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BLOOD DONORS' LONG-TERM HEALTH: IMPLICATIONS FOR TRANSFUSION SAFETY

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1. ABSTRACT

Continuous attention to transfusion safety through improvement of disease screening and donor selection has succeeded in reducing the risks of transfusion transmitted disease to practically immeasurable levels. Despite this progress, surprisingly little is known about disease occurrence among blood donors and whether there are possible long-term effects of repeated whole-blood or apheresis donation. Several investigations have addressed possible inadvertent non-infectious health effects of blood transfusions *per se*, but to date there is no conclusive evidence regarding whether blood transfusions from donors with preclinical cancer can result in cancer development in the recipient. To address such research questions, we assembled the Scandinavian Donations and Transfusions (SCANDAT) database, containing detailed data on blood donors and their donations, with transfusions and transfusion recipients encompassing more than three decades. All studies described in this thesis were based on this database.

In the first study, we describe the creation of the SCANDAT database and its contents, as well as the results from our analyses of its quality. In total, the database contains 1,134,290 donors with 15,091,280 donations, and 1,311,079 recipients with 11,693,844 recorded transfusions. Although direct evaluations of data quality were not possible, we assessed quality by various indirect methods and judged the database to be of a sufficiently high standard for epidemiological research investigations.

In the second study, we compared the mortality and cancer incidence in a cohort of 1,110,329 blood donors to the rates in background population. The relative risks were expressed as standardized mortality ratios (SMR) and standardized incidence ratios (SIR). Blood donors had an overall mortality 30% lower (99% confidence interval [CI] 29%-31%) and cancer incidence 4% lower (99% CI 2%-5%) than the background population. Furthermore, blood donors recruited in more recent years exhibited a lower relative mortality than those who started earlier.

Within the cohort of blood donors from the second study, the third study was conducted using a nested case-control design. Relative risks of cancer in relation to number of donations made, or iron loss endured, was estimated with conditional logistic regression. We identified a total of 10,866 donors who were diagnosed with a malignancy between their first recorded blood donation and study termination and selected 107,140 individually matched controls. We found no clear association between number of donations and risk of cancer overall. The risk of non-Hodgkin lymphoma was increased among frequent plasma donors, the odds ratio among donors with ≥20 plasma donations relative to those with <3 donations in the period from 3-12 years before diagnosis, was 2.00 (95% CI 1.15-3.46). Further, among male donors only, we found that the risk for selected cancers decreased with increasing iron loss in the period 3-7 years before diagnosis of the case (p<0.001).

Of the 354,094 transfusion recipients eligible for analysis in the fourth study, 12,012 (3.4%) were exposed to blood products from donors who developed cancer within 5 years. The relative risk of cancer overall comparing recipients of blood from precancerous donors to recipients of blood from non-cancerous donors was 1.00 (95% CI, 0.94-1.07). We also did not find any excess risk when we considered the site and severity of the cancer in the donor, nor when we assessed site-specific cancer risks among the recipients.

2. LIST OF PUBLICATIONS

This thesis is based on the following four publications. They will henceforth be referred to by their Roman numerals (I-IV). The publications are reproduced in this thesis with the kind permission of the publishers.

I.   Edgren G, Hjalgrim H, Tran TN, Rostgaard K, Shanwell A, Titlestad K, Jakobsson L, Gridley G, Wideroff L, Jersild C, Adami J, Melbye M, Reilly M, Nyren O. A population-based bi-national register for monitoring long-term outcome and possible disease concordance among blood donors and recipients. Vox Sanguinis 2006;91:316-23.

II.   Edgren G, Tran TN, Hjalgrim H, Rostgaard K, Shanwell A, Titlestad K, Wikman A, Norda R, Jersild C, Wideroff L, Gridley G, Adami J, Melbye M, Nyrén O, Reilly M. Improving health profile of blood donors as a consequence of transfusion safety efforts. Transfusion 2007;47:2017-2024.

III.   Edgren G, Reilly M, Hjalgrim H, Tran TN, Rostgaard K, Adami J, Titlestad K, Shanwell A, Melbye M, Nyrén O. Repeated blood donation, iron loss and risk of cancer. Manuscript submitted.

IV.   Edgren G, Hjalgrim H, Reilly M, Tran TN, Rostgaard K, Shanwell A, Titlestad K, Adami J, Wikman A, Jersild C, Gridley G, Wideroff L, Nyrén O, Melbye M. Risk of cancer after blood transfusion from donors with subclinical cancer: a retrospective cohort study. Lancet 2007;369:1724-30.

3. TABLE OF CONTENTS

4. ABBREVIATIONS

The following abbreviations have been used in this thesis and in the associated four original publications:

ALAT	Alanine aminotransferase
ATP	Adenosine triphosphate
BID	Blood component identity code
BSE	Bovine spongiform encephalopathy
CI	Confidence interval
CML	Chronic myeloid leukemia
CRS	Civil registration System
DID	Donation identity code
HB	Hemoglobin
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HGV	Hepatitis G virus
HHV	Human herpesvirus
HIV	Human immunodeficiency virus
ICD	International classification of disease
IQR	Interquartile range
IRR	Incidence rate ratio
NAT	Nucleic acid test
NHL	Non-Hodgkin lymphoma
NRN	National registration number
OR	Odds ratio
PID	Personal identifier
SCANDAT	Scandinavian donations and transfusions
SD	Standard deviation
SEK	Swedish kronor
SIR	Standardized incidence ratio
SMR	Standardized mortality ratio
TRALI	Transfusion related acute lung injury
TRIM	Transfusion related immunomodulation
TTV	Torque teno virus/Transfusion transmitted virus
US/USA	United States of America
USD	US dollar
vCJD	Variant Creutzfeldt-Jakob disease
WNV	West Nile virus

5. INTRODUCTION

In 1492 Pope Innocentius VIII received the first blood transfusion that has been recorded in history.[1, 2] In order to try to save the life of the dying pope, the blood from three young shepherd boys was drawn and somehow administered. Whether it was transfused intravenously or if the pope just drank the blood is uncertain (as is the validity of this historical event itself), but the three donors as well as the pope are recorded to have died quickly from the experiment.

Since then, the practice of transfusion medicine has developed immensely. Clinicians nowadays have at their disposal a wide range of powerful blood-derived therapeutics, from unprocessed whole-blood and common packed red cells to advanced stem cell preparations and blood for intrauterine transfusions. Meanwhile, continuous attention to transfusion safety through improvement of blood donor selection, infectious disease screening and increasingly stringent administrative processes have resulted in dramatic reductions in the risk of transfusion transmission of infectious disease,[3-7] as well as of more common non-infectious transfusion reactions.[8] Although research, in response to intense public and medial attention, primarily has been directed towards reducing the risks of transfusion transmitted infections, blood donors of today, unlike the three ten-year old voluntary blood donors that contributed to the aforementioned pope's blood transfusion, do not appear to die of their practice.[9]

While refined selection of blood donors has resulted in low risks of transfusion transmitted infections, very little is known about disease among blood donors and the possible long-term effects of repeated whole-blood or apheresis donation. Similarly, numerous investigations have addressed possible adverse health effects of blood transfusions *per se*, such as an increased occurrence of cancer overall and of non-Hodgkin lymphoma in particular, but no conclusive evidence on whether blood transfusions can result in cancer development has yet been presented. A number of specific mechanisms for why such may be the case have been suggested. Among these, transfusion associated immunomodulation, transmission of oncogenic viruses and the notion that cancer cells in a blood donor can be transferred to and continue their malignant growth in transfusion recipients have received some attention.[10]

In this thesis we have studied much-overlooked aspects of transfusion safety: the health effect of repeated blood donation in donors and the possible transmission of cancer from blood donors to transfusion recipients.

6.   BACKGROUND

6.1.   HISTORY OF TRANSFUSION MEDICINE

Although blood transfusions of some variety are suspected to have been used by Egyptian physicians in ancient time,[2] transfusion medicine as it is perceived and practiced today, a cornerstone of modern medicine, did not emerge until in 1900-01, when Karl Landsteiner derived the ABO blood group system which permitted safe use of allogenic blood transfusion.[11] However, even before Landsteiner's discovery of the ABO blood group system, the use of blood transfusions had repeatedly found its place in history.

In 1628, the English physician William Harvey described and published an account of the physiology of the circulatory system.[12] Although far from perfect in its explanation of the transfer of blood from the arterial to the venous circulation, his treatise provided the first comprehensive description of circulation. Prior to this publication, transfusions through vein-vein anastomoses are likely to have been used, reflecting some understanding of the necessary anatomy, but they were not widespread by any means. Starting in 1666, another British researcher, Richard Lower, began experimenting with transfusions between animals and animals and humans.[13, 14] However, the first recorded transfusion to a human (in this case with blood drawn from a sheep), disregarding the perhaps unreliable account of Pope Innocentius' fatal procedure, was performed somewhat successfully in Paris in 1667 by a certain Jean Baptiste Denis.[14] Unfortunately, his subsequent attempts were less successful, with several fatal attempts as a result, and consequentially the practice of transfusion therapy was rapidly banned by the French government.

Thereafter, a period of little development and activity seems to have continued until 1825 when James Blundell, an obstetrician and physiologist working in London who, acting now on seemingly steadier scientific grounds, successfully administered a transfusion to a woman who was dying of postpartum hemorrhage.[15] Following the transfusion, that was allegedly supplied by the patients' husband and was delivered via a syringe, the patient seems to have stabilized and survived her condition. During the following years Dr Blundell performed and published accounts of a total of ten transfusions, half of which were evidently successful.[16] Until James Blundell entered the field, most of the pioneers of transfusion medicine probably did more harm than good, but despite some advances that were mainly of a technical nature, an understanding of the immunologic aspect of blood transfusions and a means to keep blood from clotting during storage was still lacking. Thus followed a number of decades with few, or no, successes, as was elegantly reviewed by Reuben Ottenberg in 1908.[17]

The first of these obstacles, the immunogenicity of allogenic blood, was partially overcome by Austrian physician Karl Landsteiner who in 1901 described the ABO blood type system and thus provided a means of eliminating the major cause of transfusion complication at the time.[11] With hindsight, the initial success of such pioneers as Blundell is quite remarkable given the total lack of understanding of the immunogenicity of human blood. Following Landsteiner's discovery, several other blood group systems were discovered, and when crossmatching was introduced by Ottenberg in 1908,[17] and the second most important blood group system, the Rhesus-system, was discovered and described by Landsteiner and Wiener in 1937 (their work was not published until 1940),

most of the groundwork towards preventing immunologic reactions to blood transfusion had been laid.[18] It should be noted that in parallel with Landsteiner and Wiener's work, Phillip Levine, a student of Landsteiner's, published a case report about a woman who experienced post transfusion hemolysis after transfusion with a presumable Rh-incompatible unit already in 1939.[19]

A second obstacle, a means of anticoagulation that would allow storage of blood and thus transfusions without having a donor physically available, was overcome by Richard Lewisohn in 1915, a surgeon working at Mount Sinai in New York. His contribution, by no means very innovative, was to determine an optimal and non-toxic concentration of the already known anticoagulant sodium citrate.[20] Although his work was of great importance and, when Drs Peyton Rous and J.R. Turner at Rockefeller Institute in New York suggested glucose also to be added, thus permitting long-term storage of blood,[21] it still took almost ten years to reach widespread acceptance. Meanwhile, during the First World War, a US Army Medical Corps Officer, Oswald Robertson, developed the world's first blood bank further developing skills he had obtained as a student in said Dr Rous' laboratory.[22]

Since the advances of Blundell, Landsteiner, Lewisohn and Robertson, transfusion medicine has evolved at a steady pace. Improvements have been made in the fields of blood collection, component preparation and storage, and with the recognition of the potential for transmission of infectious disease via of blood transfusion, also in disease screening.

6.1.1.   Transfusion medicine in Sweden

The first direct blood transfusion is reported to have been performed at Hudiksvall hospital in 1916 by physician Torsten Rietz who had previously practiced extensively abroad with the Red Cross. Perhaps on account of the long Swedish tradition of peace, development of Swedish transfusion medicine was thus lagging somewhat behind other parts of the western world, such as the United Kingdom and the United States. The use of transfusion therapy did not reach any noticeable use until far later. Thereafter, however, throughout the 20th century, transfusion medicine in Sweden has largely observed the same trends as worldwide, but have differed in some notable ways.

Contrary to many countries, where blood collection is organized by the Red Cross or other voluntary organizations, Swedish transfusion services have been hospital maintained and organizationally closely tied to the public health care system, even since the beginning of organized blood collection activities in the 1950s. Thanks to a tradition of national guidelines, involving criteria for blood donor selection and very standardized technical routines for blood collection, the Swedish blood services have been and still are nationally very homogenous. Eligibility criteria for blood donation and a list of screening tests currently and historically in use in Sweden are presented in Table 1.

6.2.   TRANSFUSION SAFETY

Upholding a high level of transfusion safety involves virtually all aspects of transfusion medicine. On the first level, blood donor selection must be exclusive enough to eliminate donors with potentially harmful, transmittable, disease. Despite appropriate exclusion

Criteria	Year of introduction	Length of deferral	Note
Screening tests			
Syphilis	1948–1954	Permanent	At first registration for donation
HBsAg	1970–1972	Permanent	
Anti-HIV-1	1985	Permanent	
Anti-HBc	1989–1991	Permanent	At first registration and for donors exposed to blood transfusions or tattooing
Anti-HIV-2	1991	Permanent	
Anti-HCV	1990–1992	Permanent	
Anti-HTLV-I and -II	1994	Permanent	Only at first donation or after 5-year absence.
Medical conditions			
Heart disease	1968	Permanent	
Diabetes	1968	Permanent	Type II diabetes not requiring medical therapy is accepted
Kidney disease	1968	Permanent	
Epilepsy	1968	Permanent	
Allergy	1968	Permanent	Moderate untreated and asymptomatic allergy is accepted
Cancer history	1989	Permanent	In situ cancers and basaliomas are exempted
Infectious disease			
Malaria	1968	Permanent	
Syphilis	1968	Permanent	
Hepatitis	1968	Permanent	
Infectious risk*			
Received blood transfusion	1968	6 months	
Tattoo	1968	6 months	
Intravenous drug use	1984	Permanent	
Sexual activity*			
Male sex with male	1983	Permanent	
Sex with prostitute	1984	Permanent	
New sex partner	2001	3 months	

Table 1. Screening tests and Eligibility criteria for blood donation.
*Donors with an obvious high-risk behavior have successively been excluded from blood donation.

of unsuitable blood donors, a widely covering batch of screening tests must be employed to further reduce risk of infectious diseases to an acceptable degree. A second aspect of this level, which in the literature has been quite overlooked, the collection of blood or other blood components must be safe for the donor both in the short- and long-term.

On a second level, operating procedures and administrative routines must be impervious to human error and fail proof enough to guarantee the selection of the right blood unit for the right patient at the right indication. I will here cover some of these aspects, with an obvious emphasis on the topic of this thesis.

6.2.1. Disease among blood donors
Since all transfusion-transmitted disease invariably originates from a blood donor, there exists an inescapable, and frankly often overlooked, dependence between the health of blood donors and the safe practice of transfusion medicine. Thus, attracting, selecting and retaining healthy and motivated blood donors with a behavior that ensures a low risk of transfusion transmittable disease is a cornerstone in maintaining a safe blood

supply. Among the mechanisms that are of most importance to achieve this are naturally stringent eligibility criteria and infectious disease screening in conjunction with the altruism-linked self-selection that motivates healthy individuals to the rather unselfish act of donating blood.

Research on disease occurrence among blood donors has been both scarce and generally, with some notable exceptions, somewhat lacking in both methodological accuracy and statistical power. A series of large-scale studies of infectious disease occurrence among blood donors have demonstrated low and declining incidence of the major transfusion transmittable viral infections.[4, 23] Furthermore, studies comparing the mortality and incidence of chronic disease among blood donors to rates in the general population have implied a lower than average overall mortality,[24] as well as a lower risk of both cancer[25] and cardiovascular disease.[26-29]

6.2.1.1. *Behavioral aspects of disease occurrence in blood donors*
In addition to direct studies of disease occurrence, several investigators have studied and tried to characterize the motivational forces that provoke individuals to become and remain being blood donors.[30-34] Naturally, research has focused on how to motivate the right individuals, with a low-risk behavior, to become donors and also on how to promote donor retention in order to ensure a stable blood supply. Three rather thorough reviews have been published on donor demographics, motivation and retention of already recruited donors.[31, 32, 35] A number of key points that have a direct influence on transfusion safety are described. In all three reviews, donor motivation is characterized as mainly being driven by altruism, but donors also report to be motivated by self-esteem, a sense of social responsibility and the need for being seen as someone doing good. It is also implied that any departure from such intrinsic motivation, for example by excess pecuniary rewards or tax credits for blood donors which have been suggested historically, may increase the risk of transfusion transmitted disease. Hence, it is suggested that excess solicitation or social pressure, although effective in recruiting new donors, is a double-edged sword as it may encourage also high-risk individuals to become donors. Importantly, in the most recent of these reviews, published in 1990, the process by which first-time donors become safe repeat donors was identified as perhaps the most important aspect of donor motivation.[31]

6.2.2. Health effects of blood donation
If research on disease occurrence among blood donors leaves many unanswered questions, scarcely anything has been published on the actual health effects of blood donation beyond documentation of immediate short-term complications[9] such as nausea, bleeding and peripheral nerve damage. Even though previous studies comparing blood donors' mortality and cancer incidence to the general population have given some suggestion that blood donation is not harmful for the donor, deleterious effects of repeated letting of blood may possibly have been masked by the general healthiness of blood donors that is ensured by selection criteria, self-selection and infectious disease screening. In fact, given that a number of specific mechanisms that could potentially influence donor health (both negatively and positively) have been suggested, it is quite surprising that

to date there to date exist scarcely any dose-response comparisons of the effect of blood donations on long-term disease risk.

6.2.2.1.   Iron depletion

Iron is an essential mineral to all human tissues. Incorporated in the heme complex, it is necessary for the blood-borne transport of oxygen, generation of ATP in the electron transport chain, etc. Iron may also have toxic effects, such as generation of free radicals, and iron uptake as well as availability is consequentially tightly regulated in the human body.[36] On account of the pro-oxidative properties of free iron and the rather high prevalence of iron deficiency in women of childbearing age,[37, 38] sex-differences in iron levels were rather famously hypothesized by Jerome L. Sullivan in 1981 to be a contributor to the substantial differences between men and women in the risk of cardiovascular disease.[39]

This iron-store hypothesis has subsequently been tested in a large number of investigations with both positive,[27, 40-43] but lately mainly negative results.[44-47] In fact, a recent intervention trial where patients with known peripheral artery disease were randomized to a blood-letting scheme that was designed to safely remove a predetermined amount of iron failed to show any beneficial effects of iron depletion.[48] Although awaited by some as the definitive study on the iron store hypothesis, despite its large sample size and long follow-up it was clearly under-powered and was conducted in a very selected patient group.[49] Hence, no consensus on the long-term effects on risk of cardiovascular disease that are associated with iron depletion or overload has been established, although recent reports tend to indicate no, or at least only minor, protective effects of low iron stores.[49] In addition to being related to cardiovascular disease, the iron store hypothesis has since also been extended to include other chronic diseases such as cancer[38, 50-56] as well as type II diabetes.[57]

Since frequent and long-term blood donors, despite appropriate administration of iron supplementation, often suffer from some degree of iron depletion, it seems reasonable to suggest that, if the iron store hypothesis holds true, frequent blood donation should be protective against cardiovascular disease, and perhaps also against cancer and type II diabetes. Accordingly, such reasoning has provoked four investigations of the risk of chronic disease in relation to the frequency of blood donations.[25, 27, 58, 59] It should be noted however, that on account of the unknown extent to which frequent blood donors really do experience true iron depletion, some concern about the appropriateness of using blood donors as a test of the iron store hypothesis has been raised by the proponent of the iron store hypothesis.[60, 61]

6.2.2.2.   Mitotic stress

It has also been postulated that increased cell proliferation, itself a possible risk factor for cancer,[62, 63] caused by repeated removal of peripheral blood cells through blood donation, may increase the risk of hematopoietic malignancies.[25] There is little or no evidence regarding whether the rate of cell proliferation is really influenced by repeated removal of blood from the peripheral circulation, or if the bone-marrow stress thence possibly induced, really influences cancer risk to any noteworthy extent.[25]

6.2.2.3. Immunomodulation

Again on a strictly theoretical basis, it has been speculated that the transient, and seemingly rather unimportant, immunological effects of blood donation could be accompanied by a higher cancer risk.[64-68] Rigorous research on the long-term effects of donation induced transient immunomodulation is virtually non-existent, but as with other conditions associated with immunomodulation, one would expect blood donors to be at higher risk of non-Hodgkin lymphoma (NHL) and presumably also of non-melanoma skin cancer.[69] To our knowledge, there is no published research either supporting or refuting this hypothesis.

