Storage time of allogeneic red blood cells is associated with risk of severe postoperative infection after coronary artery bypass grafting

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Methods: The study included patients undergoing CABG with or without concomitant cardiac surgery between June 2003 and July 2008 in the North and Central Denmark regions. Data on demography, perioperative variables, allogeneic blood transfusion and severe postoperative infections (deep sternal wound infection, bacteremia or septicemia) were retrieved from medical databases and medical records. We used logistic regression analyses to compute the crude and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) for the association between storage time of transfused RBCs and the risk of severe infection. Results: A total of 4240 patients were included in the final analyses, and 1748 of these patients (41%) were transfused with RBCs. Among transfused patients, 953 were exclusively transfused with RBC stored for ≤14 days. Severe infection was identified in 165 patients (3.9%). The adjusted ORs for severe infection among all transfused patients and patients transfused with RBCs stored exclusively for either ≤14 days or >14 days were 1.6 (95% CI: 0.9–2.8), 1.1 (95% CI: 0.6–2.1), and 2.3 (95% CI: 1.2–4.2), respectively, when compared with non-transfused patients. There was a dose–response relationship between the number of transfused RBC units and the risk of severe infection. Conclusion: Although the risk of possible confounding could not be eliminated entirely in this observational study, the findings add further support for the hypothesis that storage time of RBCs is positively associated with the risk of transfusion-related severe postoperative infection in patients undergoing CABG.

Keywords: Transfusion; Red blood cell storage; Coronary artery bypass grafting; Infection

1. Introduction

A number of observational studies link allogeneic red blood cell (RBC) transfusion in cardiac surgery, including coronary artery bypass grafting (CABG), with increased morbidity and mortality [1, 2], although not all studies have been able to confirm this association [3]. A few studies have examined the role of the storage time of allogeneic RBC before transfusion, and the results indicate that storage time may be an important determinant of transfusion-related postoperative outcome, including severe postoperative infections [4, 5], which have huge economic implications in terms of increased resource use during a prolonged hospital stay [6]. This association has been ascribed to the progressive structural and functional changes that stored RBCs undergo over time [7]. However, substantial uncertainty remains regarding the importance of storage time because the available data remain sparse and inconsistent [4, 5, 7, 8].

We aimed to examine whether storage time of allogeneic RBCs is associated with the risk of severe postoperative infections following transfusion among patients undergoing CABG.
CABG with or without concomitant cardiac surgery in a large population-based follow-up study.

2. Materials and methods

This study was conducted within the population of the North and Central Denmark regions. These regions comprise approximately 1.8 million persons or 33% of the total population in Denmark.

The Danish National Health Service provides tax-supported health care for all inhabitants, guaranteeing free access to family physicians and public hospitals. All patients undergoing cardiac surgery in the North and Central Denmark regions are referred to either Aalborg or Skejby Hospital, which are both public university-affiliated hospitals. Unambiguous linkage between various population-based registers can be performed using the civil registry number, a unique permanent personal identification number given to all Danish citizens. The study was approved by The Danish Data Protection Agency (record no.: 1-16-02-1-08/017).

2.1. Identification of CABG patients

We obtained data regarding civil registry numbers, preoperative demographics, and surgical characteristics on all patients, who underwent CABG in either Aalborg or Skejby Hospital between June 2003 and July 2008, through the Western Denmark Heart Registry. The Western Denmark Heart Registry keeps records on all procedures and operations performed on patients admitted for adult cardiac surgery in the western part of Denmark. Missing data were obtained from the medical records, if possible. All patients, who underwent CABG with or without concomitant cardiac surgery, were included in the study. If patients had undergone cardiac surgery more than once during the study period, only the last operation was included in the study to reduce confounding factors related to surgery during the follow-up period.

