window, N[b]	558 032	210 663

All patients who were uncensored 180 days after their first recorded red-cell transfusion between 1970-2017 (Sweden) and 1980-2017 (Denmark) are included in this table and in the time-varying analyses. See Table 2 for the study population used in the main analyses using a 180-day exposure assessment window, which is a subset of the population in this table.
[a]Follow-up from 180-days after first transfusion to first of spontaneous ICH, censoring events, or Dec 31, 2017
[b]This differs from the total number of patients in the table due to different timings of censoring between analyses using the time-fixed 180-day exposure assessment window and the time-varying analyses
Percentages may not add up due to rounding.

512

513

Table 2. Characteristics of study population stratified by exposure during the 180-day exposure assessment window

	Sweden (Main)			Denmark (Validation)		
	Donor spontaneous ICH status at the end of follow-up					
	No ICH	Single ICH	Multiple ICH	No ICH	Single ICH	Multiple ICH
Patients	552 625	4904	503	208 432	1982	249
Male, N (%)	206 952 (37%)	2036 (42%)	194 (39%)	81 868 (39%)	856 (43%)	103 (41%)
Female, N (%)	345 673 (63%)	2868 (58%)	309 (61%)	126 564 (61%)	1 126 (57%)	146 (59%)
Age at first transfusion, Median (IQR)	65 (45-73)	66 (48-74)	65 (44-74)	63 (47-73)	65 (50-73)	64 (51-74)
Year at first transfusion, Median (IQR)	2005 (1997-2011)	1996 (1990-2001)	1994 (1988-1999)	2004 (1999-2008)	2000 (1995-2004)	1999 (1995-2004)
Number of RBC transfusions, Median (IQR)	2 (2-4)	4 (2-7)	4 (2-8)	3 (2-4)	5 (3-9)	6 (3-9)
Follow-up for patients in years, Median (IQR)[b]	6.1 (2.0-12.5)	7.1 (1.8-16.2)	8.2 (1.8-19.4)	7.3 (2.6-12.5)	6 (1-14)[a]	5 (1-15)[a]
Follow-up for blood donors in years, Median (IQR)[c]	12.7 (6.1-20.0)	20.0 (14.2-24.8)	22.2 (15.8-26.7)	13.4 (9.5-18.1)	17.2 (12.5-21.8)	17.6 (12.8-22)
Patients with single ICH, N (%)	5185 (0.9%)	67 (1.4%)	18 (3.6%)	1 901 (0.9%)	23 (1.2%)	6 (2.4%)
Age at first ICH, Median (IQR)	77 (69-81)	76 (70-82)	77 (76-81)	75 (67-80)	NA[a]	NA[a]
Time to ICH, N (%)						
<5 y	2637 (51%)	34 (51%)	11 (61%)	1 042 (55%)	NA[a]	NA[a]
5-9 y	1313 (25%)	19 (28%)	3 (17%)	535 (28%)	NA[a]	NA[a]
10+ y	1235 (24%)	14 (21%)	4 (22%)	324 (17%)	NA[a]	NA[a]
Patients with multiple ICH, N (%)	350 (0.1%)	3 (0.1%)	2 (0.4%)	123 (0.1%)	NA[a]	NA[a]
Age at second ICH, Median (IQR)	76 (67-81)	69 (65-79)	82 (80-84)	NA[a]	NA[a]	NA[a]
Time to ICH, N (%)						
<5 y	128 (37%)	1 (33%)	0 (0%)	59 (50%)	NA[a]	NA[a]
5-9 y	107 (31%)	1 (33%)	1 (50%)	38 (31%)	NA[a]	NA[a]
10+ y	115 (33%)	1 (33%)	1 (50%)	26 (21%)	NA[a]	NA[a]
Time to first ICH among donors, N (%)						
<5y		881 (18%)	123 (24%)		309 (16%)	51 (20%)
5-9y		908 (19%)	114 (23%)		492 (25%)	69 (28%)
10+y		3115 (64%)	266 (53%)		1 181 (60%)	129 (52%)
Time to second ICH among donors, N (%)						
<5y			93 (18%)			28 (11%)
5-9 y			82 (16%)			64 (26%)
10+ y			328 (65%)			157 (63%)

Only patients who have survived and were uncensored after the 180-day exposure assessment window are included.
[a]No decimals or not presented due to local data privacy regulations
[b]Follow-up from 180-days after first transfusion to first of spontaneous ICH, censoring events, or Dec 31, 2017
[c]Follow-up from first transfusion of the patient until the first of the blood donor's death, emigration, or Dec 31, 2017
Percentages may not add up due to rounding.
NA = Not Applicable

516

Table 3. Results from secondary analysis with time-varying exposure.