6.2.2.4. Other possible mechanisms

The long-term health effects of aphaeresis donation have not been carefully studied. It is also well known that plasma and platelet donors are exposed to citrate and hypothetically also to other chemicals that might be dissolved from the equipment such as plasticizers.[70, 71] It has also been suggested that apheresis donation may cause complement, leukocyte or thrombocyte activation during the extracorporeal phase of the blood collection.[71] The long-term health effects of such exposures certainly deserves more thorough investigation.

6.2.3. Disease among transfusion recipients

While blood donors can be considered to be selected for their good health, transfusion recipients in effect are selected for their failing health. The pattern of disease occurrence in transfusion recipients thus largely reflects the diseases for which they were transfused. Consequentially any non-randomized study of the health effects of blood transfusions must somehow take into account the conditions for which the patients were transfused. For example, in a case-control study of the risk of NHL after blood transfusion from the mid-1990s,[72] the researchers only studied patients who were transfused for diseases deemed themselves to be unrelated to NHL. Thereby any bias from an otherwise potentially confounding relationship between the indication for the blood transfusion and the outcome was largely eliminated.

During the last decades, studies of the deleterious health effects of blood transfusions have mainly, albeit not exclusively, been confined to infectious disease transmission. However, with increasing relative importance, recent years have seen more focus on non-infectious effects of transfusions. In fact, in most western countries the risk of more direct deleterious effects of blood transfusions currently exceeds the risk of the commonly acknowledged transfusion transmitted infections by several orders of magnitude.[8]

6.2.3.1. Transfusion transmitted infections

With the major technical and immunological obstacles hindering safe use of blood transfusions surpassed, complications in terms of spread of infectious disease were soon realized. In fact, parenteral transmission of disease had been recognized as early as 1885 when smallpox vaccination at a German shipyard caused an epidemic of viral hepatitis (at the time referred to as "catarrhal jaundice") among the vaccinated.[73, 74] Although similar observations about the transmissibility of infectious hepatitis were made throughout the following half-century, these observations were too premature as the common un-

derstanding of microcellular pathogens had perhaps not yet reached critical mass, and the today logical connection that disease transmission had indeed taken place was not fully made.[74, 75] It wasn't until 1943, when use of blood transfusions on account of World War II had become more widespread, that it was fully recognized that acute hepatitis (primarily Hepatitis A) could indeed be transmitted via blood transfusion.[76, 77] With the slow realization that also serum hepatitis (Hepatitis B and C; HBV and HCV) was of an infectious nature, which was a far more serious concern, came a long hunt for the causative agent and a suitable screening test. The serum antigen that was initially named the Australia (Au) antigen, i.e. the Hepatitis B surface antigen (HBsAg), was first discovered in leukemia, thalassemia, leprosy and hepatitis patients during the mid 1960s,[78] but was soon after its discovery realized to be associated with serum hepatitis.[79, 80] Thus screening for HBV was possible. The first Swedish HBV screening program using an HBsAg-based assay was introduced by blood banks and transfusion medicine services during 1970-1972 and similarly in Denmark between 1975-1983.

Notwithstanding the successful implementation of HBV screening, the problem of infectious hepatitis had not been fully eliminated from the blood supply. Even before the causative agent of non-A, non-B hepatitis, i.e. the hepatitis C virus (HCV), was identified in 1989,[81] the problem of HCV-transmission was largely eliminated by excluding high-risk groups (such as intravenous drug users and prior transfusion recipients) from blood donation, and by introducing surrogate screening using indirect measures of liver damage such as alanine aminotransferase (ALAT).[82] Since the implementation of anti-HCV antibody screening of all donors in Sweden in 1992, there have been very few reported cases of HCV transmission from blood donors through blood transfusions.[82]

Of graver consequence for the blood supply (and most importantly for all infected patients) was the start of the Human Immunodeficiency Virus (HIV) epidemic during the beginning of the 1980s.[83] No fewer than 85 individuals in Sweden have been confirmed to have been infected with HIV through blood transfusions.[82] Although severe, consequences of the Swedish HIV epidemic were by international standards quite limited.[84] HIV-screening was introduced in Sweden in 1985, and even as Swedish transfusion medicine services have relied on less sensitive, antibody-based screening rather than nucleic acid testing (NAT) assays, which have been introduced in many countries worldwide[5], there have been no recorded cases of HIV transmission in Sweden since 1987.[82] The success of the Swedish HIV screening can be mainly attributed to a relatively low HIV prevalence in the general population, and compared to other settings, a proportionally greater contribution from a healthier pool of (in practice) unpaid donors, who were therefore presumably driven more by altruistic rather than monetary motivations, than perhaps in other settings.[82]

Although the near-elimination of transfusion transmitted hepatitis and HIV from the blood supply can be seen as a hallmark of infectious disease screening in transfusion medicine, other infectious diseases, such as syphilis,[85] had been recognized to be transfusion transmittable at an even earlier stage. Indeed, among the soldiers used as blood donors in Oswald Robertson's World War I blood banks, only those with no history of syphilis were accepted as blood donors.[14, 86] However problematic for the practice of

medicine at a time when the prevalence of syphilis was very high and no efficient nor well-tolerated treatment existed, a shift from direct to indirect transfusion methods whereby cold storage effectively inactivated the causative spirochete, reduced the risks of syphilis transmission almost completely.[87] Accordingly, and thanks to a declining disease prevalence and implementation of efficient screening, the last cases of transfusion transmitted syphilis were observed (and described in the international literature) in Western Europe in 1977[88] and the US in 1966.[89]

During the last decade, a number of new transfusion transmittable infectious agents have been suggested, and some confirmed. Although there was initially considerable worry about the (questionably named) Hepatitis G, TT, and SEN viruses, all of which have been found to have a non-negligible prevalence among blood donors and to be readily transfusion transmittable,[90, 91] none have as yet been found to have important pathogenic effects.[5, 92-95] Recently, also the West Nile Virus (WNV) and Human Herpesvirus 8 (HHV-8; Kaposi's sarcomavirus) have been found to be transmittable by blood transfusion.[96, 97] Whereas all blood collected in the USA is currently being screened for WNV using a NAT assay,[98] it is still unclear whether WNV screening will be necessary also internationally and similarly whether universal screening will be at all necessary for HHV-8.[99] However, as a preemptive measure against WNV transmission, Swedish blood banks currently exclude donors who have visited the United States or Canada during the last 30 days.

The fear of transfusion transmission of variant Creutzfeldt-Jakob disease (vCJD; human form of Bovine Spongiform Encephalopathy [BSE]), has resulted in several countries currently excluded potential donors who have resided for extensive periods of time in the United Kingdom.[100-102] Although vCJD is decidedly transmittable by nervous tissue transplantation[103-105] and human pituitary hormone extracts,[106] solid evidence regarding transfusion transmission has for obvious reasons been slower to accumulate. However, the tissue distribution of the causative agent (PrP^{SC}),[107] successful blood-borne BSE transmission in experimental settings in sheep[108] and preliminary data from observational studies in humans[109-111] suggests such may be the case.

In summary, a list of selected infectious diseases known to be transfusion transmitted is presented in Table 2.

6.2.3.2.   Cancer occurrence among transfusion recipients

For obvious reasons, the occurrence of infectious disease among transfusion recipients has been thoroughly explored.[99] As discussed above, a rather extensive and much varied literature also exists on transfusion related immunomodulation (TRIM),[112] which like immunomodulation seen after donation among blood donors has been suspected to increase transfu-

Viruses
Colorado tick fever virus
Cytomegalovirus
Epstein-Barr virus
Hepatitis A-E
Human herpesvirus 6 and 8
Human immunodeficiency virus I and II
Human T-lymphotrophic virus I and II
Parvovirus B19
Tick borne encephalitis virus
West-Nile virus
Hepatitis G, TT and SEN viruses*
Bacteria
Bacterial contaminants
Syphilis
Rickettsia spp.
Ehrlichia spp.
Parasites
Malaria spp.
Babesia spp.
Trypanosoma spp.
Toxoplasma gondii
Microfilariasis
Other
Variant Creutzfeldt Jakob disease

Table 2. Selected Transfusion Transmitted Diseases.
*Although demonstrated to be transfusion transmittable, neither have been convincingly demonstrated to be pathogenic.

sion recipients' risk of the common immunodeficiency-related malignancies as well as bacterial infection, see 6.5.3 above.[69, 113] Although a number of studies have addressed the overall cancer pattern among transfusion recipients,[114-116] owing to immunomodulation and the near-optimal opportunity for transmission of oncogenic viruses that a blood transfusion presents, non-Hodgkin lymphomas and other virus-associated malignancies have attracted the most attention.[72, 116-120] Due to insufficient sample sizes and perhaps also the failure of most investigators to account for the transfusion indication, results have varied considerably, but the combined results seem to lean toward no association.[121]

A noteworthy example, however, is a study by Memon and Doll. They investigated the long-term cancer risk among patients who had been transfused *in utero* or shortly after birth in a creative attempt to search for unknown blood-borne oncogenic viruses.[116] Their findings essentially ruled out any dramatic effects of transfusions in the perinatal period on risk of cancer overall, but despite more than 12,000 participants who were followed for a total of 340,227 person years, the study had insufficient power to rule out any associations with NHL.

Largely due to conflicting results and results based on different patient compositions (with limited control for confounding by indication), no firm conclusion can be drawn about whether blood transfusions have a cancer promoting effect, or even any causal link with cancer. It is also unclear whether, as has been suggested before,[121, 122] subclinical cancer present among blood donors may be transmitted through blood transfusions to recipients.

6.2.3.3.   *Transfusion transmitted cancer*

As discussed above, a blood transfusion represents an optimal opportunity for the transmission of disease. Notably a large number of infectious disease have been found to be transfusion transmittable and many are consequently carefully screened for or otherwise eliminated, by exclusion of high-risk groups or pathogen inactivation, in transfusion medicine practice. However, little effort has been spent in trying to address whether any diseases traditionally considered as non-communicable can also be transmitted via blood transfusion. With the exception of case reports describing the outcome of single individuals being accidentally transfused with blood from donors who shortly after donation developed leukemia,[123-125] the only systematic attempts to study the blood-borne transmission of malignant cells have been inconclusive. In the mid-1940s, (quite obviously) prior to the pre-Helsinki declaration, the Australian researcher J.B. Thiersch conducted a series of ethically questionable, yet intriguing, experiments where otherwise moribund patients were injected with malignant cells from leukemia and lymphoma patients.[126, 127] Luckily, out of the 48 patients he meticulously inoculated (three patients with cancer of the oral cavity in the first study and 45 patients with various chronic conditions in the second study), none was confirmed to have developed a donor-derived malignancy. However, beyond their disturbingly unethical design, Dr Thiersch's studies leaves many questions unanswered regarding length of follow-up, sample size and the viability of the engrafted cells, etc. Similarly, the results from the hitherto only systematic attempt to study blood-borne transmission of malignant cells from blood donors to recipients are inconclusive, mainly for insufficiency of study subjects and lack of follow-up.[122] Using

data from the New York State cancer register and blood banks, the researchers expanded a previous effort at the University of Rochester and managed to retrospectively trace 105 transfusion recipients who had received blood from a preleukemic or prelymphomatous blood donor. During an average follow-up of 7 years, none of these recipients were found to have developed leukemia or lymphoma.

During the pioneering stages of granulocyte transfusions in the 1960s (and in the period thereafter), patients with chronic myeloid leukemia (CML) were often used as blood donors on account of their high leukocyte counts. Although blood components drawn from donors with CML are generally no longer used on account of the poor function of leukemic granulocytes, in 1984 Schiffer *et al* demonstrated sustained granulocyte count increases for as long as 11 days among 14 patients with acute leukemia who were treated for infectious episodes with transfusions from such donors.[128] Similarly, Vargas *et al* demonstrated persistence of the Philadelphia translocation for as long as 75 days, but no longer, after the accidental transfusion of blood from a donor with CML.[125]

A number of valuable lessons can also be learned from transplantation studies; transmission of both solid[129, 130] and non-solid[131-133] donor-derived malignancies have been carefully documented in both organ and bone-marrow transplant settings. Analogously, there exist scattered case reports describing cancers developing from cancer cells accidentally transplanted from patient to surgeon via surgical instruments or needle sticks.[134-136] Although only three such reports are to be found in the literature, they clearly demonstrate the ability of tumor cells to be transplanted and to survive even in seemingly immunocompetent and unrelated hosts.

In addition, there exists a series of experiments where researchers attempted allogenic transplantation of human cancer cell lines with varying degrees of success.[137] The most notorious of these experiments were conducted at Sloan-Kettering cancer institute by Dr Chester M. Southam, who later became president of the American Association for Cancer Research, and colleagues who attempted to transplant cancerous tumors to both healthy volunteers at the Ohio state penitentiary,[138] cancer patients[138, 139] and otherwise terminally ill patients with non-malignant disease.[137] The researchers found that the transplanted tumor tissue indeed produced small nodules which continued to grow for some 4-6 weeks before spontaneous and complete regression,[137-139] which was explained by an immune reaction in the transplant recipient.[140] At about the same time, or even earlier, other researchers carried out similar experiments, but with some notable differences. One such example are experiments of cancer autotransplantation where researchers purposely inoculated patients with large numbers of their own tumor cells at a site different than the original cancer site.[141-143] Surprisingly, the yield of these experiments was quite poor, perhaps indicating some level of remaining immunological reactivity to the cancer cells or, as has been suggested before, that the success of autotransplantation is somehow dependent on the capture of cancer stem cells from which the "metastasis" may develop.[144] Another important, and equally frightful, example is a case report by a Dr Edward F. Scanlon who transplanted a piece of a malignant melanoma from a female patient into the *rectus abdominis* muscle of the patient's supposedly consenting 80-year

old mother.[145] Unfortunately, the inoculated tumor cells grew and disseminated rapidly, killing the mother little more than a year after transplantation.

Disturbingly, many of the experiments described above were conducted in a post Nürnberg code era among senile or otherwise mentally incapable patients from whom no consent had been requested. Dr Southam's research has subsequently attracted considerable attention on account of its dubious ethical nature,[146, 147] and perhaps because of this, rather little attention for its scientific value. However ethically troublesome such studies are, they do convey some valuable lessons:

- Cancer cells can be transplanted between unrelated individuals, but their continued growth and spread is usually limited by the immune response in the inoculated recipient.

- The success of cancer transplantation depends both on the number of transplanted cells and presumably also on the level of genetic similarity between the donor and transplant recipient.

- Furthermore, evidence is accumulating that a third limiting factor may be the clonal nature of cancer development, i.e. that cancer and, more importantly, cancer metastases primarily develop from cancer stem cells.[144, 148]

6.2.3.4. Other noninfectious disease transmission

To date, there are no studies which clearly demonstrate transmission of a noninfectious disease, but one curious report. Only recently, Arnold *et al* presented a case where peanut hypersensitivity was passively transferred from a donor with a history of peanut allergy through a unit of fresh frozen plasma.[149] After eating a muffin with peanut butter two days after the transfusion, the patient developed a severe allergic reaction. Two months after the incident, the patient's transient peanut allergy had disappeared. No attempts have been made to systematically study the transfusion transmission of allergy, nor of other noninfectious diseases. Furthermore, on a strictly speculative note, multiple sclerosis has been (wildly?) hypothesized to be transfusion transmittable.[150]

6.2.3.5. Microchimerism

Microchimerism is defined as the prolonged persistence of small numbers of cells that are of a different genetic origin than the host. Microchimerism typically results from naturally occurring exposures such as bidirectional cell transfer between the fetus and the mother and between twins during pregnancy (fetal microchimerism), but can also be iatrogenic, resulting from organ transplantation or allogenic blood transfusions.[151]

In transfusion settings, microchimerism commonly refers to the presence of small colonies (1-5% of total white blood cell count) of donor-derived white blood cells, and has been found to occur quite frequently in severely injured, but otherwise immuno-competent, trauma patients who have received multiple transfusions.[152-154] Long-term (>25 years) microchimerism has also been found in patients who were transfused with non-filtered and non-irradiated blood *in utero*.[155] Intriguingly, it is largely unknown to what extent microchimerism is established in patient groups who have been transfused on other indications,[156] and under what conditions it occurs, e.g. whether blood filtering

or irradiation may prevent it. Also, the clinical significance of transfusion-associated microchimerism is largely unknown, but it has been suggested as a mechanism which may facilitate transplant graft acceptance.[157] Bearing in mind reports associating fetal microchimerism with various autoimmune conditions,[158, 159] it is not unreasonable to suspect that transfusion-associated microchimerism can result in the development of autoimmune disease in transfusion recipients.[156]

6.2.3.6. *Transfusion related immunomodulation (TRIM)*

It is well-established that recipients of allogenic blood transfusions experience a period where both the innate and adaptive immune responses are down-regulated. Several underlying mechanisms have been suggested, but it is generally accepted that the large amount of alloantigens the transfusion recipient is presented with is partly responsible.[160] It seems that this results in a down-regulation of especially the cellular immune defense, and concurrently, an upregulation of the humoral response, with increased antibody production.[161]

The first notion that allogenic blood transfusions implied an immunomodulation in recipients originates from a study published in 1973.[162] They reported lower renal transplant rejection rates among patients who had been transfused in conjunction with the transplantation. Subsequent studies, including a large multi-centre trial where 205 patients who were randomized to 3 allogenic transfusions had superior kidney graft survival compared to the 218 patients who did not receive any transfusions,[163] have granted further support to these findings and shown that allogenic blood transfusions may be beneficial even where modern immunosuppressive therapy is used.

There have been a number of suggestions regarding both short and long-term consequences of this immunosuppression. Several observational and randomized studies have been performed to investigate whether blood transfusions confer an increased risk of bacterial infection, and if possible tumor growth promoting effects result in an increased risk of postoperative rumor recurrence.[113] However, no conclusive results have been presented and more research on the matter is probably warranted. As mentioned above, there also exists varied literature on the risk of NHL following blood transfusions.

7. SPECIFIC AIMS

The overall objective of this thesis was to characterize the long term health patterns of blood donors and the consequences thereof on transfusion safety.

Specifically, our aims were:
- To assemble and evaluate the quality of a binational donation and transfusion database with long-term follow-up of health outcomes for both blood donors and transfusion recipients.

- To characterize in detail the occurrence of cancer and cause-of-death of Swedish and Danish blood donors relative to the background population.

- To investigate to what extent cancer occurrence and mortality of Swedish and Danish blood donors relative to the background population has changed over time.

- To investigate whether there is any excess risk of cancer associated with the repeated donation of blood.

- To investigate whether iron loss from repeated blood donation is associated with a decreased risk of cancers of the lung, liver, esophagus, stomach and colon.

- To investigate whether there is any excess risk of cancer associated with receiving a blood transfusion originating from a blood donor who later developed cancer.