2.2. Clinical procedures and indications for allogeneic blood transfusions

Standard surgical and cardiopulmonary bypass techniques as well as postoperative care were used in the two hospitals. The use of prophylactic antibiotic regimens and type of cardioplogia differed between the hospitals. Patients operated in Aalborg Hospital received 1.5 cefuroxime t.i.d. together with gentamicin 240 mg q.d. for 48 h. Patients operated in Skejby Hospital received dicloxacillin 1 g t.i.d. and one dose of teicoplanin for 24 h, according to body weight. Cold blood cardioplegia was used in Aalborg Hospital, whereas cold crystalloid cardioplegia was used in Skejby Hospital. Postoperative direct reinfusion of unwashed mediastinal blood was used as a routine during most of the study period in both centers but was abandoned as a routine at Aalborg Hospital from September 2007 onward. Preoperative autologous blood donation and prophylactic transfusions of plasma and platelets (PLTs) were never used. Acute normovolemic hemodilution, cell saving, and hemofiltration were rarely used.

RBC products transfused during the study period were buffy-coat-reduced RBCs suspended in saline—adenine—glucose—mannitol unless white blood cell-depleted products filtered before storage were specifically ordered. Whole blood was never used. PLTs transfused were predominantly pooled units of random-donor whole blood-derived PLT concentrates. Apheresis PLT derived from a single donor was seldom used (<1.5% of PLT transfusions).

Indications for per- and postoperative allogeneic RBC transfusions were at the discretion of the attending surgeon and anesthesiologist. No practice guidelines or recommendations for transfusions were used rigorously.

2.3. Data on blood transfusions

We obtained data regarding the number of allogeneic RBC and PLT units transfused to the patients during hospitalization for CABG through local databases maintained by the blood banks in the two hospitals. In addition, we obtained information about storage time of the RBCs and ABO type of transfused blood products from the blood banks. We classified patients as having received none or ≥1 unit of allogeneic RBC. Furthermore, we classified transfused patients according to the number of transfused RBC units as well as the storage time of transfused RBC (<14 days vs ≥14 days). This cutoff point was chosen because the mean storage time of transfused RBC was 14 days, and because storage lesions become more prominent over time

2.4. Data on severe postoperative infections

The primary endpoint of this study was a composite of severe postoperative infections comprising deep sternal wound infection, septicemia, and bacteremia. Patients who developed deep sternal wound infections within 90 days postoperatively were identified through the National Registry of Patients as patients who underwent reoperation due to purulent infection involving the sternum and/or the mediastinum. The National Registry of Patients was established in 1977 and includes data on all hospitalizations from non-psychiatric Danish hospitals, including dates of admission and discharge, surgical procedure(s) performed, and up to 20 discharge diagnoses and procedures coded according to the International Classification of Diseases (8th revision (ICD-8) until the end of 1993, and 10th revision (ICD-10) thereafter). Patients with postoperative bacteremia and/or septicemia within 90 days after surgery were identified through local databases at the departments of clinical microbiology as patients having positive blood cultures postoperatively. A complete registration of all bacteremias in the North Denmark Region has been undertaken since 1996 using the North Denmark Bacteremia Research Database [9]. A similar local database was used in Skejby Hospital, thus ensuring that all cases of bacteremia/septicemia were identified during the study period.