	Sweden (Main)				Denmark (Validation)			
	Single spontaneous ICH as outcome		Multiple spontaneous ICH as outcome		Single spontaneous ICH as outcome		Multiple spontaneous ICH as outcome	
Donor status at end of follow-up	**Events / Person years; IR**	**Adjusted HR[a]**	**Events / Person years; IR**	**Adjusted HR[a]**	**Events / Person years; IR**	**Adjusted HR[a]**	**Events / Person years; IR**	**Adjusted HR[a]**
No spontaneous ICH	7686 / 6014477; 1.28	1.00 (ref)	597 / 6035297; 0.10	1.00 (ref)	3080 / 2427772; 1.27	1.00 (ref)	231 / 2435233; 0.09	1.00 (ref)
Single spontaneous ICH	114 / 73562; 1.55	1.01 (0.84-1.23)	9 / 73869; 0.12	0.97 (0.50-1.89)	36 / 27926; 1.29	0.75 (0.48-1.16)	3 / 28023; 0.11	0.39 (0.05-2.87)
Multiple spontaneous ICH	21 / 8067; 2.60	1.95 (1.26-3.03)	2 / 8109; 0.25	2.14 (0.53-8.64)	10 / 3378; 2.96	1.74 (0.72-4.19)	0 / 3409; 0.00	NA[b]
With 5-year latency								
No spontaneous ICH	7745 / 6041764; 1.28	1.00 (ref)	600 / 6062657; 0.10	1.00 (ref)	3101 / 2439984; 1.27	1.00 (ref)	233 / 2447479; 0.10	1.00 (ref)
Single spontaneous ICH	64 / 48799; 1.31	0.96 (0.75-1.25)	6 / 49043; 0.12	1.01 (0.45-2.27)	21 / 17045; 1.23	0.73 (0.40-1.32)	1 / 17114; 0.06	0.69 (0.10-5.02)
Multiple spontaneous ICH	12 / 5543; 2.16	1.74 (0.96-3.15)	2 / 5576; 0.36	3.24 (0.80-13.09)	4 / 2047; 1.95	2.00 (0.64-6.23)	0 / 2073; 0.00	NA[b]

[a] Adjusted for sex, age, sex and age interaction, calendar year, number of transfusions, hospital/geographical location

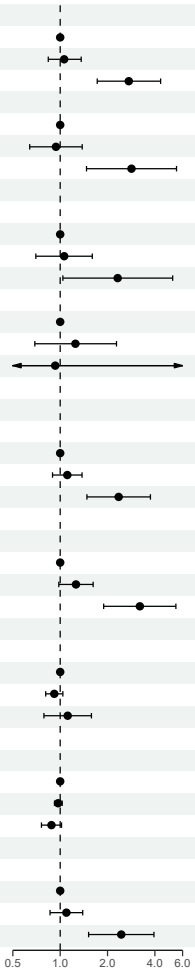
[b] Not estimable due to insufficient events

IR = Incidence rate per 1000 person years, NA = Not applicable

Figure 1. Adjusted hazard ratios for the development of spontaneous ICH from main and sensitivity analysis using a 180-day exposure assessment window.

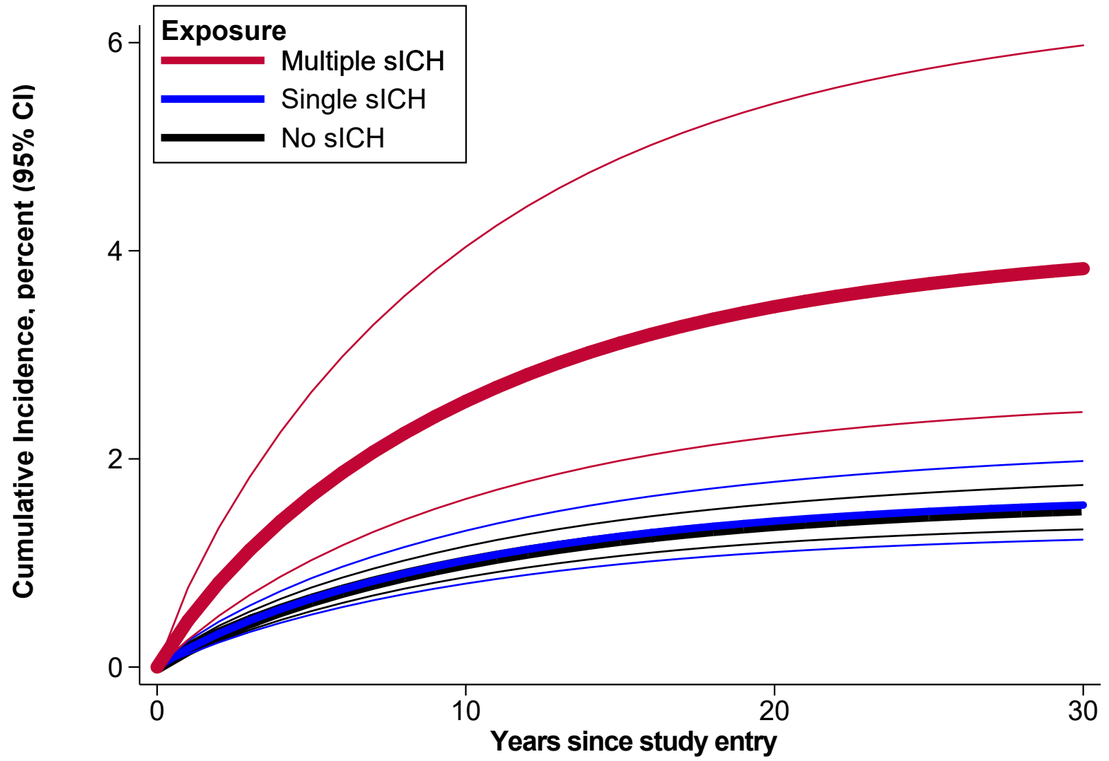
Figure 2. Cumulative incidence for single spontaneous ICH as outcome using a 180-day exposure assessment window (main Swedish cohort).