8. METHODS AND MATERIALS

8.1. DATABASE ASSEMBLY

8.1.1. National registration numbers

Since 1947, all Swedish residents are assigned a unique national registration number (NRN), which consists of a 6 digit date of birth and an additional 4 digits. Initially the NRNs were nine digits long, but in 1967 a tenth check digit was added. The NRNs are assigned immediately after birth, or at immigration. The NRNs are used extensively, both by official authorities and by health care providers, banks as well as businesses, and can consequently be used to uniquely link population and health registers.[164]

The Danish Civil Registration System (CRS) was established on April 1, 1968, at which time all persons alive and living in Denmark were registered. It was initially created by compiling and computerizing information that was previously kept separately in administrative regions. The CRS has since allocated unique 10-digit national registration numbers (civil registration number to all residents in Denmark. Similarly as in Sweden, the first 6 digits of the person-number indicate the day, month, and year of birth of the person. The next 3 digits constitute a serial number with the first indicating the century of birth of the person and the 10th digit as a check digit as well as an indicator of the sex.[165, 166]

8.1.2. Donation and transfusion register data

8.1.2.1. History

Sweden was among the first countries in the world that kept computerized records of both blood donors and recipients as well as all their donations and transfusions. Starting in 1965 the first population-based computerized transfusion registers were established in Sweden.[167-169] Initially these registers covered about 35 percent of the Swedish population, but gradually increased their coverage.[170] The registers were based on NRNs from the start and were created mainly for blood-safety and administrative purposes. Although originally not primarily intended for research, such applications were also envisioned.

Since the systems were developed and put into practice at a time when microcomputers were not yet widespread, they were very centralized both with regards to location and administration. Data about blood donors, donations, transfusions and patients was recorded in blood banks on standardized punch cards. These punch-cards were then mailed to the central computer facility on a weekly basis, whereupon any changes or new records entered locally could be updated in the central computer. Processing of data and printing of donor cards for the regular call-up of donors due for donation was also done at the central computer facility on a weekly basis. By performing these tasks for all blood centers at the same time, the total run time on the central computer was minimized, and costs were kept low. The data was stored on standard IBM half-inch magnetic tapes. The tapes that still remain today date back as far as 1966, but the computer systems were not fully implemented until 1968 when their use became widespread.

Similar computer systems were established also in Denmark during the mid-1960s, but unfortunately these utilized storage media that were reused in a cyclic manner,[171] alas, no data from the oldest Danish computer systems have survived for use in this project.

During the 1980s, with the advent of affordable microcomputers, an increasing number of Swedish hospitals established locally administered donation and transfusion registries.[172] By 1992 such systems had been implemented in most major hospitals and blood banks, and were reported to cover approximately 90 percent of the Swedish population. Further development has resulted in complete coverage of the Swedish population sometime during the mid-late 1990s.

As in Sweden, Danish blood banks and transfusion medicine clinics also started implementing local microcomputer-based donation and transfusion medicine systems during the early 1980s. However, the Danish implementation was somewhat slower to start. Approximately 90 percent coverage of the Danish population was attained only by 1997 and complete coverage in 2002, when the last blood bank introduced a computerized system.

8.1.2.2. Survey of existing systems

In order to create a database compatible with the existing material as well as with the research questions we were aiming to address, a number of questions needed to be answered: where computerized data was being recorded, what data was recorded, how it was stored, in what formats and in what volumes. To address these issues, we conducted a thorough survey among the key individuals responsible for computer systems in all transfusion medicine clinics and blood banks in Sweden and Denmark. The survey was conducted during the middle of 2003.

8.1.2.3. Core database design

The results from the survey were then used to align the different computer systems to each other and to decide on a database design. Based on the availability and formatting of data that was reported through the survey we constructed a database model containing all universally available data that was deemed to be of interest for the project. The full database model is depicted in Figure 1.

All tables in the database are linked using the personal identifier (PID), the donation identity codes (DID), or the blood component identity code (BID) as unique key variables. The core of the database consists of four tables – one for donations, one for blood components, one for transfusions, and one for persons. The *donation table* holds information on all registered donations with the PID of the blood donor, donor's hemoglobin concentration, donation date, and DID for every donation. The *component table* holds more detailed information about type of component, manufacturing date, volume, and fate (i.e., whether pooled, transfused, or discarded) of all components manufactured from the blood donations. The *component table* is linked to the *donation table* by the DID. Each blood component is uniquely identified with a BID. The *transfusion table* contains data on all transfusions with the recipient's PID and the transfusion date as well as the DID and BID of the transfused component. This table is linked to the *component table* by the BID and to the *donation table* by the DID. Hence, transfusions can be connected to all donors who contributed to the transfused component. The *person table* holds information about all individuals in the database (PID, birth date, sex, blood group and, if applicable, also dates of death and/or emigration). This table is connected to the *donation* and
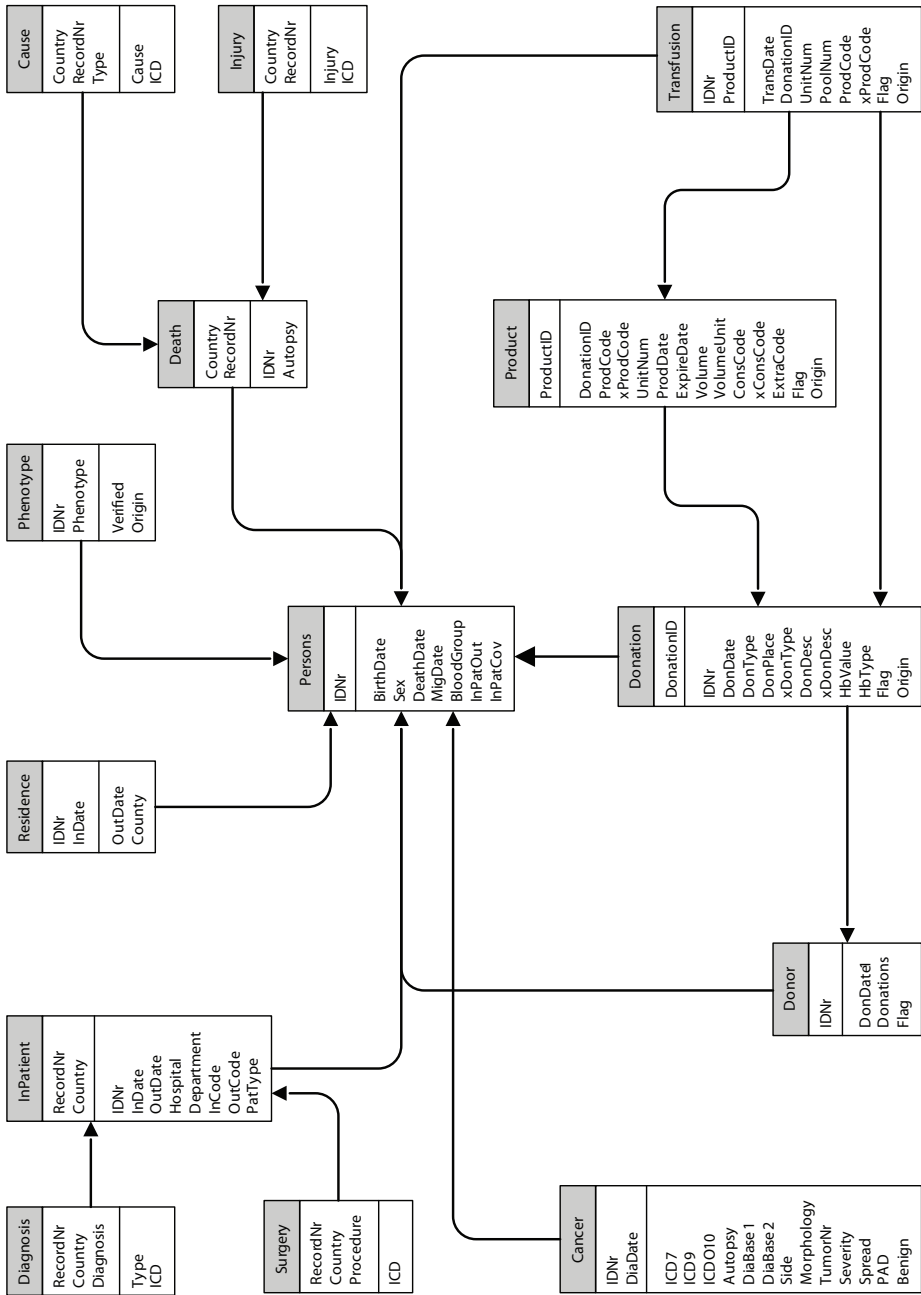
Figure 1. Full database structure.

transfusion tables by the PID. In separate tables, the database also contains information on surface antigens and erythrocyte antibodies that were measured and recorded for a large proportion of transfusion recipients.

8.1.2.4. Data collection

Using the database model, data was requested from the respective register keepers and after extraction, it was delivered by means of registered mailing of encrypted CDs. Since data was ordered to fit our database model, data processing was for the most part kept at a minimum. After a test run with one of the major Swedish contractors during the summer of 2003, data was delivered throughout the rest of the year. However, shortly after initial data management and cleaning, it was discovered that a substantial amount of data from the blood banks in Stockholm county was missing, whereby the data extractions for that county had to be remade during the spring of 2004.

The data from the oldest tape-based systems (which was still being kept in the archives of the original computer system developer, Databyrån AB) was no longer available on a modern medium. Since the blood banks no longer had the equipment that was necessary to read this data, it was read by experts at the Swedish National Archives (Riksarkivet).

Whereas the newer systems successively had adapted a modern relational database structure for data storage, where data was stored in separate interconnected tables (as in our database model), the old systems used a data storage model that was based on the different types of punch cards that had been used. In effect this meant data was stored in large files where sequential observations could contain information on a blood donation, component preparation and transfusion. Thus, extensive data processing and restructuring was necessary to achieve data that was compatible with our predefined data model. After the data cleaning had been finished in 2004, it was discovered that there was a substantial gap in the data. Data from the Uppsala and Stockholm counties that were recorded in the old tape-based systems during the mid-to-late 1980s were seemingly missing. Upon manually searching the archives of Databyrån AB, a number of additional magnetic tapes were discovered and read using the same procedures.

The assembly of the Danish portion of the database followed much the same procedures as the Swedish. The ordering of data and data processing was based on the same database model as was devised using the Swedish survey. In some instances the available data did not match the database model, whereby discrepancies and extensive data remodeling was inevitable.

The Danish database assembly was largely based on work conducted during the mid-1990s, as described in a thesis defended by Dr Henrik Hjalgrim at Copenhagen University.[173]

8.1.2.5. Data management and cleaning

The data management was performed in a number of sequential steps. As the data from the respective blood banks arrived, the crude data was first entered into an Oracle database. Thereafter the data from each individual blood bank was carefully scrutinized, cleaned and, where necessary, transformed to fit the predefined database model. Since data originating from blood banks utilizing the same computer systems was associated

with much the same problems, the data cleaning and reformatting processes were to a large extent automated.

The data from the oldest systems, which were based on punch-cards and magnetic data tapes, naturally incurred the most manual work. Firstly, in some instances, some of the data tapes had been stored in a basement for more than three decades. Therefore we encountered some difficulty when the tapes were read, but overall very few tapes proved unreadable. Since the original data storage model was based on the types of punch cards that had been used for each entry of a piece of information, the transformation required to fit our relational database structure was extensive. A number of different punch card types had been used, each type containing different pieces of information. In order to obtain all the information prescribed in our database model, several cards (observations) had to be identified and read to avoid missing data. In addition to difficulties reading and converting the tapes, we also encountered a number of problems with date variables when we interpreted the data on the tapes.

At the time when the systems were developed and implemented, data storage often came at a very high cost. In order to reduce storage space and thus cost, programmers often developed very creative strategies to limit the number of bytes used for each variable. This was especially common for date variables which instead of being recorded with four positions for year, two for month and two for day (i.e. YYYYMMDD), were commonly recorded with only one position for year, two for week number and one for day (i.e. YWWD), thus reducing the total number of storage positions by half. In some instances, such as when referring to component manufacturing and expiry dates, the year was omitted and thus using only three positions (i.e. WWD). Although probably necessary at a time when storage space was scarce and very expensive, these formats have been quite difficult to decipher and their interpretation has therefore been somewhat prone to error. Dates recorded without a position for decade were decoded using the year the tape was written, whereas dates written without any mention of neither decade nor year were decoded using both on the decade the tape was written and the year the rest of the dates recorded in that observation. Unfortunately the format with which the tapes were written (EBCDIC; a proprietary IBM format) cannot be converted to the more common ASCII format without loss of some information. Therefore, in order to permit manipulation and storage of those variables that had been written using binary bit-coding (such as was the case for the variable holding the card type identifier), we had to impute the value of these variables using other information contained in each observation.

After all the data had been converted to fit the database model and entered into the database, several data cleaning steps were performed. Firstly, all key identifiers (NRN, DID and BID) were checked for validity, and observations that were incorrect were flagged accordingly. Thereafter we removed all perfect duplicates (i.e. multiple observations that contained the exact same information) and identified all non-perfect duplicates (i.e. observations where the DID and/or BID had been reused). All such observations were kept in the database but were flagged for removal where appropriate. The data cleaning steps were first run on data from all blood banks individually, then on individual blood bank data that had been aggregated by type of system, and finally on all the data when

it had been combined. Since in some instances neighboring blood banks were running computer systems that had been shared and later separated, the number of perfect duplicates identified in the second and third layers of data processing was substantial.

8.1.3. Record linkages

After the data collection and cleaning had been finished, we extracted all available NRNs from the two databases and sent them to the respective population and health data register holders for register data extraction. Thereafter, before addition of the population and health register data, both databases were de-identified by exchanging the original NRNs for unique, yet non-identifiable, random numbers (PID). Record linkages were performed with both the Danish and Swedish population, cause of death, cancer, hospital, and medical birth registers.

8.1.4. Cause of death registers

8.1.4.1. Sweden

The Swedish National Cause-of-Death Registry is held and maintained by the Swedish National Board of Health and Welfare and is updated on an annual basis. Since 1961 It records data on the deaths of all individuals who have died while being residents of Sweden. In addition, it also contains data of somewhat lesser quality and completeness, for all individuals who died between 1952 and 1960.

All recorded deaths are identified by the NRN of the deceased, the date of death and the underlying cause of death. In addition, it also has the possibility to record a number of contributory causes of death which has ranged from 6 before 1986 to the current 20. The validity of the Swedish Cause of Death Registry has been evaluated a number of times.[174-176] Generally, the quality has been found to be quite satisfactory.

8.1.4.2. Denmark

Since 1943, completion of death certificates including information on cause of death has been mandatory for all persons dying in Denmark. Upon completion, death certificates are sent to the National Board of Health, where the cause of death is recorded using the ICD. The part of the register used in our database is coded according to ICD-8 (1970-1993) and ICD-10 (1994-2002). Since 1968, deaths have been registered using the CRS number of the deceased individual.[177]

Since completion of death certificates is mandatory by law, underreporting is very uncommon, and accordingly the Danish Cause of Death Registry is considered to be of high validity. The register has also been evaluated a number of times with excellent results.[178]

8.1.5. Cancer registers

8.1.5.1. Sweden

The Swedish Cancer Registry has been in nationwide operation since 1958. Notification of all diagnosed malignant conditions to one of six regional cancer register is mandatory by law for all physicians. The register is compiled annually by the Swedish National Board of Health and Welfare by merging data from six regional cancer registers. All cancers in the registers are recorded according to the current revision of the ICD, together with a

translation into a Swedish adaptation of ICD-7 for complete historical compatibility. The register holds special variables to indicate benign conditions, the basis for the diagnosis, as well as an indication of whether the malignancy was discovered during autopsy, and if so, if it was an expected or unexpected finding. With respect to different histological cancer types, a special histological coding, based on the World Health Organization C24 system,[179] has been in use since 1958.

The proportion of tumors that have histological confirmation has generally been high, and has improved with time.[180, 181] The Swedish Cancer Registry covers the entire Swedish population. Compliance with the reporting requirements is excellent, and the register has been found to have a high level of completeness.[182] Since the NRNs are thoroughly checked, the proportion with incorrect NRNs is negligible.

8.1.5.2. Denmark

The Danish Cancer Registry was established in 1943, and has since registered essentially all cancers diagnosed in Denmark. Until 1987 reporting of cancer was voluntary, but has since been mandatory by law. It therefore records virtually all malignancies diagnosed in Denmark. Until 1977 all cases of cancer were coded according to a locally modified and extended version of ICD-7, but since 1978 cancers have been coded according to the ICD-O maintaining automated translation into ICD-7 codes. The fact that reporting to the register is mandatory by law in combination with the government-provided healthcare for the entire population, ensures a high level of completeness. The Danish Cancer Registry is considered to be of high standard with close to 100 percent coverage for cancers at most anatomical sites.[183] In recent years a very high proportion of cases of cancer recorded in the Danish Cancer Registry have been verified histologically.

8.1.6. Hospital registers

8.1.6.1. Sweden

In 1964-1965, the Swedish National Board of Health and Welfare started collecting data on individual hospital discharges in the Swedish Inpatient Registry. At discharge from hospitals, a special form is completed for each patient. These forms are computerized locally and delivered annually to the Swedish National Board of Health and Welfare for compilation into a national register. Each record represents one in-hospital episode.

In addition to the NRN of the hospitalized individual, dates of admission and discharge, as well as hospital and department codes, it records a number of discharge diagnoses and surgical procedures (coded according to the Swedish Classification of Operations and Major Procedures) and anesthesiological procedures. The discharge diagnoses are coded according to ICD-7 through 1968, according to the ICD-8 until 1986, ICD-9 until 1996, and ICD-10 thereafter. The number of hospitals delivering data to the register has increased steadily over time: the register covered approximately 60 percent of the Swedish population in 1969, 75 percent in 1978, 85 percent by the end of 1983, and 100 percent from 1987 and thereafter.

The proportion of patients recorded in the register with erroneous NRNs has varied with time, county, and diagnosis. This proportion was highest during the 1970s, but has dropped substantially during recent years. In a study performed by the register holder,

the overall proportion of incorrect or missing NRNs was 7 percent in 1977, but dropped to less than 2 percent in 1983. The degree of underreporting to the register has also been addressed. In one detailed review of 900 case records reveled that the number of false negative cases of trauma, ischemic heart disease, and malignant tumors ranged from 3-5 percent. Underreporting of surgical procedures was noted in 8 percent of the cases. The proportion of false positive cases was found to be very low.[184]

8.1.6.2. Denmark

The Danish National Hospital Discharge Registry was established by the Danish National Board of Health on January 1, 1977. Since then, information on all somatic hospitalizations has been recorded on an individual level (using patient NRNs), for each hospital admission encompassing data on hospital, department, dates of admission and discharge together with as many as 20 diagnoses per hospitalization and up to six surgical procedures per diagnosis. From 1994 there were no limits on the number of diagnoses and procedures. Since 1995, the register also records data from outpatient clinics.

Since very few hospital departments do not participate in the registration, the coverage of the register is estimated to be almost 100 percent. Discharge diagnoses are classified according to ICD-8 until, and including, 1993 and since then according to ICD-10, while surgical procedures were coded according to the classification of Surgical Procedures and Therapies.

The quality of the Danish Inpatient Registry has also been assessed in a number of studies.[46, 185-188] The level of completeness and correctness has been found to vary both between groups of diseases and over time, but overall the quality of the register has been found to be satisfactory.

8.1.7. Swedish-Danish database assembly

Upon addition of the data from the record linkages, Swedish and Danish databases were aligned and merged to create one homogenous Swedish-Danish database. While trying to retain the original structure of the health data registers, some changes were necessary to make the Swedish and Danish databases fully compatible and suitable for the intended research purposes.

We identified all core variables where a corresponding variable existed from both countries. Based on these variables, and the structure of the respective databases, a common database structure was agreed upon, and the databases were merged.