2.5. Data on possible confounding variables

To take into account factors associated with both use of allogeneic blood transfusion or bleeding and the risk of
severe postoperative infection, we obtained the following data from the National Registry of Patients, Western Denmark Heart Registry, laboratory databases, and medical records: age, gender, body mass index (BMI), preoperative hemoglobin, preoperative creatinine, ABO blood group of patients and ABO blood group of transfused blood products (RBCs and PLTs), reoperation due to bleeding, use of extracorporeal circulation, concomitant cardiac surgery, and comorbidity. The patients were classified as having received a major ABO mismatched PLT transfusion if at least one PLT transfusion was classified as major ABO mismatched. The patients were classified as having received minor ABO mismatch if at least one PLT transfusion was classified as minor ABO mismatched and major mismatch did not occur. Charlson comorbidity index score was calculated for each patient as a summary measure of the patients’ comorbidity profile. The Charlson index covers 19 major disease categories, including acute myocardial infarction, congestive heart failure, cerebrovascular disease, and cancer, weighted according to their prognostic impact on patient survival [10]. The index was adapted for use with hospital discharge registry data in ICD databases [11], and has previously been used to adjust for comorbid diseases in relation to bacteremia [12]. A weight was assigned to each comorbid disease category and the score is the sum of these weights. We defined three levels of comorbidity for each patient, based on the Charlson comorbidity index score. The Charlson comorbidity index score was calculated from the entire available hospital discharge history preceding the date of surgery: score of 0 (no recorded underlying diseases included in the Charlson index); score of 1—2, and >2. Diabetes was excluded from the index and categorized as a separate variable. To optimize the sensitivity of the diabetes diagnoses, we combined information from the National Registry of Patient with data from population-based prescription registries. Prescription databases maintained in the regions retain information on all redeemed prescriptions for refundable drugs, including type of drug and date of prescription. We identified all patients with either a hospital discharge diagnosis of diabetes with or without end-organ disease or at least one filled prescription for insulin or an oral antidiabetic drug. The predictive value of a diagnosis of diabetes identified by this approach has been estimated to be 97% (95% confidence interval (CI): 89—100) [13].

### 2.6. Statistical analyses

Initially, we compared baseline characteristics, at the time of operation, among transfused and non-transfused patients using the Likelihood ratio test from a logistic regression with group as response and all variables from Table 1 included against the model where all variables are excluded. We then used logistic regression to compute the odds ratio (OR) with 95% CI for development of a severe infection according to transfusion of allogeneic RBCs and storage time of the blood. Instead of testing patient groups against controls who did not receive transfusions, a common model was applied using two indicator variables encoding RBC < 14 days and ≥ 14 days. Patients who received a mixture of blood stored for < 14 days and ≥ 14 days were excluded. We adjusted for place of surgery, age, gender, BMI, preoperative hemoglobin concentration, diabetes mellitus, reoperation due to bleeding, use of cardiopulmonary bypass, concomitant valve surgery, Charlson comorbidity index score (modified by excluding diabetes from the index), number of transfused RBC and PLT units, ABO blood group of the patient, and major- and minor PLT ABO mismatch. ABO blood group was included as a categorical variable with five categories: O, A, B, AB, and unknown. We also did an alternative analysis using a stepwise backward procedure with the significance level for removal from model set to 20%. We did not adjust for creatinine values because too many values were missing. We performed adjustment with and without excluding patients among whom data on preoperative hemoglobin value were available. The proportions of heavily transfused patients defined as patients transfused with >5 units of RBC were compared using the Pearson’s chi-square test. The dose—response relationship was assessed using the number of RBC transfusions as a continuous variable. A possible trend was assessed by including the category of transfusion (0: non-transfused, 1: storage time < 14 days, and 2: storage time ≥ 14 days) as a continuous variable. Statistical analyses were