Exposure	Events / Personyears; IR	Adjusted Hazard Ratio (95% CI)
A Sweden (Main)		
No sICH	5185 / 4,611,866 ; 1.12	1.00 (ref)
Single sICH	67 / 49,583 ; 1.35	1.06 (0.84 to 1.36)
Multiple sICH	18 / 5704 ; 3.16	2.73 (1.72 to 4.35)
5-year delay		
No sICH	2190 / 2,662,568 ; 0.82	1.00 (ref)
Single sICH	27 / 32,191 ; 0.84	0.94 (0.64 to 1.38)
Multiple sICH	9 / 3952 ; 2.28	2.84 (1.47 to 5.49)
B Denmark (Validation)		
No sICH	1901 / 1,751,337 ; 1.09	1.00 (ref)
Single sICH	23 / 16,916 ; 1.36	1.06 (0.70 to 1.60)
Multiple sICH	6 / 2127 ; 2.82	2.32 (1.04 to 5.19)
5-year delay		
No sICH	751 / 998,857 ; 0.75	1.00 (ref)
Single sICH	11 / 10,375 ; 1.06	1.25 (0.69 to 2.28)
Multiple sICH	1 / 1306 ; 0.77	0.93 (0.13 to 6.62)
C Sensitivity analyses (main Swedish cohort)		
Without censoring for additional transfusions		
No sICH	5996 / 5,000,303 ; 1.20	1.00 (ref)
Single sICH	85 / 56,345 ; 1.51	1.11 (0.89 to 1.38)
Multiple sICH	18 / 6209 ; 2.90	2.35 (1.48 to 3.75)
Only considering ICH if it is the primary diagnosis[a]		
No sICH	4596 / 4,621,577 ; 0.99	1.00 (ref)
Single sICH	63 / 44,735 ; 1.41	1.26 (0.98 to 1.62)
Multiple sICH	14 / 4400 ; 3.18	3.21 (1.89 to 5.44)
Ischemic stroke in patients as the outcome[b]		
No sICH	23,837 / 4,458,442 ; 5.35	1.00 (ref)
Single sICH	248 / 48,222 ; 5.14	0.92 (0.81 to 1.04)
Multiple sICH	32 / 5552 ; 5.76	1.12 (0.79 to 1.58)
Ischemic stroke in donors as exposure and in patients as outcome[c]		
No stroke	22,591 / 4,234,502 ; 5.33	1.00 (ref)
Single stroke	1339 / 237,163 ; 5.65	0.97 (0.92 to 1.03)
Multiple Stroke	187 / 39,577 ; 4.72	0.88 (0.76 to 1.02)
Adding dementia in donors as exposure[d]		
No sICH	5184 / 4,611,556 ; 1.12	1.00 (ref)
Single sICH	69 / 49,859 ; 1.38	1.09 (0.86 to 1.39)
Single sICH + Dementia	17 / 5738 ; 2.96	2.44 (1.52 to 3.94)



[a]Excluding hospital episodes with another primary diagnosis
[b]As a negative control, the exposure is transfusions from blood donors that develop spontaneous ICH and the outcome is the first ischemic stroke in transfused patients.
[c]As a negative control, the exposure is transfusions from blood donors that develop ischemic stroke and the outcome is the first ischemic stroke in transfused patients.
[d]The exposure is transfusions from blood donors that develop no spontaneous ICH, single spontanous ICH, or single spontaneous ICH and dementia. Tthe outcome is the first spontaneous ICH in transfused patients.

IR = Incidence Rate per 1000 person years
sICH = spontaneous ICH



No. at risk by exposure

Multiple sICH	502	228	115	39
Single sICH	4889	2019	841	265
No sICH	15331	6496	2740	839

Cumulative incidence difference, percent (95% CI)

Donor No sICH	0.0 (ref)	0.0 (ref)	0.0 (ref)
Single sICH vs No sICH	0.0 (-0.3–0.3)	0.0 (-0.3–0.4)	0.0 (-0.4–0.5)
Multiple sICH vs No sICH	1.6 (0.4–2.7)	2.1 (0.5–3.6)	2.3 (0.6–4.0)

Median (IQR) follow-up was 8.3 (1.8–19.4) years for recipients of donors with multiple sICH, 7.1 (1.8–16.3) years for recipients of donors with single sICH, and 7.6 (2.0–16.8) years for recipients of donors with no sICH.

sICH = spontaneous ICH