The major changes that were necessary pertained mainly to the cause-of-death and hospital registers, where the Danish registers had a more modern, highly normalized structure. To accommodate this, the Swedish cause-of-death and hospital registries were normalized according to the Danish database design. This restructuring was performed without compromise of the original content or validity, but enabled the combined bi-national database to be queried more efficiently. In addition, the coding of a number of variables, both in the donation-transfusion data as well as in the data provided by the additional record linkages, were recoded uniformly to make the Swedish and Danish data compatible.

8.2.1. Study I – Assembly of binational donation and transfusion database

The overall aim of Study I was to describe the assembly and quality evaluation of a historical binational donation and transfusion database with long-term follow-up of health outcomes for both blood donors and transfusion recipients. The assembly of the Scandinavian Donations and Transfusions (SCANDAT) database is described in detail in section 8.1 above.

Since third-party historical data about blood donations is virtually nonexistent, and since transfusions that have been administered are notoriously poorly recorded on patient charts and in other medical records,[72] we were not able to conduct direct evaluation of the donation and transfusion registration. Rather, we had to resort to a number of indirect measures of data quality. Firstly, comparisons were made with official statistics of the annual number of donations and transfusions per administrative region, that are published by the Swedish National Board of Health and Welfare,[82] and the Danish Medicines Agency.[189] Secondly, we also made internal consistency checks within the database, such as the proportion of donors and patients with correct NRNs, the proportions of donated units which ended up being transfused and the proportion of transfusions for which it was possible to trace the originating blood donor. Finally, as a further insight into the quality of the transfusion registration, we also made comparisons with the respective hospital registers, i.e. assessed whether a transfused individual was admitted to an inpatient clinic at the time of the transfusion.

In addition to the quality analyses, we also performed a series of analyses of the divergence and convergence of blood products. We both calculated the number of recipients that were exposed to blood products emanating from a single donor and the number of donors whose blood was transfused to a single recipient.

8.2.2. Study II – Health profile of blood donors

The aims of Study II were firstly to characterize in detail the mortality and cancer incidence among Swedish and Danish blood donors relative to the background population, and secondly to investigate to what extent cancer occurrence and mortality of Swedish and Danish blood donors have changed over time.

In order to do this, we established a cohort of all individuals with a valid NRN who were recorded to have performed at least one whole blood, plasma or platelet donation between 1968 and 2002. All blood donors were followed from their first recorded whole-blood, plasma or platelet donation until death, emigration or end of follow-up, whichever occurred first. For cause-specific mortality analyses, end of follow-up was 31st of December, 2002 in Sweden and 31st of December, 2000 in Denmark. The shorter follow-up time was necessary as the Danish Cause-of-Death register had not been updated further at the time when the record linkages were performed. For cancer incidence analyses, follow-up ended on December 31, 2002 in both countries. All cancers were considered in the analyses.

The respective cause-of-death registers provided the cause-of-death, according to revisions 7 through 10 of the ICD. These were grouped according to the seventeen chapters

of ICD-7 through 9. Since ICD-10 is divided into nineteen major chapters, chapters VI (Diseases of the nervous system), VII (Diseases of the eye and adnexa) and VIII (Diseases of the ear and mastoid process) of ICD-10 were combined to be compatible with chapter VI (Diseases of the central nervous system and sensory organs) of the previous revisions. Similarly, from the respective countries' cancer registers, we extracted the date of diagnosis and anatomical classification, according to the 7th revision of the ICD, of any recorded malignancies for these individuals. The diagnoses from the cancer registers were reclassified into 46 groups of malignancies.

8.2.3.   Study III – Health effects of blood donation

The aims of Study III were firstly to investigate whether there is any excess risk of cancer associated with donation frequency or intensity, and secondly to investigate whether iron loss due to repeated blood donation is associated with a decreased risk of cancers of the lung, liver, esophagus, stomach and colon. In contrast to Study II, above, the aim was not to investigate the health effects of being a blood donor, but rather to perform dose-response comparisons within the donor cohort.

Within the cohort used for Study II, described in section 8.2.2 above, we conducted a matched nested case-control study. We defined as cases all blood donors who were diagnosed with a first primary cancer (excluding non-melanoma skin cancer) between their first recorded blood donation and end of follow-up (i.e. date of death, emigration or 31st of December 2002, whichever occurred first). Since the introduction of the computerized blood donation registration was gradual, register coverage was not complete until towards the end of the study period. A sizeable number of donors will therefore have been blood donors prior to computerization and as such may have performed more donations than those we have recorded. To avoid this exposure misclassification, we used residence data from the Swedish and Danish registers of internal migration and the start up dates of the blood donation registers to compute the date from which each donor could be considered entirely "in view" of the computerized system. The cases were required to have at least seven years of register coverage and, to allow meaningful comparisons, to have performed at least one donation in the previous 12 years. For each eligible cancer case we then randomly selected, from the same cohort of blood donors, ten controls who at the time of diagnosis of the index case were cancer free and living in the same county. The controls were also required to have at least seven years of register coverage and to have donated in the previous 12 years. The controls were selected using incidence density sampling and were matched to be of the same sex and age (±180 days) as the cases, as well as to be living in the same county. For all matched sets of cases and controls we counted the number of donations they had performed prior to diagnosis of the case. Using hemoglobin concentration measurements that are recorded for all blood donations, and information on donation type (i.e. whole-blood, plasma and platelets) and the corresponding volume of whole blood-loss (450 ml for whole blood, 50 ml for plasma and platelets), we also estimated the iron loss for each donor.

Since donors with an incipient malignancy can be assumed to donate less often we wanted to identify the time point from which the cases' donation activity first started to decline. We divided data from the five years immediately before diagnosis into six-month

segments. For each of these segments, we calculated a relative donation intensity as the ratio of the sums of the cases' observed to expected donation frequencies had they followed the same average frequency as one randomly selected control from their matched set. Confidence intervals were calculated for these relative donation intensities under the assumption that the number of donations followed a Poisson distribution. Based on these calculations, which revealed decreasing donation intensity among cases starting as early as two years before the index date (Figure 2), we decided to exclude from the analyses the two years immediately before diagnosis.

To account for possibly varying induction periods, the information about number of donations and iron loss was divided into three windows: 3-12 years, 3-7 and 8-12 years before the diagnosis of the case.

8.2.4. Study IV – Risk of transfusion transmitted cancer

The aim of Study IV was to investigate the possibility of transfusion transmitted cancer. Hence, we wanted to compare the incidence of cancer among transfusion recipients who had been transfused with at least one unit of blood from a blood donor who later developed cancer, to the incidence of cancer among transfusion recipients who had not received such an exposed blood unit.

We carried out a standard cohort study of all individuals who were recorded to have received at least one unit of whole-blood, erythrocytes, plasma or platelets between 1968 and 2002. All recipients with a known history of malignant disease were excluded. For all these individuals we considered all transfusions that had been administered during the first 30 days following the first recorded blood transfusion and identified the contributing blood donors. Using the respective cancer registers we then matched all malignancies that were recorded for the contributing donors to their respective transfusion recipients. All blood units coming from a donor who had been diagnosed with a malignancy (excluding
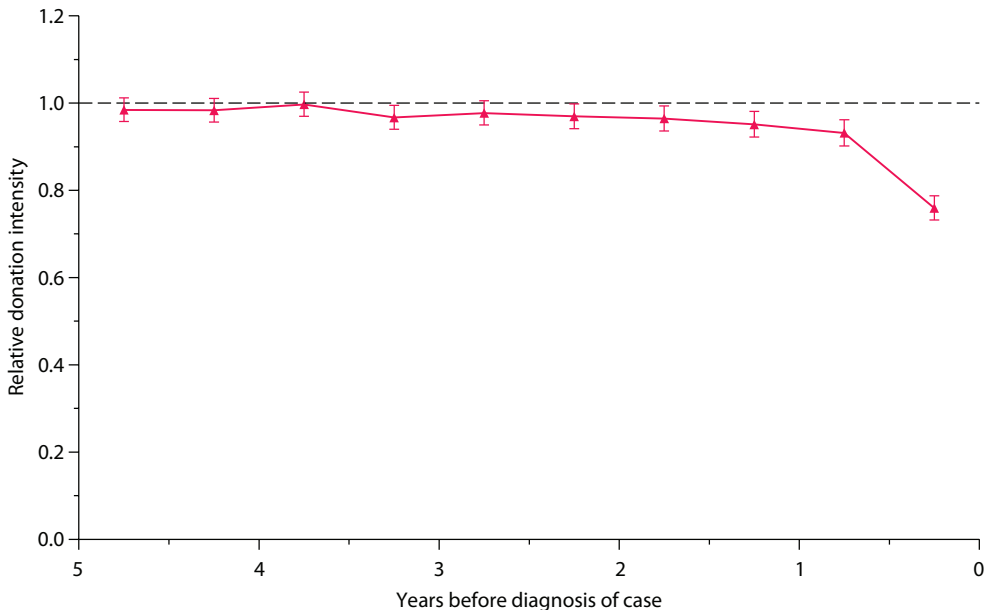

Figure 2. Donation intensity among cases relative to controls by time to diagnosis.

non-melanoma skin cancer) within five years after the donation was performed were classified as precancerous blood units and all units that were decidedly not from a donor who developed cancer within five years were classified as non-precancerous. In order to correctly classify donors as either precancerous or non-precancerous, we therefore also excluded recipients of blood from unknown donors or donors for whom we did not have at least five years of follow-up.

In the overall analyses we also excluded all recipients of blood from donors with a prior cancer diagnosis, and to be able to adjust for all important potential confounders, also recipients for whom the area of residence at the time of first transfusion could not be ascertained. Since unspecified anemia, a frequent complication of impending malignant disease,[190-192] is one of the most common indications for blood transfusion,[193] reverse causation (see section 10.4.1.1 below) is an important consideration in this setting. Therefore, start of follow-up was delayed until 180 days after the first recorded transfusion. The recipients were followed until date of first malignant cancer diagnosis (again excluding non-melanoma skin cancer), death, emigration, end of follow-up (31st of December 2002), or exposure to a precancerous blood unit, whichever came first. We also censored recipients who later received blood from an unknown donor, or from a donor for whom we did not have sufficient follow-up.

Since we had little support in the literature for our perceived definition of exposure, i.e. cancer in the donor within 5 years, we also ran a series of sensitivity analyses where we progressively shortened the exposure period from 5 to 1 year, thus also relaxing the exclusion criteria involving follow-up in all contributing donors.

8.3. STATISTICAL ANALYSES

In all four studies all data processing and statistical analyses were performed using statistical analysis software (SAS) version 8 or higher (SAS Institute, Cary, NC, USA).

8.3.1. Study I – Analyses of connectivity

We first explored the distribution of number of recipients of blood components originating from a single blood donor (i.e. the number of recipients per donor), which is an important aspect of the potential for disease spread from donors with subclinical disease. All blood donors recorded to have performed at least one whole-blood, plasma or platelet donation were eligible, but donors who had performed autologous or non-standard donation types, such as peripheral stem cell or granulocyte donations were excluded. To quantify the potential for diseases of different latencies to spread, we counted the number of recipients per donor, using two, five, and ten years of follow-up from the first recorded donation. Since the SCANDAT database covers donations and transfusions through 2002, the entries into the studied donor cohorts were stopped on December 31st, 1992, 1997, and 2000 for the 10-, 5-, and 2-year follow-up analyses, respectively. The analyses were also stratified by age and calendar period of first donation.

We also investigated the convergence of blood components, i.e. the number of donors whose blood each recipient was exposed to. We identified all patients who had received at least one blood unit and grouped all transfusions into transfusion episodes, which were defined as a period of transfusions that were separated from previous or

subsequent transfusions by intervals of at least seven days with no transfusions. For each transfusion episode we then counted the number of donors who contributed and, from the respective nations' inpatient registers, identified the type of ward the patient had first been admitted to during the episode. The analyses were conducted both overall and stratified by sex, age, calendar period of first transfusion in the episode and type of ward (Internal medicine, Surgery, Orthopedic surgery, Thoracic surgery, Gynecology/Obstetrics, Multiple types and Other).

8.3.2.   Study II – External comparison of mortality and cancer incidence

The relative risks of death and cancer among blood donors compared to the general population were expressed as standardized mortality ratios (SMR) and standardized incidence ratios (SIR), respectively. These ratios were calculated by dividing the observed number of deaths or cancers that occurred among the blood donors by the number expected if the donor cohort had experienced the same mortality and cancer incidence rates as the background population. The expected number of deaths or cancers were calculated by multiplying the corresponding follow-up time in the donor cohort by the incidence and mortality rates specific to each stratum defined by country, 5-year calendar period, 5-year age group, and sex in the general population, and summing these products. Ninety-nine percent confidence intervals were calculated for the SMRs and SIRs using a Poisson distribution approximation.[194] We chose a more stringent alpha level for the confidence interval calculations to, at least partly, account for the number of statistical tests performed.

To examine the relationship between when the blood donor was recruited and the mortality and cancer incidence relative to the background population, we plotted the SMRs and SIRs by year of first recorded donation. Since such analyses could possibly be biased by an interaction between age and calendar period, we also conducted analyses of relative mortality and cancer incidence by calendar period of first donation, stratified by age at first donation and attained age.

8.3.3.   Study III – Conditional logistic regression

We analyzed the association between the number of donations and cancer risk using conditional logistic regression with the number of donations performed in each exposure window categorized using the median, upper quartile and 90th percentile as cut-off points, to allow for non-linear relationships, and as a discrete numerical variable, to test for linear trends. After examination of all-site cancer risk, further analyses were performed for three broad groups of malignancies: solid tumors, non-Hodgkin lymphomas (including chronic lymphocytic leukemia), and other hematological or lymphatic malignancies.

We then considered whole blood and plasma donations separately, again both as categorical and discrete numerical variables. Both the number of whole blood and the number of plasma donations were categorized using the categorization scheme described above, but for plasma donations the middle two categories were collapsed. Since type of donation was available to us with less precision (or not at all) in the Danish data,

the analyses where we considered whole blood and plasma donations separately were restricted to the Swedish data.

We also conducted analyses investigating the association between iron loss and risk of selected solid tumors (lung, liver, colon, stomach and esophageal cancers) for which high iron levels have previously been implicated.[50, 51, 54-56] Iron loss was categorized, again roughly according to the median, upper quartile and 90th percentile as cut-off points.

To assess the potential for confounding by lifestyle factors, we used the Swedish and Danish inpatient registers to investigate, among the controls only, the incidence of hospitalization for alcoholism, alcoholic hepatitis and chronic obstructive pulmonary disease by donation frequency. These diseases are associated with tobacco and alcohol use. The control donors were followed from the index date until first occurrence of either of these diseases, death, emigration or end of follow-up. We also assessed the prevalence of smoking in relation to number of donations in the period from 3-12 years before the index donation among all female donors who had been pregnant since 1983, for whom smoking habits had been recorded in the Swedish medical birth register.

8.3.4.   Study IV – Poisson regression

We calculated the incidence rate ratios (IRR) of cancer in the exposed group relative to the unexposed group using multivariate log-linear Poisson regression. The analyses were adjusted for sex, attained age, area of residence, ABO-blood type, number of transfusions during the first 30 days, calendar period and time since first transfusion. All variables were analyzed as categorical variables. Also, attained age, calendar period and time since first transfusion were treated as time-dependent covariates, allowing individuals to move between categories with time.

Since immune responses and thus the possible susceptibility to engraftment of foreign cells may vary by sex and age, and since the prerequisites for transmission (such as the use of filtered blood and different types of blood components) have changed considerably during the study period, we conducted sub-analyses stratified by recipient sex and age, as well as calendar period of transfusion, number of transfusions and component type. Further, to test whether the risk of cancer in the recipient in any way depended on what type of cancer the precancerous donor later developed, we also conducted analyses with multiple exposure categories corresponding to 15 broad groups of anatomical cancer sites. Correspondingly, we compared site-specific cancer risks among exposed and unexposed transfusion recipients.

Provoked by a study describing transfusion transmission of HHV-8 that was published while our work was in progress for Study IV, we also specifically investigated whether possible HHV-8 transmission would result in Kaposi's sarcoma concordance between blood donors and recipients.

8.4.   ETHICAL CONSIDERATIONS

The conduct of all studies upon which this thesis is based and the creation of the SCANDAT database were approved by all regional ethics committees in Sweden (reference number 02-416), the Danish Scientific Ethics Committee and the Danish Data Protection Agency.

9. RESULTS

9.1. STUDY I

9.1.1. Data cleaning process

In total the material we received from the donation and transfusion register holders contained 7,793,132 unique NRNs, 18,776,851 donation records and 14,657,489 transfusion records. We removed 925,670 (4.9%) perfect duplicate donation records and flagged, but kept in the database, 805,567 records from after December 31st, 2002 which could not be checked through record linkages.

Out of the remaining 17,045,614 donation records, 274,955 (1.5%) were marked as invalid because they contained an erroneous NRN, an invalid date or an NRN which did not match a valid person upon record linkage. Out of the remaining 16,771,792 valid records, a further 1,679,379 (10.0%) did not correspond to valid donations or had an unknown or missing donation type.

Of the 14,657,489 transfusion records that we received initially we removed 1,328,006 (9.1%) records which were perfect duplicates and flagged 740,070 (5.0%) records from after December 31st, 2002. Of the remaining 12,589,413 (7.1%) transfusion records 895,569 were flagged because of obviously erroneous NRNs, erroneous dates or NRNs which did not correspond to a valid person.

Finally, out of the original 7,793,132 NRNs in the *persons table*, 285,152 (3.7%) could immediately be flagged because of illegal values or characters. Record linkages further revealed that another 72,322 (0.9%) NRNs did not correspond to a valid person.

9.1.2. Database contents and quality

The SCANDAT database ultimately contained 15,091,280 valid donations, 11,693,844 valid transfusions and 7,435,658 individuals with valid NRNs. These individuals included
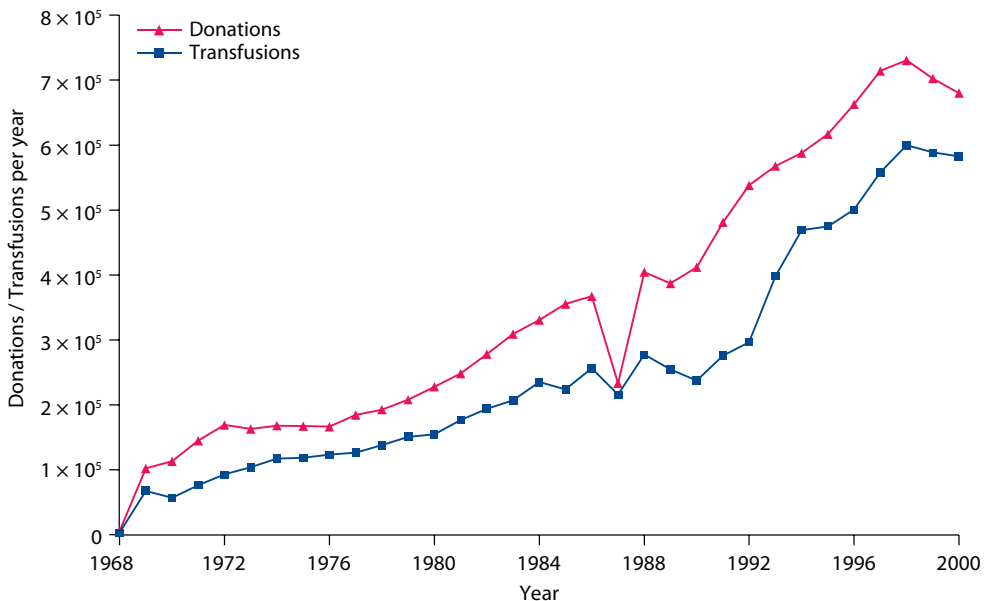
Figure 3. Number of whole blood donations and transfusions of any type per year in Sweden.