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### Table 1. Baseline demographical and surgical characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-transfused (red blood cells) (n = 2492)</th>
<th>Transfused (red blood cells) (n = 1748)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD, years</td>
<td>66 ± 10</td>
<td>70 ± 9</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>2197 (88)</td>
<td>1190 (68)</td>
</tr>
<tr>
<td>BMI (kg/m²), mean ± SD</td>
<td>28 ± 6</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>446 (18)</td>
<td>338 (19)</td>
</tr>
<tr>
<td>Hemoglobin (mmol/l), mean ± SD</td>
<td>7.8 ± 1.5 [106]</td>
<td>6.9 ± 1.4 [68]</td>
</tr>
<tr>
<td>Creatinine (µmol/l), mean ± SD</td>
<td>85 ± 45 [416]</td>
<td>99 ± 70 [255]</td>
</tr>
<tr>
<td>Comorbidity score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0, n (%)</td>
<td>1113 (45)</td>
<td>569 (33)</td>
</tr>
<tr>
<td>1—2, n (%)</td>
<td>1191 (48)</td>
<td>899 (51)</td>
</tr>
<tr>
<td>&gt;2, n (%)</td>
<td>188 (8)</td>
<td>280 (16)</td>
</tr>
<tr>
<td>Place of surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aalborg Hospital, n (%)</td>
<td>942 (38)</td>
<td>834 (48)</td>
</tr>
<tr>
<td>Skejby Hospital, n (%)</td>
<td>550 (62)</td>
<td>914 (52)</td>
</tr>
<tr>
<td>Use of ECC, n (%)</td>
<td>1896 (76)</td>
<td>1443 (83)</td>
</tr>
<tr>
<td>Concomitant cardiac surgery, n (%)</td>
<td>357 (14)</td>
<td>535 (31)</td>
</tr>
<tr>
<td>Reoperation due to bleeding</td>
<td>65 (3)</td>
<td>269 (15)</td>
</tr>
</tbody>
</table>

BMI: body mass index; Comorbidity score: Charlson comorbidity index score; ECC: extracorporal circulation. The number of patients with missing values on hemoglobin and creatinine are mentioned in square brackets.
performed using Stata version 10.1 (StataCorp LP, College Station, TX, USA).

3. Results

We identified 4279 patients who underwent CABG during the study period. Thirty-four patients were excluded because they died within 48 h postoperatively. Furthermore, five patients were excluded due to missing values regarding BMI leaving 4240 patients available for the analyses. Baseline and operative characteristics of the patients are shown in Table 1. The single P-value comparing baseline characteristics between transfused and non-transfused patients derived from the Likelihood ratio test is <0.001. A total of 1748 patients (41%) were transfused with allogeneic RBCs. The transfusion rates differed between the two participating hospitals. A total of 47% and 37% of the patients received transfusion of RBCs in Aalborg and Skejby Hospital, respectively (P < 0.001). Transfused patients tended to be older on average, had more comorbid illnesses, and more often concomitant cardiac surgery. The storage time of RBC transfused during the study period is shown in Fig. 1. The mean storage time of RBC transfused to the patients was 14.0 days (range: 1—35 days). A total of 953 patients exclusively received RBC stored for <14 days and 548 patients exclusively received blood stored for ≥14 days. The transfused RBC units were predominantly ABO blood group O (42%) and ABO blood group A (42%).

A total of 165 patients (3.9%) developed severe postoperative infection. Deep sternal wound infection was identified in 96 patients (2.2%). Twenty-one of these patients (1.2%) also experienced septicemia/bacteremia and 69 patients (2.1%) developed septicemia/bacteremia without deep sternal wound infection. Among patients who developed severe infection were 44 patients who exclusively were transfused with blood stored for <14 days and 38 who exclusively were transfused with blood stored for ≥14 days. Crude and adjusted ORs with 95% CI for the development of severe postoperative infections according to transfusion and storage time of RBC are shown in Table 2. The OR estimates were similar whether or not patients with missing hemoglobin values were excluded from the analyses. Allogeneic RBC transfusion was associated with an increased risk of developing severe infection, although the overall risk did not reach statistical significance. When stratifying the analysis according to storage time of RBC, it appeared that the increased risk was restricted to the patients who received RBC stored for ≥14 days. These patients were 2.3 times more likely to develop severe infection compared with those not transfused. By contrast, transfusion with RBC exclusively stored for <14 days was not associated with any increased risk for severe infection. In the alternative analysis using stepwise backward selection, the adjusted ORs for severe infection among all transfused patients and patients trans fused with RBC stored exclusively for either <14 days or ≥14 days were 1.3 (95% CI: 0.8—2.2), 0.8 (95% CI: 0.5—1.5), and 1.7 (95% CI: 1.0—2.9), respectively, when compared with non-transfused patients.