1,134,290 donors, 1,311,079 recipients and 5,045,707 other individuals who either registered for blood donation without actually performing a proper donation, or were blood typed while pregnant or prior to surgery without receiving any transfusion. Figure 3 shows the number of whole blood donations and transfusions of any type in Sweden per year and Figure 4 in Denmark per year.

Disregarding autologous donors, in all, 55,418 individuals had records as both a donor and as a recipient. Of these, 10,630 (19%) were recorded to have received at least one transfusion before becoming a blood donor. For a total of 11,060,023 (87.5%) transfusion records, we were able to find a corresponding hospitalization in either of the inpatient registers.
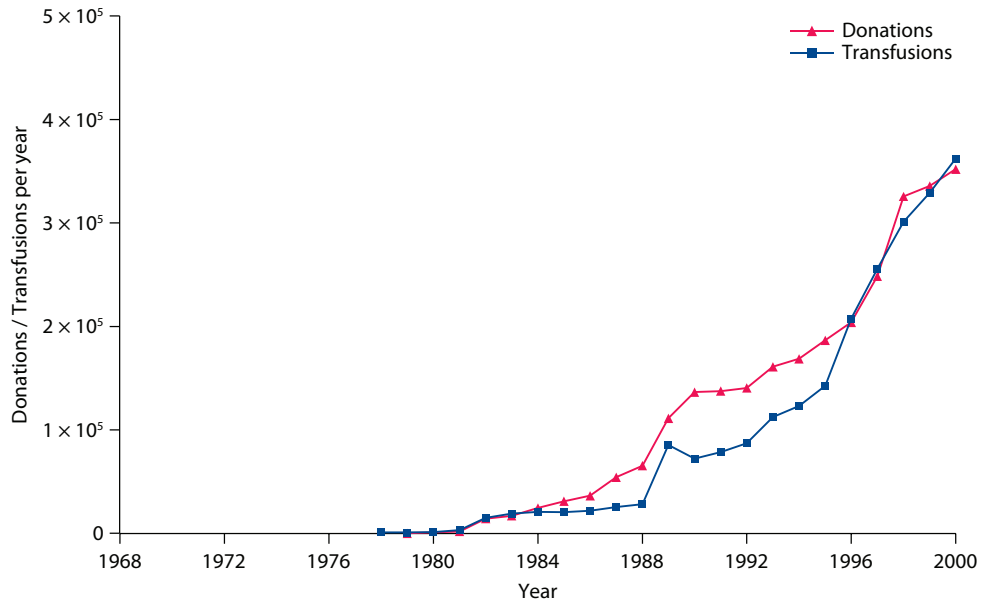
Figure 4. Number of whole blood donations and transfusions of any type per year in Denmark.

9.1.3.   Connectivity analyses

Results from the analysis of number of recipients per donor are presented in Table 3. With a 2-year follow-up from the first recorded blood donation, the average number of recipients per donor was 4.5. With a 5-year follow-up it was 8.3 and with 10 years, the average was 12.3. As demonstrated in Figure 5, the divergence of blood products emanating from a single donor remained relatively stable across calendar time.

Similarly, results from the analyses of the divergence of blood products are presented in Table 4. We identified a total of 2,557,307 transfusion episodes with an average of 5.2 donors contributing to each of them. Swedish recipients were exposed to blood from

more donors than Danish donors (5.3 versus 4.9), and male recipients were exposed to blood components from more donors than were female recipients (6.0 versus 4.5). This measure of blood product convergence also varied considerably with calendar year and type of ward.

Stratum	Length of Follow-up								
	2 years			5 years			10 years		
	Mean, median (interquartile range)								
Overall	4.5	4	(2-6)	8.3	7.0	(3-12)	12.3	8.0	(3-18)
Country									
Denmark	4.2	4	(2-6)	8.1	7.0	(4-11)	12.0	10.0	(5-17)
Sweden	4.6	4	(2-6)	8.4	6.0	(2-12)	12.3	8.0	(2-19)
Sex									
Female	4.1	4	(2-6)	7.2	6.0	(3-10)	10.4	7.0	(3-15)
Male	4.8	4	(2-7)	9.1	7.0	(3-13)	13.4	9.0	(3-20)

Table 3. Average number of recipients exposed to blood from a single blood donor, stratified by sex of donor, country and length of follow-up.

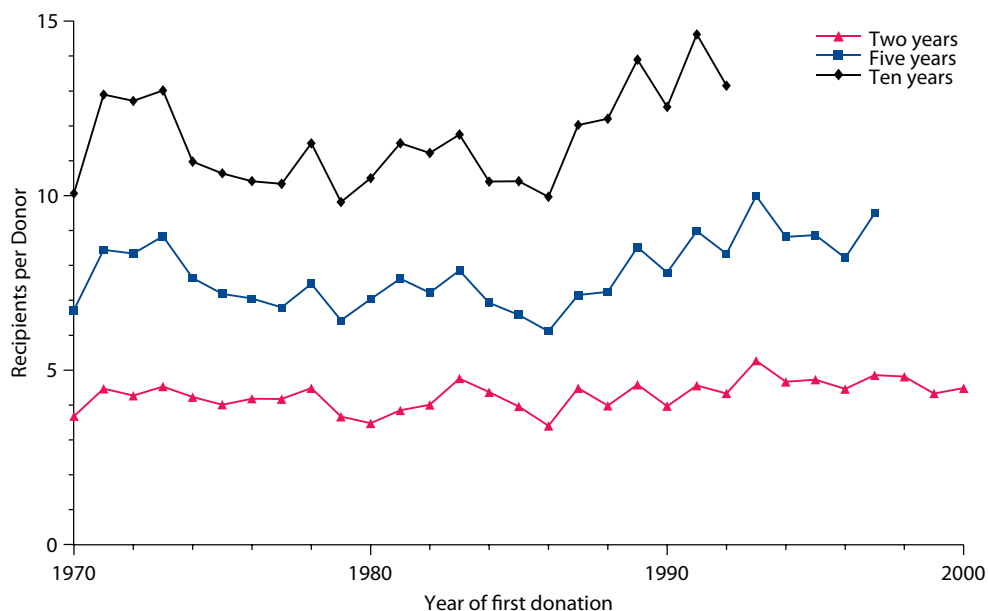**Figure 5.** Average number of recipients exposed to blood components from a single blood donor followed for 2, 5 or 10 years after first donation, stratified by year of first donation.

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Stratum	Number of episodes	Number of contributing donors		
		Mean, median (interquartile range)		
Overall	2,557,307	5.2	3	(2-5)
Country				
Denmark	693,655	4.9	3	(2-5)
Sweden	1,863,652	5.3	3	(2-5)
Sex				
Female	1,373,059	4.5	3	(2-4)
Male	1,184,248	6.0	3	(2-5)
Type of ward				
Internal medicine	777,632	5.3	3	(2-5)
Surgery	673,627	5.6	3	(2-5)
Orthopedic surgery	318,764	3.9	3	(2-4)
Thoracic surgery	91,454	8.0	4	(2-9)
Gynecology/obstetrics	115,273	3.5	2	(2-4)
Other ward	15,723	3.8	3	(2-4)
Unknown ward	363,741	3.5	3	(2-3)
Multiple different wards	201,093	8.5	4	(3-8)

Table 4. Number of contributing blood donors by recipient episode stratified by sex of recipient, country and type of ward.

9.2. STUDY II

9.2.1. Study population

We extracted from the database a total of 1,110,329 donors who were recorded to have performed 14,796,856 whole-blood, plasma and platelet donations (disregarding 294,424 donations of other type and 23,961 donors who had only performed such donations). Detailed characteristics of the donor cohort are presented in Table 5. The donors were

	Sweden	Denmark	Total
Number of subjects	777,386	332,943	1,110,329
Sex			
Female	329,290	160,003	489,293
Male	448,096	172,940	621,036
Age at entry into cohort (years), N (%)			
< 30	381,523 (49.1)	98,151 (29.5)	479,674 (43.2)
30-39	196,961 (25.3)	91,025 (27.3)	287,986 (25.9)
40-49	132,642 (17.1)	82,243 (24.7)	214,885 (19.4)
50-59	58,626 (7.5)	50,258 (15.1)	108,884 (9.8)
≥60	7,634 (1.0)	11,266 (3.4)	18,900 (1.7)
Mean age at entry into cohort, years (SD)	31.9 (10.7)	37.2 (11.6)	33.5 (11.2)
Median length of follow-up, years (range)	12.0 (0-35)	6.4 (0-22)	10.3 (0-35)
Total length of follow-up, years	10,396,083	2,646,154	13,042,237
Average number of donations (SD)	14.9 (23.2)	9.7 (8.9)	13.3 (20.1)

Table 5. Characteristics of donor population in Study II.
Note: SD denotes standard deviation

on average 33.5 years (standard deviation [SD], 11.2) upon entry into the cohort and the median follow-up time was 10.3 years. The mean age of active blood donors increased from 37 years in 1985 to 42 years in 2000, while concurrently, the proportion of male donors decreased from 65 to 57 percent. The cohort was followed for a total of 13,042,237 person years.

9.2.2. Mortality analyses

During follow-up, we observed 32,640 deaths which compared to 46,586 deaths expected produced an SMR of 0.70 (99% confidence interval [CI], 0.69-0.71). The SMR was marginally lower in female donors than in male (0.67 vs. 0.71). As is presented in Table 6, the SMRs varied greatly between the different chapters of the ICD. Donors were at a considerably reduced risk of dying from a number of classes of diseases, most notably endocrine and nutritional diseases (SMR, 0.37; 99% CI, 0.31-0.44) and infectious diseases (SMR, 0.44; 99% CI, 0.36-0.53). The risk of dying from diseases of the circulatory system (SMR, 0.68; 99% CI 0.67-0.70) or diseases of the respiratory system (SMR, 0.54; 99% CI 0.50-0.58) was also reduced.

Cause of death (ICD-chapter)	Both sexes	Women	Men
	Standardized mortality ratio (95% confidence interval)		
All causes†	0.70 (0.69-0.71)	0.67 (0.65-0.69)	0.71 (0.70-0.72)
Infectious diseases (I)	0.44 (0.36-0.53)	0.42 (0.27-0.63)	0.45 (0.36-0.55)
Neoplasms (II)	0.80 (0.78-0.82)	0.78 (0.75-0.82)	0.80 (0.78-0.83)
Endocrine and nutritional diseases (III)	0.37 (0.31-0.44)	0.27 (0.17-0.41)	0.40 (0.33-0.47)
Diseases of the blood and blood-forming organs (IV)	0.38 (0.32-0.46)	0.27 (0.17-0.40)	0.42 (0.35-0.51)
Mental and behavioural disorders (V)	0.65 (0.60-0.71)	0.61 (0.48-0.76)	0.66 (0.60-0.73)
Diseases of the central nervous system and sensory organs (VI)	0.60 (0.54-0.67)	0.50 (0.40-0.62)	0.64 (0.57-0.73)
Diseases of the circulatory system (VII)	0.68 (0.67-0.70)	0.61 (0.57-0.65)	0.70 (0.68-0.72)
Diseases of the respiratory system (VIII)	0.54 (0.50-0.58)	0.49 (0.41-0.57)	0.55 (0.51-0.60)
Diseases of the digestive system (IX)	0.57 (0.52-0.61)	0.44 (0.36-0.53)	0.60 (0.55-0.65)
Diseases of the genitourinary system (X)	0.47 (0.38-0.58)	0.42 (0.26-0.64)	0.49 (0.39-0.61)
Diseases of the pregnancy and childbirth (XI)	0.74 (0.21-1.81)	0.74 (0.21-1.81)	–
Diseases of the skin and subcutaneous tissue (XII)	0.36 (0.12-0.80)	0.14 (0.00-1.01)	0.45 (0.15-1.05)
Diseases of the musculoskeletal system and connective tissue (XIII)	0.39 (0.28-0.52)	0.33 (0.19-0.53)	0.44 (0.28-0.64)
Other diseases (XVI)	0.51 (0.45-0.57)	0.52 (0.40-0.67)	0.50 (0.44-0.57)
External causes of mortality (XVII)	0.79 (0.76-0.82)	0.78 (0.72-0.85)	0.79 (0.76-0.82)

Table 6. All-cause and cause-specific standardized mortality ratios among Scandinavian blood donors, presented overall and stratified by sex.
Note: ICD denotes international classification of disease.

As a validation of the success of the progressively more stringent selection for blood donation, analyses stratifying by year of first recorded donation showed a trend towards lower mortality relative to the background population among blood donors who started donating more recently (Figure 6). The downward trend remained even
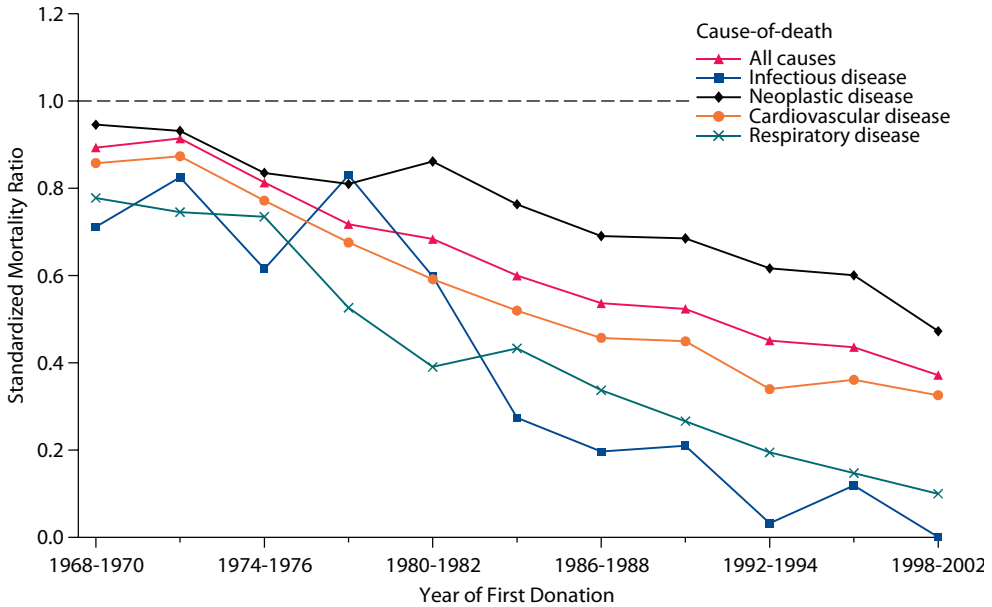
Figure 6. Overall and cause-specific standardized mortality ratio, by year of first recorded donation.

when stratified by calendar period of first donation, age at first donation and attained age (data not shown).

9.2.3. Cancer analyses

We observed a total of 38,169 cancers during follow-up, compared to 39,650 cancers expected (SIR, 0.96; 99% CI 0.95-0.98). There was little variation with calendar period, sex or attained age. Table 7 presents the relative risk of cancer overall and in 46 groups. The site-specific cancer risks varied little with calendar period, sex or age. For most sites, the SIRs were relatively close to 1.00, but markedly reduced risks were observed for some cancers, most notably those of the lung (SIR, 0.77; 99% CI 0.73-0.81) and liver (SIR, 0.66; 99% CI 0.55-0.78). No significant departures from the expected incidence was observed for any of the hematological or lymphatic cancers, separately or combined.

The number of observed cancers was significantly higher than expected for four groups of malignancies: breast cancer (SIR, 1.08; 99% CI 1.04-1.12), prostate cancer (SIR, 1.21; 99% CI 1.16-1.26), testicular cancer (SIR, 1.20; 99% CI 1.09-1.32), and malignant melanoma (SIR, 1.17; 99% CI 1.11-1.24). Contrary to the mortality analyses, the SIR for cancer overall was only marginally lower among blood donors who started donating more recently (Figure 7). However, the site-specific pattern was quite different. Whereas the SIR for lung cancer was almost half among donors who began donating only recently compared to those who began donating in 1968-1970, the SIR for prostate cancer remained consistently well above 1.00 for all sub-cohorts, and increased rather dramatically with the

Site (ICD-7 code)	Both sexes	Women	Men
	Standardized incidence ratio (99% confidence interval)		
All cancers	0.96 (0.95-0.98)	0.97 (0.95-0.99)	0.96 (0.94-0.98)
Lip (140)	0.71 (0.55-0.89)	1.17 (0.68-1.88)	0.63 (0.47-0.82)
Tongue (141)	0.78 (0.62-0.98)	0.92 (0.55-1.44)	0.75 (0.57-0.96)
Salivary glands (142)	1.05 (0.81-1.35)	1.24 (0.80-1.83)	0.96 (0.68-1.31)
Mouth (143-144)	0.66 (0.54-0.81)	0.87 (0.56-1.28)	0.61 (0.48-0.77)
Pharynx (145-148)	0.78 (0.66-0.91)	0.60 (0.37-0.91)	0.82 (0.69-0.97)
Oesophagus (150)	0.75 (0.64-0.87)	0.49 (0.27-0.80)	0.79 (0.67-0.93)
Stomach (151)	0.79 (0.71-0.86)	0.74 (0.59-0.91)	0.80 (0.72-0.89)
Small intestine (152)	0.80 (0.63-0.99)	0.87 (0.57-1.26)	0.77 (0.58-0.99)
Colon, including recto-sigmoid joint (153)	0.94 (0.89-1.00)	0.96 (0.87-1.06)	0.93 (0.87-1.00)
Rectum, excl. anus (154)	0.95 (0.88-1.01)	0.92 (0.80-1.05)	0.95 (0.88-1.03)
Liver (155.0)	0.66 (0.55-0.78)	0.75 (0.51-1.05)	0.63 (0.51-0.77)
Gallbladder, biliary passages, ampulla *Vateri* (155.1)	0.66 (0.55-0.79)	0.68 (0.51-0.90)	0.65 (0.50-0.82)
Liver, not specified as primary (156)	0.58 (0.44-0.76)	0.60 (0.34-0.97)	0.57 (0.41-0.78)
Pancreas (157)	0.86 (0.78-0.94)	0.88 (0.73-1.04)	0.85 (0.75-0.95)
Peritoneum and unspecified (158-159)	0.77 (0.45-1.23)	0.82 (0.35-1.61)	0.74 (0.35-1.36)
Nasal cavities, and sinuses (160)	0.84 (0.59-1.17)	1.32 (0.71-2.23)	0.69 (0.43-1.04)
Larynx (161)	0.68 (0.57-0.80)	0.75 (0.41-1.24)	0.67 (0.56-0.80)
Lung primary, tracheae (162.0, 162.1)	0.77 (0.73-0.81)	0.88 (0.80-0.96)	0.73 (0.69-0.78)
Pleura (162.2)	1.06 (0.84-1.31)	1.00 (0.35-2.22)	1.06 (0.84-1.33)
Lung, not specified as primary (163)	0.85 (0.56-1.25)	0.78 (0.25-1.80)	0.87 (0.54-1.32)
Mediastinum (164)	1.68 (0.95-2.72)	0.83 (0.09-3.03)	1.93 (1.05-3.23)
Breast (170)	1.08 (1.04-1.12)	1.08 (1.04-1.12)	1.13 (0.73-1.67)
Cervix uteri (171)	0.77 (0.69-0.85)	0.77 (0.69-0.85)	–
Corpus uteri (172)	0.83 (0.75-0.92)	0.83 (0.75-0.92)	–
Uterus, other parts and unspecified (173-174)	0.76 (0.55-1.03)	0.76 (0.55-1.03)	–
Ovary, fallopian tube, broad ligament (175)	0.93 (0.85-1.02)	0.93 (0.85-1.02)	–
Other and unspecified female genital organs (176)	0.79 (0.57-1.05)	0.79 (0.57-1.05)	–
Prostate (177)	1.21 (1.16-1.26)	–	1.21 (1.16-1.26)
Testis (178)	1.20 (1.09-1.32)	–	1.22 (1.09-1.32)
Other and unspecified male genital organs (179)	0.86 (0.63-1.15)	–	0.86 (0.63-1.15)
Kidney (180)	0.84 (0.77-0.91)	0.82 (0.68-0.98)	0.84 (0.76-0.92)
Bladder including papilloma (181)	0.98 (0.92-1.04)	0.93 (0.79-1.10)	0.99 (0.92-1.05)
Melanoma of skin (190)	1.17 (1.11-1.24)	1.14 (1.05-1.24)	1.19 (1.11-1.27)
Other skin (191)	1.02 (0.97-1.07)	1.01 (0.93-1.09)	1.03 (0.97-1.09)
Eye (192)	0.96 (0.74-1.21)	1.00 (0.63-1.51)	0.94 (0.69-1.25)
Brain and nervous system (193)	0.94 (0.89-1.00)	1.02 (0.93-1.12)	0.90 (0.82-0.97)
Thyroid (194)	1.05 (0.92-1.19)	1.04 (0.88-1.23)	1.05 (0.84-1.29)
Endocrinal glands (195)	0.92 (0.83-1.03)	0.91 (0.78-1.07)	0.93 (0.80-1.08)
Bone (196)	1.02 (0.77-1.32)	1.11 (0.65-1.77)	0.98 (0.69-1.34)
Connective tissue (197)	1.02 (0.88-1.19)	0.98 (0.75-1.27)	1.05 (0.86-1.25)
Metastases (198)	0.72 (0.54-0.94)	0.52 (0.29-0.86)	0.84 (0.60-1.13)
Other and unspecified sites (199)	0.77 (0.69-0.84)	0.72 (0.61-0.86)	0.79 (0.70-0.88)
Non-Hodgkin lymphoma (200, 202, 205.99)	0.96 (0.89-1.03)	0.90 (0.77-1.04)	0.98 (0.90-1.07)
Hodgkins disease (201)	0.98 (0.84-1.13)	1.07 (0.82-1.39)	0.93 (0.77-1.12)
Multiple myeloma (203)	0.91 (0.80-1.03)	0.96 (0.74-1.23)	0.89 (0.77-1.03)
Leukemia (204-207)	0.97 (0.89-1.06)	0.95 (0.80-1.12)	0.98 (0.89-1.09)

Table 7. All-cancer and site-specific standardized incidence ratios for cancer among Scandinavian blood donors, presented overall and by sex.
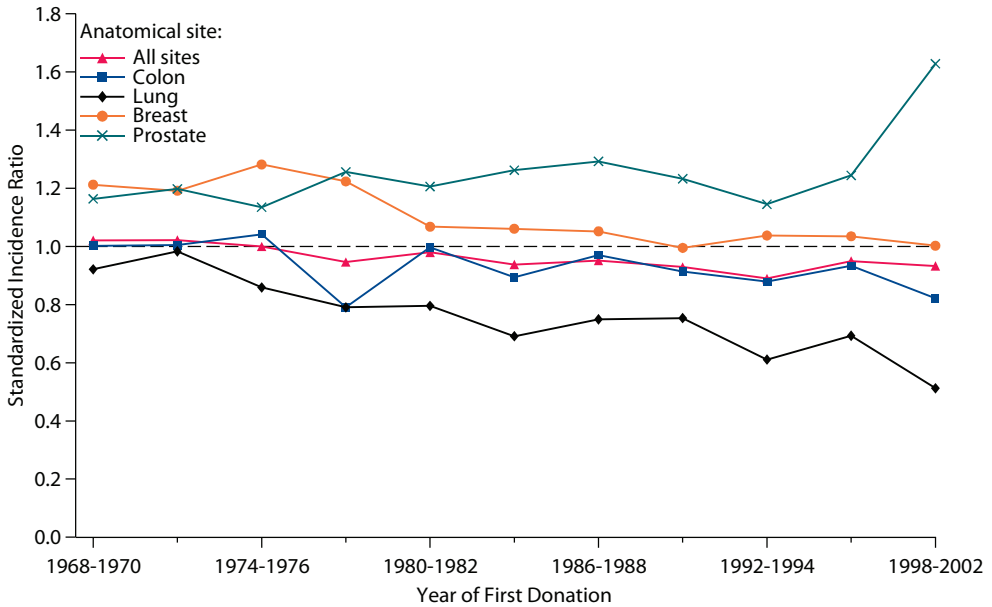Note: ICD denotes international classification of disease

Figure 7. All-site and site specific standardized incidence ratio for cancer for selected sites, by year of first recorded donation.

last cohorts, donors who began in 1995-1997 and 1998-2002. For breast cancer, the SIR was initially approximately 20 percent elevated, but became progressively lower with each successive sub-cohort, almost reaching unity in the last cohorts.

9.3. STUDY III

9.3.1. Study population

We found a total of 30,729 first primary cases of cancer among the donors. Of these, we excluded 13,810 who did not have at least 7 years of donation register coverage and another 6,053 who had not donated in the window from 3-12 years before diagnosis. Thus remained 10,028 donors with solid cancers, 482 with NHL and 356 with other hematological/ lymphatic malignancies. For the remaining 10,866 cases we selected 107,140 controls who had also had at least 7 years of register coverage and who had donated in the window from

	Cancer cases	Matched controls
Number of subjects	10,866	107,140
Site of case cancer, N (%)		
Solid cancers	10,028 (92.3)	98,871 (92.3)
Non-Hodgkin lymphomas	482 (4.4)	4,757 (4.4)
Other Hematological/lymphatic malignancies	356 (3.3)	3,512 (3.3)
Female sex, N (%)	4,720 (43.4)	46,492 (43.4)
Swedish, N (%)	7,949 (73.2)	78,553 (73.3)
Median age (IQR)	57 (48-64)	57 (48-64)
Number of recorded donations		
<5	2,071 (19.1)	19,579 (18.3)
6-15	3,942 (36.3)	38,232 (35.7)
16-50	3,667 (33.7)	37,895 (35.4)
>50	1,186 (10.9)	11,434 (10.7)
Median number of donations (IQR)		
In window 3-7 years	4 (0-9)	4 (0-9)
In window 8-12 years	5 (1-10)	5 (1-10)

Table 8. Characteristics of the study subjects in Study III
Note: Cases and controls (10 per case, alive and free of cancer on the date of the respective case's cancer diagnosis) were matched on sex, age (±180 days) and county of residence; IQR denotes interquartile range

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3-12 years before diagnosis, but who at the time of the diagnosis of the case, were alive, cancer free and had not emigrated.

Characteristics of the study population are presented in Table 8. The proportion of women was the same among both cases and controls (43.4 percent), as was the median age at diagnosis (57 years). Almost three quarters (73%) of both cases and controls were Swedish. The average annual number of previous donations was similar for cases and controls (the median of these individual averages was 0.8 per year for cases and 0.9 for controls).

9.3.2.   Main findings

The overall results are presented in Table 9. For cancer overall, we did not find any clear dose-dependent relationship with the number of donations in any of the exposure windows. Donors who belonged in the higher exposure categories, i.e. who had made more than 3 donations in the window from 3-12 years or at least 2 donations in the shorter windows, were generally at a lower risk, albeit not statistically significantly so, than donors with fewer donations. Analyses stratified by sex did not reveal any conspicuous deviation from the general pattern. In no window did we find an increased cancer risk associated with a higher donation intensity.

Number of donations					
Odds ratio (95% confidence interval)					
3-12 years before diagnosis	1-2	3-10	11-20	>20	P for trend
Both sexes	1.00 (ref)	0.95 (0.88-1.03)	0.96 (0.88-1.05)	0.97 (0.88-1.07)	0.84
Women	1.00 (ref)	0.94 (0.84-1.05)	0.98 (0.86-1.11)	0.98 (0.84-1.14)	0.78
Men	1.00 (ref)	0.96 (0.85-1.08)	0.94 (0.83-1.07)	0.96 (0.84-1.09)	0.68
3-7 years before diagnosis	0-1	2-5	6-10	>10	
Both sexes	1.00 (ref)	0.95 (0.90-1.00)	0.94 (0.89-0.99)	0.94 (0.89-1.00)	0.46
Women	1.00 (ref)	0.94 (0.87-1.02)	0.97 (0.90-1.06)	0.96 (0.87-1.06)	0.93
Men	1.00 (ref)	0.95 (0.88-1.02)	0.91 (0.84-0.98)	0.93 (0.86-1.00)	0.39
8-12 years before diagnosis	0-1	2-5	6-10	>10	
Both sexes	1.00 (ref)	0.99 (0.91-1.07)	1.01 (0.93-1.10)	0.98 (0.90-1.08)	0.72
Women	1.00 (ref)	0.96 (0.86-1.07)	1.00 (0.89-1.13)	0.98 (0.85-1.13)	0.76
Men	1.00 (ref)	1.03 (0.91-1.16)	1.02 (0.91-1.15)	1.00 (0.89-1.13)	0.82

Table 9. Relative risks, expressed as odds ratios, for all cancers combined in relation to number of whole blood and plasma donations, by latency and stratified by sex.
Note: The analyses considering the two exposure windows from 3-12 and 8-12 years were restricted to the 5319 cases and 52,369 controls for whom we had at least 12 years of donation register coverage.

The analyses considering solid cancers, NHL and other hematological/lymphatic malignancies are presented in Table 10. For solid cancers and other hematological/ lymphatic malignancies, the results followed essentially the same pattern as for cancer overall. For NHL, however, we found a tendency for a moderately increased risk in the highest exposure category, but the elevation was not statistically significant.

	Number of donations				
	Odds ratio (95% confidence interval)				
3-12 years before diagnosis	1-2	3-10	11-20	>20	P for trend
Solid cancer	1.00 (ref)	0.95 (0.87-1.04)	0.96 (0.88-1.05)	0.96 (0.87-1.07)	0.73
Non-Hodgkin lymphoma	1.00 (ref)	1.04 (0.70-1.55)	1.06 (0.69-1.64)	1.18 (0.75-1.86)	0.07
Other	1.00 (ref)	0.82 (0.53-1.27)	0.77 (0.47-1.27)	0.79 (0.45-1.40)	0.16
3-7 years before diagnosis	0-1	2-5	6-10	>10	
Solid cancer	1.00 (ref)	0.94 (0.89-0.99)	0.94 (0.89-1.00)	0.93 (0.88-0.99)	0.37
Non-Hodgkin lymphoma	1.00 (ref)	0.98 (0.75-1.28)	1.01 (0.77-1.33)	1.12 (0.86-1.46)	0.16
Other	1.00 (ref)	1.08 (0.80-1.44)	0.78 (0.56-1.08)	0.93 (0.66-1.31)	0.29
8-12 years before diagnosis	0-1	2-5	6-10	>10	
Solid cancer	1.00 (ref)	0.99 (0.91-1.08)	1.01 (0.93-1.11)	0.99 (0.90-1.09)	0.71
Non-Hodgkin lymphoma	1.00 (ref)	1.12 (0.76-1.66)	1.01 (0.68-1.49)	1.04 (0.69-1.58)	0.16
Other	1.00 (ref)	0.79 (0.50-1.25)	0.87 (0.55-1.38)	0.76 (0.45-1.30)	0.09

Table 10. Relative risks, expressed as odds ratios, for solid cancers, non-Hodgkin lymphoma and other hematological/lymphatic malignancies in relation to number of donations, by latency.
Note: The analyses considering the two exposure windows from 3-12 and 8-12 years were restricted to the 5319 cases and 52,369 controls for whom we had at least 12 years of donation register coverage.

Table 11 presents, for the Swedish donors only, the risks of solid cancers, NHL and other hematological/lymphatic malignancies in relation to the number of whole blood or plasma donations considered as separate variables. Except for a small decreased risk among frequent donors in the window 3-7 years before the index date, the analyses revealed no noteworthy associations with the risk of solid cancers. However, in all three windows, we found moderate positive associations between the number of plasma donations and the risk of NHL. Donors with more than 20 plasma donations in the window 3-12 years were at higher risk than those with none (odds ratio, 2.00; 95% CI 1.15-3.26). Also donors in the highest exposure categories in the windows 3-7 and 8-12 years before diagnosis were at increased risk.

Further exploring the association between number of plasma donations and risk of NHL we stratified the analyses by calendar period of first donation. While donors who made more than 20 plasma donations in the window 3-12 years who began donating before 1986 were at increased risk (odds ratio, 2.36; 95% CI 1.22-4.55), this association did not appear among those who began later (odds ratio, 1.11; 95% CI 0.33-3.71). The same pattern, with excess risks only among donors who began before 1986, appeared also in the windows from 3-7 and 8-12 years (data not shown).

The results of analyses of iron loss in relation to risk of cancers of the liver, lung, colon, stomach and esophagus are presented in Table 12. In the exposure window 3-7 years before the index date we noted an inverse association between iron loss and risk. Although the P-value for linear trend was highly significant (<0.001), our analysis of categorical data suggested a threshold effect with the largest drop between the lowest and second lowest exposure categories. The inverse association was seemingly driven by male donors. Men who lost >2.5 g of iron were at 34% lower risk compared to those

Table 11.

	Number of whole-blood donations					Number of plasma donations			
	Odds ratio (95% confidence interval)					Odds ratio (95% confidence interval)			
3-12 years before diagnosis	1-2	3-10	11-20	>20	P for trend	0-2	3-20	>20	P for trend
Solid cancer	1.00 (ref)	0.96 (0.88-1.06)	0.99 (0.89-1.10)	0.98 (0.87-1.11)	0.49	1.00 (ref)	0.96 (0.83-1.11)	1.01 (0.87-1.18)	0.89
Non-Hodgkin lymphoma	1.00 (ref)	1.26 (0.82-1.94)	1.21 (0.75-1.97)	1.29 (0.76-2.19)	0.69	1.00 (ref)	0.80 (0.36-1.79)	2.00 (1.15-3.46)	0.05
Other	1.00 (ref)	0.78 (0.48-1.29)	0.84 (0.47-1.50)	1.06 (0.53-2.09)	0.85	1.00 (ref)	1.04 (0.48-2.23)	0.41 (0.12-1.36)	0.18
3-7 years before diagnosis	0-1	2-5	6-10	>10	P for trend	0-1	2-10	>10	P for trend
Solid cancer	1.00 (ref)	0.95 (0.89-1.02)	0.95 (0.89-1.02)	0.92 (0.85-0.99)	0.02	1.00 (ref)	0.95 (0.83-1.10)	0.98 (0.87-1.10)	0.99
Non-Hodgkin lymphoma	1.00 (ref)	1.00 (0.75-1.35)	0.95 (0.70-1.30)	1.05 (0.77-1.43)	0.80	1.00 (ref)	1.84 (1.06-3.20)	1.59 (1.01-2.50)	0.08
Other	1.00 (ref)	0.97 (0.69-1.37)	0.82 (0.55-1.21)	1.04 (0.69-1.55)	0.90	1.00 (ref)	1.08 (0.55-2.12)	0.43 (0.19-0.95)	0.24
8-12 years before diagnosis	0-1	2-5	6-10	>10	P for trend	0-1	2-10	>10	P for trend
Solid cancer	1.00 (ref)	0.98 (0.89-1.08)	0.99 (0.90-1.10)	1.01 (0.90-1.13)	0.85	1.00 (ref)	0.96 (0.80-1.15)	1.00 (0.85-1.18)	0.72
Non-Hodgkin lymphoma	1.00 (ref)	1.17 (0.76-1.82)	1.19 (0.77-1.84)	1.03 (0.63-1.68)	0.72	1.00 (ref)	0.86 (0.34-2.19)	1.83 (1.00-3.34)	0.12
Other	1.00 (ref)	0.73 (0.42-1.26)	0.93 (0.54-1.60)	1.03 (0.55-1.95)	0.95	1.00 (ref)	0.62 (0.19-2.04)	0.32 (0.08-1.35)	0.15

Table 11. Relative risks, expressed as odds ratios, for solid cancers, non-Hodgkin lymphoma and other hematological/lymphatic malignancies among Swedish blood donors in relation to number of whole blood and plasma donations, presented by latency.
Note: The analyses considering the two exposure windows from 3-12 and 8-12 years were restricted to the 3941 cases and 38,883 controls for whom we had at least 12 years of donation register coverage.

who lost <0.25 g (odds ratio, 0.66; 95% CI 0.55-0.79). Contrastingly, in the exposure window 8-12 years before index date, point estimates were suggestive of increased risks among donors losing 0.5-2.5 g of iron. The dose-risk curve was seemingly bell-shaped and there was no obvious linear trend (P=0.73). Again, the phenomenon was most marked among male donors. In order to shed light on the possibility that effect modification by calendar period, in turn possibly reflecting changing self-selection and increasingly strict deferral policies,[1] would explain the unexpected difference by latency, we fitted models with interaction terms. The parameter for an interaction between calendar period and iron loss did not attain statistical significance (data not shown). When studying the associations in a wider exposure window (3-12 years) that encompassed both of the 5-year windows described above, the net associations were unimpressive. They were all statistically non-significant, but the point estimates among donors with the greatest iron loss (>5.0 g) suggested an 18-26 percent lower risk than among those with the smallest iron loss (<0.5 g).

9.3.3. Supplementary analyses

Among the controls, there were only minor variations in the incidence of alcoholism and alcoholic hepatitis with donation activity, but whereas the incidence of chronic obstructive pulmonary disease was similar in the three lowest exposure categories, 1-2, 3-10 and 11-20 donations per year, the incidence in the highest exposure category was nearly 50 percent lower (see Table 13). Among the 23,471 female donors who had been pregnant in or after 1983, those who reported

Iron loss through blood donation				
Odds ratio (95% confidence interval)				
3-12 years before diagnosis	<0.5g	0.5-2.0g	2.1-5.0g	>5g	P for trend
Both sexes	1.00 (ref)	0.97 (0.79-1.19)	0.95 (0.77-1.16)	0.79 (0.61-1.03)	0.06
Women	1.00 (ref)	1.00 (0.73-1.38)	1.14 (0.83-1.57)	0.82 (0.48-1.41)	0.99
Men	1.00 (ref)	0.94 (0.72-1.22)	0.84 (0.64-1.09)	0.74 (0.54-1.02)	0.03
3-7 years before diagnosis	<0.25g	0.25-1.0g	1.1-2.5g	>2.5g	
Both sexes	1.00 (ref)	0.85 (0.74-0.98)	0.87 (0.77-0.99)	0.72 (0.62-0.85)	<0.001
Women	1.00 (ref)	0.84 (0.66-1.07)	1.04 (0.85-1.28)	0.94 (0.69-1.28)	0.87
Men	1.00 (ref)	0.85 (0.72-1.01)	0.79 (0.67-0.92)	0.66 (0.55-0.79)	<0.001
8-12 years before diagnosis	<0.25g	0.25-1.0g	1.1-2.5g	>2.5g	
Both sexes	1.00 (ref)	1.27 (1.03-1.57)	1.21 (1.00-1.47)	0.97 (0.76-1.23)	0.73
Women	1.00 (ref)	1.17 (0.84-1.62)	1.31 (0.97-1.76)	0.83 (0.50-1.37)	0.53
Men	1.00 (ref)	1.34 (1.01-1.76)	1.16 (0.90-1.50)	1.00 (0.75-1.33)	0.46

Table 12. Relative risks, expressed as odds ratios, for cancers of the lung, liver, esophagus, stomach and colon in relation to iron loss, by latency and stratified by sex.
Note: The analyses considering the two exposure windows from 3-12 and 8-12 years were restricted to the 945 cases and 9312 controls for whom we had at least 12 years of donation register coverage.

being smokers had on average made 5.0 donations in the previous 10 years compared to 6.6 among non-smokers.

Number of previous donations				
Incidence rate (95% confidence interval)				
Condition	1-2	3-10	11-20	>20
Alcoholism	78.6 (51.3-115)	60.1 (42.7-82.1)	62.2 (41.3-89.9)	136 (98.5-183)
Alcoholic hepatitis	15.1 (4.92-35.3)	24.7 (14.1-40.1)	17.8 (7.69-35.1)	19.1 (7.00-41.5)
Chronic obstructive pulmonary disease	244 (194-303)	249 (212-290)	228 (186-276)	142 (104-190)

Table 13. Incidence of hospitalization for selected diagnoses associated with alcohol and smoking, in relation to number of donations in the window 3-12 years before the index date.