Fig. 2 shows a histogram displaying the distribution of numbers of RBC units transfused to each patient. The proportion of heavily transfused patients defined as patients receiving less than 5 units of RBCs differed significantly between the compared groups (P < 0.05). Twelve percent of the patients who exclusively received blood stored for <14 days and 4% of the patients who exclusively received blood stored for ≥14 days were transfused with ≥5 units of RBCs. There was an adjusted dose—response relationship between the numbers of transfused RBC units and the risk of developing severe infection. The unadjusted risk of severe infection was increased by 17% for each transfused unit of RBC among patients who exclusively received blood stored for ≥14 days (OR 1.17; 95% CI: 1.07—1.27), and, after confounder adjustment, increased by 23% (OR: 1.23; 95% CI: 1.11—1.35) for each unit of RBCs transfused. The dose—response relationship was weaker among patients exclusively

### Table 2. Crude and adjusted odds ratios (OR) with 95% confidence intervals (CI) for the development of severe postoperative infection according to transfusion and storage time of transfused red blood cells.

<table>
<thead>
<tr>
<th></th>
<th>All patients (n = 4240)</th>
<th>Patients developing severe infection (n = 165)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-transfused</td>
<td>2492</td>
<td>55</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Transfused (red blood cells)</td>
<td>1748</td>
<td>110</td>
<td>3.0 (2.1—4.1)</td>
<td>1.6 (0.9—2.8)</td>
</tr>
<tr>
<td>Storage time ≤14 days</td>
<td>953</td>
<td>44</td>
<td>2.1 (1.4—3.6)</td>
<td>1.2 (0.6—2.1)</td>
</tr>
<tr>
<td>Storage time &gt;14 days</td>
<td>548</td>
<td>38</td>
<td>3.3 (2.6—5.4)</td>
<td>2.5 (1.2—4.2)</td>
</tr>
</tbody>
</table>

The ORs were adjusted for age, gender, body mass index, preoperative hemoglobin concentration, diabetes mellitus, reoperation due to bleeding, use of extracorporal circulation, concomitant cardiac surgery, hospital of surgery, Charlson comorbidity index score, number of transfused red blood cell units, number of transfused platelet units, ABO blood group of the patient, minor and major ABO-incompatibility of platelet transfusions.

Fig. 1. Distribution of storage time of red blood cell units transfused to 1,748 patients undergoing coronary artery bypass grafting with or without concomitant cardiac surgery.

![Figure 1](image-url)
have been interesting to perform stratified analyses, storage time in the blood banks [19] and it would therefore in infection, in cardiac surgery [18].

transfusions have been associated with immune-complex incompatible PLT transfusions because ABO-incompatible PLT transfusions, we extend their findings. We adjusted for ABO-transfusions and ABO major- and minor mismatched PLT transfusions, however, were not accounted for in the study by Koch et al. and by adjusting, for example, for the number of PTL transfusions and ABO major- and minor mismatched PLT transfusions, we extend their findings. We adjusted for ABO-incompatible PLT transfusions because ABO-incompatible PLT transfusions have been associated with immune-complex formation and increased morbidity, including bleeding and infection, in cardiac surgery [18].

ABO groups are not normally distributed according to storage time in the blood banks [19] and it would therefore have been interesting to perform stratified analyses, according to ABO blood groups. However, the outcome numbers of severe infections were too small to allow conclusive stratified analyses.

We only focused on severe infections because milder kinds of postoperative infections may not have been sufficiently registered. The validity of a diagnosis of deep sternal wound infections is assumed to be high in the present study because, in contrast to superficial sternal wound infections, which can be handled in local hospitals, all patients with deep sternal wound infection are returned to either Skejby or Aalborg Hospital for further surgical treatment.