9.4. STUDY IV

9.4.1. Study population

From the SCANDAT database we identified a total of 1,311,079 transfusion recipients. After exclusion of 373,014 with a previous diagnosis of cancer, 208,692 who died, emigrated or were diagnosed with cancer before start of follow-up, 91,959 who received blood from an unknown donor, 230,076 who received blood from a donor who was followed for less than five years, 9,377 who received blood from a donor with a prior cancer diagnosis, 3,760 recipients of autologous transfusions, and 40,107 with an unknown area of residence at the time of first transfusion, no less than 354,094 recipients remained for analysis. The general characteristics of the study population are presented in Table 14.

	Unexposed cohort	Exposed cohort	All participants
Number of subjects	342,082	12,012	354,094
Sex			
Female	206,899	6,551	213,450
Male	135,183	5,461	140,644
Country			
Denmark	54,848	2,686	57,534
Sweden	287,234	9,326	296,560
Age at entry into cohort (years), N (%)			
< 40	81,183 (23.7)	2,419 (20.1)	83,602 (23.6)
40-59	68,199 (19.9)	2,444 (20.3)	70,643 (20.0)
60-69	55,882 (16.3)	2,046 (17.0)	57,928 (16.4)
≥70	136,818 (40.0)	5,103 (42.5)	141,921 (40.1)
Mean age at entry into cohort, years (SD)	57.7 (22.7)	59.6 (21.4)	57.8 (22.6)
Median length of follow-up, years (range)	7.0 (0-34)	6.2 (0-33)	7.0 (0-34)
Median year of first recorded transfusion (IQR)	1992 (1986-1995)	1993 (1989-1996)	1992 (1986-1995)
Median number of transfused units (range)*	2 (1-136)	4 (1-285)	2 (1-285)

Table 14. Characteristics of study participants in Study IV.
Note: SD denotes standard deviation; IQR denotes interquartile range. *During initial 30 days following first blood transfusion.

9.4.2. Main findings

During the study period, a total 3,200,800 person-years of follow-up accrued. We observed 29,651 primary cancers. In total, 12,012 recipients (3.4%) were exposed to blood from precancerous donors. During follow-up for a total of 90,928 person-years among the exposed recipients, we observed 978 incident cancers. Table 15 presents results from overall and stratified multivariate Poisson regression analyses of cancer incidence. We found no excess risk of cancer overall among recipients who had received one or more blood products from a precancerous blood donor, compared with recipients who had only received blood from non-cancerous donors (incidence rate ratio [IRR], 1.00; 95% CI, 0.94-1.07). Stratified analyses revealed that the risk did not differ significantly with sex, age, calendar period or number of transfusions. Further stratification, however, revealed a significantly increased cancer risk among exposed male recipients in the period between 5 and 10 years following the first transfusion (IRR, 1.19; 95% CI, 1.03-1.38). There was no indication of a corresponding excess risk for exposed women in the same follow-up period (IRR, 0.93; 95% CI, 0.76-1.12), nor was there any evidence of excess risks in any of the other follow-up intervals for either sex.

When receipt of blood from donors with cancers at different anatomical sites were considered as separate exposure categories, we did not find any variable potential for cancers at different sites (Table 16). Also, there was little variation in site-specific cancer risk between exposed and unexposed recipients (Table 17). Finally, combining the anatomically most plausible sites (lung, liver, skeleton and central nervous system) also did not show any excess risk (IRR, 1.00; 95% CI, 0.85-1.17).

Stratum	Number of cancers/ person-years among exposed	Number of cancers/ person-years among unexposed	Incidence rate ratio (95% CI)
Overall	978/90,928	28,673/3,109,872	1.00 (0.94-1.07)
Sex			
Female	425/51,298	14,290/1,991,245	0.98 (0.89-1.08)
Male	553/39,630	14,383/1,118,627	1.03 (0.94-1.12)
Age at first transfusion			
<40	64/29,040	2,450/1,201,474	1.08 (0.84-1.38)
40-59	183/23,023	6,589/778,005	0.91 (0.78-1.06)
60-69	267/16,028	7,561/482,795	1.03 (0.91-1.16)
≥70	464/22,837	12,073/647,599	1.02 (0.93-1.12)
Calendar period of first transfusion			
1968-1979	74/14,095	4,944/891,022	0.91 (0.71-1.13)
1980-1989	283/30,589	8,716/1,036,966	0.96 (0.85-1.08)
1990-2002	621/46,244	15,013/1,181,885	1.03 (0.95-1.12)
Time since first transfusion			
0-4 years	511/45,181	13,412/1,313,197	0.99 (0.90-1.08)
5-9 years	297/26,585	7,705/877,132	1.08 (0.96-1.21)
10-19 years	148/16,387	5,874/733,825	0.96 (0.81-1.13)
20-34 years	22/2,775	1,682/185,718	0.90 (0.59-1.38)
Number of transfusions			
1-2	258/26,845	15,765/1,851,497	1.01 (0.89-1.14)
3-4	238/21,703	6,936/701,640	1.02 (0.90-1.16)
5-9	252/21,166	4,244/397,543	1.05 (0.92-1.19)
≥10	230/21,215	1,728/159,193	0.94 (0.81-1.07)

Table 15. Incidence rate ratios of cancer in recipients of precancerous blood, relative to recipients of non-cancerous blood; overall and stratified by sex, age at transfusion, calendar period of transfusion, time since exposure and number of transfusions.

Note: For each stratum, the reference group is recipients of non-cancerous blood. CI denotes confidence interval; Adjusted for sex, attained age, calendar period of observation, number of transfusions, area of residence, ABO blood type and follow-up time.

9.4.3. Supplementary analyses

We also conducted a number of supplementary analyses. Analyses according to type of blood component, storage time and time to cancer death of the donor revealed no notable variation. Also, we found no conspicuous pattern when successively reducing the maximum time span allowed between donation and cancer in the donor for the blood to be considered precancerous. The risk associated with transfusions from donors who received blood within four (IRR, 1.00; 95% CI, 0.93-1.07), three (IRR, 1.00; 95% CI, 0.92-1.07), two (IRR, 0.93; 95% CI, 0.84-1.02) and one year (IRR, 0.93; 95% CI, 0.81-1.05), were all non-significant. Using Danish data only, where we had access to information on tumor stage at diagnosis, we compared recipients of blood from donors who presented with metastatic cancer within 5 years after the donation to unexposed recipients. The risk was not significantly elevated (IRR, 0.99; 95% CI, 0.48-1.79).

In the analysis of Kaposi's sarcoma concordance, we identified 14 donors who later were diagnosed with Kaposi's sarcoma. Among the donors, the time from last donation to diagnosis averaged 6.5 years (range from 0 to 10 years). However, during a total follow-

Anatomical site of donor cancer	Number of cancers/ person-years among recipients	Incidence rate ratio (95% CI)
No cancer (i.e. unexposed recipient)	28,673/3,109,872	1.00 (ref)
Oral cavity and upper gastrointestinal tract	37/3,614	1.05 (0.75-1.43)
Lower gastrointestinal tract	76/7,642	0.94 (0.74-1.17)
Liver and gallbladder	6/405	1.26 (0.50-2.56)
Respiratory organs	35/3,088	1.11 (0.78-1.52)
Breast and mammary gland	160/14,857	0.98 (0.84-1.14)
Female genital organs	57/6,243	0.91 (0.69-1.17)
Male genital organs, incl. prostate	143/12,756	1.06 (0.90-1.25)
Urinary organs	72/7,458	0.91 (0.72-1.14)
Melanoma of skin	101/8,387	1.13 (0.92-1.36)
Eye and nervous system	48/4,092	1.07 (0.79-1.40)
Endocrinal glands	37/3,515	1.20 (0.85-1.63)
Bone and connective tissue	11/1,689	0.72 (0.37-1.23)
Malignant lymphomas	38/4,164	0.91 (0.65-1.24)
Leukemia and myeloma	30/3,003	0.98 (0.67-1.38)
Other and unspecified	127/10,016	1.01 (0.84-1.20)

Table 16. Incidence rate ratios of cancer at all sites in recipients of precancerous blood from donors with cancers at different anatomical sites; relative to recipients of non-cancerous blood.
Note: CI denotes confidence interval; Adjusted for sex, attained age, calendar period, number of transfusions, area of residence, ABO blood type and follow-up time.

up of 390 years (range from 0 to 26 years), none of the 55 patients that received a blood component from these donors developed Kaposi's sarcoma.

Finally, the cancer incidence among the 9,377 recipients of blood from donors with a prior cancer diagnosis did not differ from that among recipients of blood from non-cancerous donors (IRR, 0.94; 95% CI, 0.86-1.02).

Anatomical site of recipient cancer	Number of cancers among exposed	Number of cancers among unexposed	Incidence rate ratio (95% CI)
Oral cavity and upper gastrointestinal tract	64	1,789	1.04 (0.80-1.32)
Lower gastrointestinal tract	138	3,577	1.13 (0.95-1.34)
Liver and gallbladder	27	762	0.96 (0.63-1.38)
Respiratory	88	2,363	1.02 (0.82-1.26)
Breast	89	3,597	0.90 (0.72-1.11)
Female genital organs	49	1,645	1.02 (0.76-1.34)
Male genital organs, incl. prostate	160	4,165	1.06 (0.90-1.24)
Urinary organs	85	2,240	1.07 (0.85-1.33)
Melanoma of skin	80	2,418	1.04 (0.82-1.29)
Eye and nervous system	27	649	1.32 (0.87-1.92)
Endocrinal glands	14	603	0.82 (0.46-1.34)
Bone and connective tissue	9	176	1.51 (0.71-2.84)
Lymphomas	30	938	0.92 (0.62-1.31)
Leukemia and myeloma	38	1,056	1.05 (0.74-1.43)
Other and unspecified	80	2,695	0.78 (0.61-0.96)

Table 17. Incidence rate ratios of site-specific cancer in recipients of precancerous blood, relative to recipients of non-cancerous blood. For each anatomical site, the reference group is recipients of non-cancerous blood.

Note: CI denotes confidence interval; Adjusted for sex, attained age, calendar period, number of transfusions, area of residence, ABO blood type and follow-up time.

10. METHODOLOGICAL CONSIDERATIONS

10.1. BIAS

The epidemiologist's definition of bias is "any systematic error in an epidemiologic study that results in an incorrect estimate of the association between exposure and risk of disease". Although there are many types of bias, three main types are commonly acknowledged: selection bias, information (or measurement error) bias and confounding bias (or simply confounding), which is discussed in section 10.2 below. A near-complete list of different types of bias has been assembled by Delgado-Rodriguez *et al.*[195]

10.1.1. Selection bias

Selection bias refers to the type of systematic error that primarily occurs in a case-control study when the probability of being selected as a case or as a control is somehow related to the exposure status. Although supposedly less common, selection bias can also occur in a cohort study when the occurrence of the disease of interest differentially influences the probability of the selection of exposed or unexposed study participants. Whereas in prospective cohort studies the exposure ascertainment is done before the disease occurs, making selection bias even more unlikely in such designs, this is not the case in retrospective cohort studies. With retrospective cohort designs, selection bias can indeed occur if the study population is not stringently defined or if the material from which the cohort was retrospectively defined, was somehow incomplete or excluded (i.e. truncated) individuals who had died or emigrated before the start of the study period.

Another common cause of selection bias in cohort studies may occur when cohorts are defined from hospitalized patients, or as in Study IV, transfused populations. In

such cohorts, elevated rates of diseases are commonly observed during the year/years following entry into the cohort. In the setting where these studies were performed, one of the most common indications for blood transfusion is, according to a recent study, anemia of unknown cause.[193] Since anemia is a frequent complication of cancer, in fact being present as often as in 39 already upon diagnosis,[190, 192] blood transfusions are frequently administered to patients with a prevalent, yet undetected, malignancy only on the grounds of a clinical anemia. As such, the malignancy can be said to have caused the transfusion despite seemingly being diagnosed even long after the transfusion was administered. For this very reason, the occurrence of cancer is greatly inflated during the time directly following a blood transfusion, as is demonstrated in Figure 8, a diagram of the SIR for leukemia and non-melanoma skin cancer by time since first transfusion. The relative risk of cancer, comparing transfused individuals to the general population, remains statistically significantly elevated for both malignancies in all follow-up periods (i.e. even twenty years after the first blood transfusion). However, there are substantial differences between different cancer sites. More exact, Table 18, presents the SIR for all cancers combined, breast, colon, prostate cancer, and leukemia. Whereas the relative risk of leukemia, a malignancy that is frequently associated with anemia, is greatly elevated in the follow-up periods closest to the first transfusion and then decreases, the relative risk of non-melanoma skin cancer, where no association with anemia is plausible, remains at a constant 30-40 percent elevated level throughout follow-up. The excess risk of non-melanoma skin cancer is probably best explained by systematic differences between transfusion recipients and the general population.

Beyond the obvious example of reverse causation in the study of disease among transfusion recipients, reverse causation must also be considered when investigating the health effects of blood donation. Since the decision to prematurely cease donating
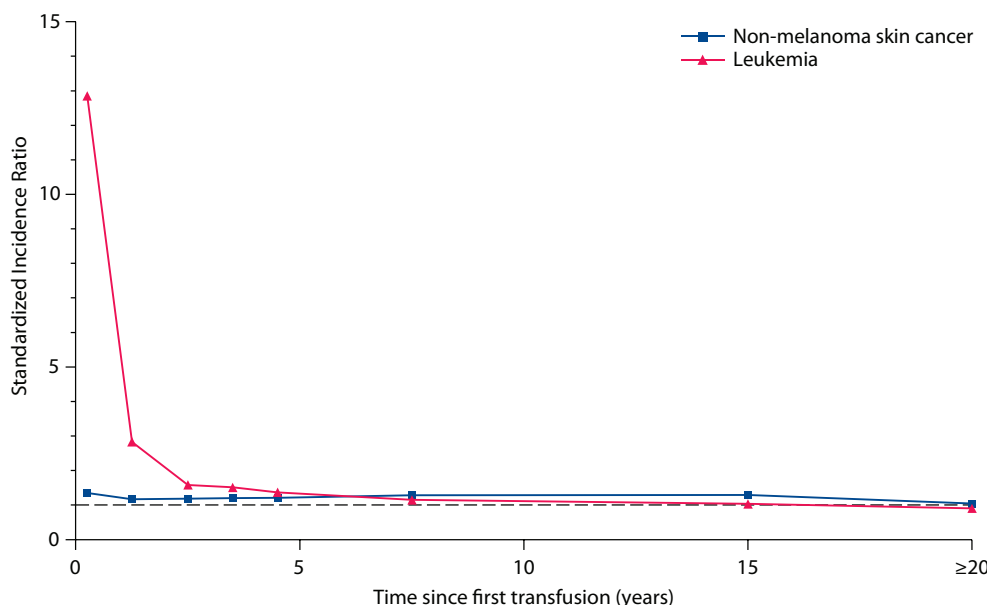

Figure 8. Standardized incidence ratio for leukemia and non-melanoma skin cancer among transfusion recipients, presented by time since first recorded transfusion.

	Time since first transfusion			
	<1 year	1–4 years	5–9 years	≥10 years
	Standardized incidence ratio (95 percent confidence interval)			
All sites	2.49 (2.48-2.51)	1.10 (1.09-1.11)	1.09 (1.08-1.10)	1.10 (1.09-1.11)
Breast	1.11 (1.06-1.16)	0.93 (0.88-0.97)	0.95 (0.90-1.00)	1.07 (1.02-1.12)
Colon	4.85 (4.74-4.97)	1.07 (1.02-1.13)	1.02 (0.96-1.08)	1.07 (1.00-1.13)
Leukemia	5.54 (5.31-5.78)	1.50 (1.38-1.63)	1.15 (1.03-1.27)	1.01 (0.89-1.13)
Non-melanoma skin cancer	1.21 (1.13-1.30)	1.20 (1.12-1.28)	1.28 (1.20-1.38)	1.24 (1.15-1.33)
Prostate	1.43 (1.38-1.47)	0.91 (0.88-0.95)	0.99 (0.94-1.03)	1.02 (0.98-1.07)

Table 18. Standardized incidence ratios in transfusion recipients for cancer overall, breast, colon and prostate cancer as well as leukemia, presented by time since first recorded transfusion.

blood may be a symptom of imminent health problems, one would expect that blood donors who are diagnosed with disease will have performed fewer donations during the time period directly before the diagnoses, than blood donors who are still in good health. The frequency of blood donations in the period before diagnosis will therefore seem inversely correlated with risk of the disease. This problem is readily illustrated with data from the nested case-control study of health effects of blood donation (Study III), where the declining rate of donation among cases as early as two years before diagnosis can probably be attributed to the incipient malignancy and is thus another example of reverse causation (see Figure 1).

Another special type of selection bias which most commonly occurs in prospective studies of occupational health is the *healthy worker effect*. Because workers with disease are typically not available for occupational surveys, or may simply not hold employment, the active workforce therefore appears healthier. A similar phenomenon, the *healthy donor effect*, can be assumed to partly account for the health differences between blood donors and the general population described in Study II. (It should be noted that in this study we do not consider the dramatic differences between blood donors and the general population as bias, *per se*, but rather as a measure of the success of blood donor selection.)

10.1.2. Information bias

Information bias, or observation bias, occurs when the accuracy of measurement of the exposure or outcome somehow differs between comparison groups. Unlike selection bias, which typically occurs in retrospective studies, information bias may occur in both cohort and case-control studies. Typically, information bias occurs in a cohort study when a different technique for ascertaining the outcome is used for exposed and unexposed resulting in different accuracy for exposure ascertainment. Similarly, it occurs in a case-control study when the accuracy with which the exposure is measured differs between cases and controls.

Several different classes of information bias have been identified, all of which may lead to misclassification of either the exposure or outcome. Among the most commonly acknowledged are loss to follow-up, surveillance bias, recall bias and interviewer bias. In addition to the possible effects of misclassification itself, this thesis will mainly cover

the classes that are most likely to have had an impact on the results here presented, namely surveillance bias and loss to follow-up.

Misclassification (or perhaps more intuitively, measurement error) is a more or less ever-present form of epidemiologic bias. It occurs in all epidemiologic studies due to the inability to perfectly enumerate the types of factors we are interested in, such as exposure variables, other explanatory variables or the outcome of interest. Most commonly, misclassification is of a random (or non-differential) nature, which tends to cause a bias towards a null effect. An important example of such measurement error may be taken from Study IV, where the contribution of blood from unknown donors or donors with less than 5 years of follow-up will lead to misclassification of the exposure. Since we deemed this misclassification to be a serious threat to the validity of the results, and since a negative study is especially sensitive to non-differential misclassification, we went to great lengths to exclude all recipients of such unknown donors or donors with insufficient follow-up from the study cohort. We also censored all recipients who, outside of the initial 30-day exposure period, were transfused with an unknown blood unit or unit with less than five years of follow-up of all contributing donors. These exclusions reduced the number of study participants by more than half,[196] but as was noted in an accompanying editorial comment the exclusions helped "refine rather than obfuscate the comparison of interest".[197]

Contrary to non-differential misclassification, which is more or less ignored by many investigators, differential misclassification on the other hand is a serious threat to any epidemiologic investigation. It typically occurs when the outcome is measured with different accuracy in exposed and unexposed in a prospective study, or conversely when the exposure is measured with different accuracy among cases and controls in a case-control study. Examples may be differential loss to follow-up and recall bias. Although it is unlikely that differential misclassification has affected the final results in any of the Studies I-IV to any great extent, differential misclassification was a possible issue in Study III, where left truncation which was caused by the gradual introduction of the computerized donation registers may have lead to considerable exposure misclassification. Since the registers were introduced in a geographically systematic manner, and since cancer incidence varies between different regions in both Sweden and Denmark,[179, 198] this misclassification may indeed have been differential. We therefore combined data from Swedish and Danish registers of internal migration and the start-up dates of the different donation registers to compute the dates from which point all study participants were fully covered. Using these start up dates we then excluded all participants who were not completely in view for the full duration of the considered exposure windows. Again, this exclusion took a heavy toll on the number of study participants, but ensured a minimal exposure misclassification.

As noted above, misclassification may also be caused by loss to follow-up, which itself may be caused by for example due to the emigration of study participants. However, thanks to the outstanding population, death, emigration and health data registers that are available in both Sweden and Denmark, any outcome misclassification thus introduced should be minimal.

Another special case of information bias is surveillance bias. For instance, in the study of health effects of blood donation (Study III), every blood donation represents a contact with the health care system. Frequent blood donors may therefore, from a short-term perspective at least, be more likely to be diagnosed with cancer and an artificial association between donation frequency and cancer may therefore appear. As such, caution is further warranted when interpreting the transient effects of recent blood donations on disease risk. Similarly, in the study of disease occurrence among transfusion recipients, part of the excess cancer risk among the transfusion recipients that we have demonstrated during the immediate period after a blood transfusion (see Figure 8, above) can be attributed to increased detection rates while the patient is in hospital or in contact with the healthcare system.

10.2. CONFOUNDING

Confounding may arise when an independent determinant of the outcome, other than the factor of interest, is unevenly distributed among different comparison groups (i.e. exposed and unexposed), and if this determinant is not an intermediate in the causal pathway between the factor of interest and the outcome. Thus, confounding occurs when the true effect of the exposure of interest is mixed with the effect of the other determinant, i.e. the confounder.

For reasons of self-selection, healthy lifestyle, stringent eligibility criteria and infectious disease screening, as reviewed in section 6.3 above, in the study of health effects of blood donation, confounding is an obvious issue that needs to be carefully considered and dealt with accordingly. In a comparison of the health of blood donors and the health of non-donors, diseases that are linked to these selection forces or to the presumably healthier lifestyle of the donor population will naturally occur less frequently among blood donors than non-blood donors. Therefore, such a comparison cannot be expected to reveal any deleterious health effects of blood donation, as such effects are likely to be masked by the sheer healthiness of blood donors. However, since the same selection forces apply to all blood donors alike, in a dose-response comparison of health effects of blood donation conducted among blood donors only, the effects of such confounding should be far less dramatic. As presented previously (Table 13, above), the incidence of a number of diagnoses strongly related to smoking and alcohol consumption, are also related to prior number of donations. These incidence rate estimates were obtained from a cohort analysis conducted among the controls in Study III. Although other explanations for the observed pattern are possible, it seems reasonable to suggest that there is a clear gradient of smoking habits among the donors with the lowest rate of exposure among the most frequent donors.

Conversely, in the study of health among transfusion recipients, who in effect are selected for their poor health, confounding by indication and unhealthy lifestyle is similarly inevitable. Therefore smoking and alcohol related cancers such as lung cancer and stomach cancer are over-represented among the cancers for which we saw an excess risk in the long-term perspective (see Table 18).

Whereas confounding by lifestyle (be it healthy or unhealthy), occupation, socioeconomic status or indication in the study of health effects of blood donations or transfusions

is more or less inevitable, for the study of transfusion transmission of disease, the case is quite different. Since at the time of the transfusion, the future disease occurrence of the blood donor is unknown, the exposure to blood from a donor who later will develop a certain disease, be it cancer or an infectious disease, can be considered a random event. Thus, only factors that are directly related to the exposure (i.e. cancer occurrence among the contributing blood donors), or to the probability of being exposed, while being independently associated with the outcome (i.e. the future cancer occurrence of the recipient), are possible confounders. In addition to the number of transfusions administered, which of course is directly proportional to the probability of exposure as well as being related to the indication for the transfusion and thus also to the outcome, only factors that influence donor and recipient health concurrently, such as calendar period and area of residence, or are consciously considered when a blood unit is selected, as are factors such as blood type, are possible confounders. See Figure 9 for a schematic representation of the issue of confounding in Study IV. Since all these possible confounding factors are easily measured, with minimal misclassification, and adjuste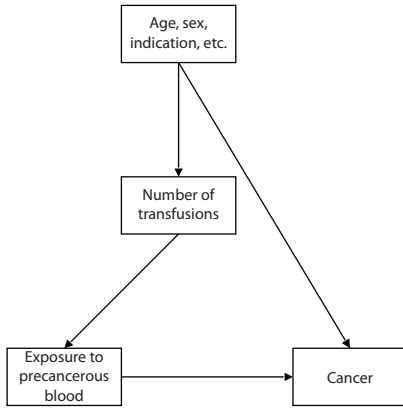d for in a multivariate analysis, in the case of the study of transfusion transmitted cancer, comparing cancer incidence among recipients exposed to precancerous blood units to the cancer incidence of those unexposed, should be equivalent to an experiment where the same exposure was randomly allocated.

Figure 9. Schematic representation of confounding in Study IV.

10.3.   PRECISION

Although chance is an ever-present, and largely untamable, force in epidemiology, in the studies that are presented in this thesis, we had the good fortune to only seldom be hindered by lacking statistical precision. The connectivity analyses presented in Study I, for example, were based on such large number of occurrences that we chose not to calculate any standard measures of statistical uncertainty. That said, however, some of the site-specific analyses in Study II-IV were clearly hampered by a lack of power and should consequently be interpreted with some caution.

11.   FINDINGS AND IMPLICATIONS

11.1.   STUDY I

In Study I, we describe the creation of the SCANDAT database and the results from the data quality assessment of this database. Our main finding was that it is possible to build a nationwide long-term hemovigilance system using preexisting computerized data collected in routine health care. Comparisons with external data sources and within the database, as well as an assessment of the overall condition of the data as received from the blood banks and transfusion medicine clinics, revealed that the overall quality of the data was very good. As an example, of the data received, only 1.6, 6.4, and 5.3 percent of the person, donation and transfusion records, respectively, had to be discarded after quality assessment or comparisons with independent data sources. Furthermore, using the respective countries' inpatient registers we were able to find a matching hospitalization for 88 percent of all transfusions. Thus, our conclusion from the quality assessment was that the database we had constructed was of sufficiently high quality for the research purposes intended.

In addition, Study I also provided valuable lessons about the creation of computerized hemovigilance systems. Firstly, in our data, no less than 21.3 percent of all donors and 8.9 percent of all recipients donated blood or were transfused in more than one county. Similarly, 19.1 percent of all blood components were eventually transfused in a different county than where it was originally drawn. Thus, long-term hemovigilance systems should, if possible, be implemented on a nationwide basis. Secondly, the utility, completeness of follow-up and to some extent also the data quality will be highly dependent both on the availability of some form of unique personal identification number (such as NRN) and the existence of population and health data registers. Naturally, the prerequisites in both Sweden and Denmark are excellent in this regard, but systems similar to ours have been implemented also elsewhere,[199] or could be. Thirdly, through perseverance and luck we were able to salvage data from registers dating back as far as the mid-1960s which enabled us to follow individuals over more than three decades. The feasibility of using historical data for these purposes must therefore be emphasized. Further, the necessary tools for reading the magnetic tapes on which the data was stored was no longer available at the blood banks. Future systems should for that reason be designed with platform independence and forward compatibility in mind.

Study I also provided further insight into donation and transfusion habits that can be used for more refined modelling of the risk of disease transmission. In both countries, we found the average number of recipients exposed to blood from a single donor to be quite low. We also found that the convergence of blood products, i.e. the average number of donors whose blood each recipient was exposed to, to be low. For future simulation studies the SCANDAT database, and similar systems, can provide detailed measures of disease incidence among blood donors, patterns of donation habits and inter-relatedness of donors and recipients.

11.2. STUDY II

In Study II, we observed considerably lower mortality, both overall and for broad groups of diseases, among blood donors compared to the general population. The overall mortality in the cohort was approximately 30 percent lower than in the background population, but whereas the mortality among donors who began their donating career in the late 1960s or early 1970s was only marginally lower than in the background population, among more recently recruited donors it was almost halved. The decreasing relative mortality was most pronounced for infectious diseases and respiratory disease, presumably due to the introduction of successively more stringent eligibility criteria and a declining prevalence of smoking among the donors. The successive enrichment of an increasingly healthy donor population has been achieved without an obvious compromise of the blood supply.

Contrastingly, we observed that for cancer, blood donors had only a marginally lower incidence than in the background population. In fact, the overall SIR was reduced by only 4 percent. For the majority of anatomical sites, we observed small to moderately reduced cancer incidence, but for breast, prostate and testicular cancer and for malignant melanoma the SIRs were increased, most likely reflecting the selection of persons of high socioeconomic status and health awareness into the blood donor population.[33, 34, 200] For other sites, etiologically linked to smoking and alcohol consumption such as the liver and lung, the SIRs were reduced by 34 percent and 23 percent, respectively. We did not see a marked overall decreasing cancer incidence over time corresponding to the pattern of decreasing mortality. In fact, for some anatomical sites, such as prostate cancer, the SIR was higher among more recently recruited donor cohorts.

11.3. STUDY III

In Study III, we conducted dose-response analyses of blood donation or iron loss through blood donation and the risk of cancer. The analyses were conducted for cancer overall and for specific predefined groups. Three different exposure windows, with different latencies, were used: 3-7 years before the index date, 8-12 years and the combination of the two, 3-12 years before the index date. In the overall analyses of number of donations we did not find any important effects on the risk of cancer overall in any of the exposure windows, neither for men or women. Neither did we find any associations between number of donations and risk of solid cancers, NHL or all other hematological/lymphatic malignancies combined. However, imprecise data for NHL showed that the risk might be moderately increased among frequent plasma donors. The association remained statistically significant and relatively consistent in all exposure windows, but appeared to be confined to the donors who began their donation career before 1986. We cannot readily explain this finding, but a number of mechanisms may have been influential. For example, whole blood donation has been found to cause a transient, yet detectable immunomodulation.[64, 65, 67] The same may apply also to plasma donation. Also, the association was apparently confined to donors who began in 1985 or earlier, i.e. before donor selection had started to adapt to the increasing prevalence of HIV in the donor population by implementing increasingly selective donor eligibility criteria. Thus, and since plasma donor compensation (60 SEK at the time) is likely to have been

a stronger motivational force, the association can conceivably have been confounded by lifestyle-related risk factors. Although strictly speculative, technical aspects of plasma donations such as chemical additives and chemicals dissolved from blood collection equipment may possibly also have been an important factor,[70, 71] as may the mechanical effects of especially older apheresis systems.

We found a moderately decreased risk of pulmonary, hepatic, esophageal, gastric and colonic cancer among the donors with the most pronounced donation-associated iron loss. However, in the light of the inverse relationship between the number of previous donations and risks of smoking and alcohol related diagnoses seen in the supplementary analyses, it seems plausible that the association between iron loss and cancer was confounded by such life-style factors. Furthermore, the association was inconsistent across latency times and between men and women which indicates that some form of bias, such as a residual effect of the obvious reversed causation (see section 10.1.1 above) may have been important. Naturally, the male-female inconsistency can also be seen as biologically plausible as iron loss may have a lesser effect in women who are commonly already depleted of iron from menstural iron loss. We therefore cannot draw any firm conclusions on whether iron loss indeed protects the frequent donor against the aforementioned malignancies.

11.4. STUDY IV

The overall conclusion of Study IV is that blood components originating from precancerous blood donors do not confer an increased cancer risk on the recipients. Notwithstanding the slight excess risk seen among male recipients in one of the follow-up strata, this held true irrespective of calendar period, recipients' age and sex, and the overall number of transfusions received in addition to the exposed blood component. We also did not find any evidence to support the hypothesis that different anatomical sites in donors modified the risk of cancer transmission to any important extent, nor did the type of unit through which the recipient was exposed to precancerous blood. Although the overall results strongly indicate that transmission of cancer from blood donors to transfusion recipients is unlikely to have any noteworthy impact on a population level, we have by no means shown that it does not occur. It is still quite possible that blood transfusions can provide a means for cancer transmission in individual cases.

In our analyses we also found no evidence to support the hypothesis that recipients exposed to blood from donors with a previous cancer history are at increased risk of cancer. It would thus seem that long-term cancer survivors are a safe donor group. Naturally, since the group of donors with a cancer history who were still allowed to donate, despite cancer survivors normally being deferred, may not be representative for all cancer survivors. Therefore, some reservation must be made for these results.

Our results from the analysis of concordance of Kaposi's sarcoma must also be carefully interpreted. We did not by any means attempt to show that HHV-8 is not transfusion transmittable, only that such transmission, if it occurs, only rarely (at least in this setting) can be detected to result in the development of Kaposi's. Due to the exclusion of donors with HIV or HIV-risk factors we only found 14 donors with a subsequent Kaposi's sarcoma diagnosis. Our power to detect such transmission was thus clearly inadequate.

12. CONCLUSIONS

- With the creation of the SCANDAT database, we have demonstrated the feasibility of creating a database for research and transfusion safety monitoring using preexisting, routinely collected administrative data. By indirect comparisons and internal consistency checks, we have found that the database thence created is of sufficient quality for research purposes.

- From analyses of the inter-relatedness of blood donors and transfusion recipients, we have found that the theoretical potential for wide dissemination of communicable diseases, even with extended latencies, through blood transfusion is moderate.

- Scandinavian blood donors enjoy lower than average mortality and cancer incidence. More recently recruited donors depart further from the population average with regards especially to mortality. It thus appears that the gradual reinforcement of selection mechanisms for blood donors (with the introduction of screening tests, exclusion of high-risk groups, further medical history taking, and relative reduction of the economic compensation) have succeeded in selecting a particularly healthy group of people for blood donors.

- Repeated blood donation does not seem to be associated with any increased or decreased risk of cancer overall.

- Among donors who began their donating career before 1986, when the selection criteria for blood donation was tightened on account of the HIV epidemic, frequent plasma donation was associated with an increased risk of NHL.

- Among men, there was a moderate, and by latency somewhat inconsistent, protective association between iron loss through blood donation and the risk of cancers previously associated with high iron stores.

- There was no evidence that blood transfusions from precancerous blood donors are associated with increased cancer risk among recipients. Cancer, irrespective of anatomical site and disease severity, therefore does not seem to be transfusion transmittable.

13. FUTURE STUDIES

- Work done internationally, e.g. in Finland,[199] has demonstrated that an undertaking such as the creation of the SCANDAT database, while keeping the database identifiable and possible to continuously update, is possible. Since the SCANDAT database in its current form is static and de-identified, addition of additional data through record linkage or updating the database with more transfusion register data is not possible. Thus, to improve its versatility, it should be recreated in an identifiable format.

- Although we have in Study II and Study III essentially ruled out any strong deleterious associations between blood donations and the risk of cancer, the abysmal lack of research on the health effects of repeated letting of blood in healthy individuals must be attended to. There exists a number of mechanisms whereby donor health could be modified by blood donation. Therefore the risks of other chronic diseases such as cardiovascular disease in relation to blood donation intensity should be properly investigated. Furthermore, the unexpected excess risk of NHL associated with plasma donation we found in Study III deserves replication.

- It is quite probable that a non-negligible proportion of the more than three percent of recipients who were exposed to blood units from precancerous blood donors in Study IV actually did receive an inoculation of malignant cells. Considering the repeated demonstration of long-standing colonies of circulating cells of donor origin in studies of microchimerism, the fate of these transfused cancer cells may deserve further investigation using molecular methods. Using the SCANDAT database it is possible to identify donor-recipient pairs where both developed a malignancy of the same histological type. Tissue samples from these tumors can then be identified and their genetic origin compared.

- A wide range of epidemiological studies have been conducted in the field of transfusion medicine, but these have mainly focused on transfusion transmitted infections. In this thesis we have tried to widen the understanding of the possible long-term non-infectious complications of both blood donations and transfusions, but many knowledge gaps persist.

- Thanks to a declining risk of contracting a transfusion transmittable infection, administrative errors and non-infectious transfusion-related causes of morbidity and mortality such as TRALI have relatively speaking become more important.[201] In a further strive to improve transfusion safety, more effort should therefore be directed towards understanding and eliminating such conditions and to further improve administrative routines in transfusion services to avoid life-threatening human errors.

14. SVENSK SAMMANFATTNING

De senaste årens intensiva arbete med transfusionssäkerhet, genom förbättrad infektionsscreening och förbättrat blodgivarurval, har lett till att riskerna för transfusionsöverförda infektioner idag nått näst intill omätbart låga nivåer. Trots detta är förvånansvärt lite känt om sjuklighetspanoramat hos blodgivare och de möjliga långtidseffekterna av upprepade helblods- och aferestappningar. Tidigare undersökningar har studerat möjliga icke-infektiösa risker med blodtransfusioner, men evidens om huruvida blodtransfusioner från blodgivare med preklinisk cancer kan leda till att cancer utvecklas hos berörda blodmottagare saknas. För att fylla några av dessa kunskapsluckor, sammanställde vi detaljerade data om blodgivare, blodtappningar, transfusioner och transfusionsmottagare i databasen Scandinavian Donations and Transfusions (SCANDAT). Alla här presenterade studier är baserade på denna databas.

I det första arbetet beskriver vi sammanställningen av SCANDAT-databasen och dess innehåll, samt resultaten från våra kvalitetsanalyser. Totalt innehåller databasen information om 1,134,290 blodgivare med 15,091,280 blodtappningar och 1,311,079 transfunderade patienter med 11,693,844 registrerade blodtransfusioner. Då direkta undersökningar av databasens kvalitet ej befanns vara möjliga, gjordes en serie indirekta jämförelser med externa datakällor som alla tydde på att kvalitén var tillräckligt hög för databasens avsedda syfte.

I det andra arbetet gjordes en kohortstudie där vi jämförde mortalitet och cancerincidens bland 1,110,329 blodgivare med bakgrundsbefolkningen. Relativa risker för död och cancer uttrycktes som standardiserade mortalitets- och incidensratior. Blodgivarna visade sig ha 30 % lägre mortalitet (99 % konfidensintervall [KI] 29 %-31 %) och 4 % lägre cancerincidens (99 % KI 2 %-5 %). Vidare uppvisade blodgivare som rekryterats sent i studieperioden en relativt sett lägre mortalitet än de som rekryterats tidigt.

Inom blodgivarkohorten från studie nummer två genomförde vi i studie tre en nestad fall-kontrollstudie. Den relativa risken för cancer i relation till antal tidigare blodtappningar, eller av dessa orsakad järnförlust, uppskattades med logistisk regression. Totalt identifierades 10,866 givare som drabbats av cancer mellan deras första registrerade blodtappning och studiens slut, till vilka 107,140 kontrollgivare utan cancer matchades. Vi fann inget klart samband mellan antal blodtappningar och cancerrisk överlag. Risken för non-Hodgkinlymfom var ökat bland frekventa plasmagivare; oddskvoten bland givare med ≥20 plasmatappningar i perioden 3-12 år före fallets diagnos jämfört med de med färre än 3 tappningar var 2.00 (95 % KI 1.15-3.46). Vidare fann vi bland manliga givare att cancerrisken minskade med ökande järnförluster i perioden 3-7 år före fallets diagnos (p<0.001).

Av de 354,094 transfunderade patienter som deltog i analyserna i den fjärde studien var 12,012 (3.4%) exponerade för blodprodukter från givare som diagnosticerades med cancer inom fem år (precancerösa givare). Den relativa risken för cancer bland mottagare som fått blod från precancerösa givare jämfört med de som fått blod som ej kom från precancerösa givare var 1.00 (95 % KI, 0.94-1.07). Vi fann ej heller någon överrisk när vi tog hänsyn till tumörlokalisationen eller allvarlighetsgraden hos givaren. Ej heller visade sig risken för specifika tumörformer skilja sig åt mellan exponerade och oexponerade patienter.

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