Studies regarding the association between transfusion and the development of severe infection have also been performed outside cardiac surgery [20]. A recent systematic literature review of 24 studies concluded that it is difficult to determine whether there is a relationship between the storage time of RBCs and outcome in adult patients, except for a possible association between transfusion of blood stored for > 14 days in trauma patients receiving massive transfusion and the risk of increased morbidity or mortality [21]. The studies were very heterogeneous with respect to study populations, outcome measures, storage time of the RBCs, and other methodological aspects.

Biomechanical and biochemical changes of RBCs during storage have been proposed as a biologically plausible explanation for the negative postoperative outcomes associated with allogeneic RBC transfusions. These storage lesions include a reduction in RBC deformability, altered RBC adhesiveness and aggregability, reduction in intracellular 2,3-diphosphoglycerate and adenosine triphosphate (ATP), and accumulation of immunomodulating bioactive substances released from leukocytes to the storage medium [7]; however, there is no clear evidence to support a causal role of these mechanisms in relation to the development of severe postoperative infections.

Our study has a number of strengths, including the population-based study design, the use of prospectively collected and detailed data, the complete follow-up, and the relatively large sample size. Further, we were able to adjust for several confounders not accounted for in previous studies.

The study also has some limitations. First of all, it is an observational study comparing two groups with all the inherent risks of residual or unaccounted confounding. We did not adjust for all factors that may be associated with the risk of bleeding/transfusions, for example, use of antifibrolytic drugs, time on cardiopulmonary bypass, duration of surgery, preoperative hemoglobin, surgeon’s experience, and urgency of surgery. However, it is less likely that any of these factors are associated with storage time of RBCs and we consider the impact of these factors on our findings to be most likely modest.

The transfusion of white blood cell (WBC)-containing allogeneic RBC products has been associated with an increased risk of postoperative infection in cardiac surgery [22]. Most of the patients in the present study received buffy-coat-reduced RBCs. Therefore, the results cannot necessarily be generalized to clinical settings where WBC-reduced blood products are used. In addition, these results cannot be applied to centers transfusing predominantly apheresis PTLs, which are derived from a single donor, because pooled units

![Fig. 2. Number of red blood cell units transfused to each of 1,748 patients undergoing coronary artery bypass grafting with or without concomitant cardiac surgery.](image)
of random-donor whole blood-derived PTL concentrates were predominantly transfused in the present study.

Studies on the impact of storage time of RBCs may be carried out comparing different groups with respect to either mean storage time of transfused blood, storage time, amounts of the oldest blood, or different mix of storage time. We decided to divide transfused patients into two groups that exclusively received either blood stored for either <14 days or ≥14 days because outcome following transfusion based on a mixture of blood with different storage times may be even more difficult to interpret when observational studies are performed.

The transfusion of other blood products, that is, PLT and plasma, may also be associated with adverse outcomes, including infections. Only few studies have investigated the possible association between PLT transfusion and post-operative adverse outcomes, including postoperative infections in cardiac surgery. The results are conflicting but taken together they do suggest that transfusion of PLT does not confer increased risk for morbidity or mortality [23,24]. We did not examine the risk experienced by patients, who were exposed to PLT and plasma in addition to RBC transfusions, except for adjusting for the number of PLT transfusions and ABO PLT mismatch.

In our opinion, there is currently not sufficient evidence for surgeons and anesthesiologists to request ‘fresh blood’ for cardiac surgical patients, but results from observational studies, including our study, highlight the need for randomized studies in this field. Randomized studies investigating the impact of storage time on outcome following transfusion seem feasible [25] and the results from ongoing randomized clinical studies are eagerly awaited.

In conclusion, bearing the inherent risk of confounding in mind, this large population-based follow-up study strongly supports the hypothesis that transfusion of allogeneic RBCs stored exclusively for ≥14 days is associated with a dose-dependent increased risk of severe postoperative infection in patients undergoing CABG compared with non-transfused patients and patients transfused with RBCs exclusively stored for <14 days. These findings warrant further attention to the role of storage time of transfused RBCs, preferably by clarifying the impact of storage time in randomized clinical trials.

References


Appendix A

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