Patient Blood Management in Elective Orthopaedic Surgery

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Aan mijn ouders

Contents

Chapter 1

General Introduction

Current transfusion alternatives in elective orthopaedic surgery

Total hip and knee prosthesis surgery is performed worldwide about two million times annually (approximately 50.000 in the Netherlands). These surgeries may result in significant intra- and postoperative blood loss (800 to1500mL) with a potential need for allogeneic Red Blood Cell (RBC) transfusions to compensate for the resulted anaemia. Although blood transfusions are relatively safe, transfusion reactions such as fever, haemolysis, antibody formation, Transfusion Associated Circulatory Overload (TACO), Transfusion Related Acute Lung Injury (TRALI), or transmission of infectious agents may occur. Furthermore, Natural Killer (NK) activity was found to be decreased in patients after an allogeneic RBC transfusion compared to no RBC transfusion or peri-operative autologous wound blood re-infusion [1]. It has been postulated that, immuno-modulatory effects of blood transfusions may result in an increased susceptibility for infections in the postoperative period [2-7]. In the field of orthopaedic surgery, there is an ongoing trend to aim for optimal Patient Blood Management (PBM). PBM is a new concept in Transfusion Medicine that is based on three approaches (pillars): 1. optimising the patient's own blood; 2. minimising surgical blood loss and bleeding; and 3. harnessing and optimising the patient-specific physiological reserve of anaemia (including restrictive transfusion thresholds) (http://www.health.wa.gov.au/bloodmanagement/home.health_professionals.cfm). This approach includes pre-operative, intra-operative and post-operative strategies for managing the patient, such as alternatives for RBC transfusions, but also surgical and anaesthesiological strategies. A comprehensive overview of blood conservation strategies in major orthopaedic surgery in the European setting is published in 2009 by Munoz et al [8]. In this overview, several of these strategies are discussed, such as correction of perioperative anaemia, pharmacological and non-pharmacological measures to reduce blood loss, preoperative autologous donation, and perioperative blood salvage. Based on the efficacy and safety of these strategies in literature, recommendations are offered. However, some recommendations are not supported by a high level of evidence due to a lack of appropriate data.

TRANSFUSION PRACTICE

A large variation in transfusion practice is present. Recently, in the Austrian benchmark study, Gombotz and colleagues found a transfusion rate varying from16 to 85% for patients undergoing primary total hip replacement (THR) and a 12 to 87% transfusion rate for patients undergoing primary total knee replacement (TKR) surgery [9]. The Orthopaedic Surgery Transfusion Haemoglobin European Overview (OSTHEO) study of Rosencher and co-workers assessed standard practice in blood management in six countries in Europe (225 centres, n=3996 patients) and found that, despite existing guidelines, a large percentage (21%) of the pre-transfusion Hb levels were greater than or equal to 10 g/dL and 10% even exceeded the level of 13.0 g/dL [10]. An additional problem is the implementation of

guidelines in daily practice, which is often difficult to achieve. This is not only relevant for implementing transfusion thresholds, but also for the number of transfusions at each event to reach a particular target Haemoglobin (Hb) level. Barr and co-workers investigated red blood cell transfusion practice in Northern Ireland in 2005 and still found a two-unit instead of single-unit transfusion practice in medical and surgery patients (n=1474) [11].

BLOOD TRANSFUSION PROTOCOLS

An important blood saving strategy is the use of a restrictive transfusion protocol. In 1988, the NIH published consensus guidelines for red blood cell transfusions [12]. Since then, several guidelines have been published, recommending that a range of Hb levels between 6 and 10 g/dL can be used, depending on the presence of serious co-morbidity [13-15]. These clinical practice guidelines, however, have based their recommendations on data from published reports on series of patients for whom red cell transfusions were withheld (for instance Jehovah's witnesses), and observational studies, rather than on the results of clinical trials. Since June 2004, the 4-5-6 Flexinorm transfusion trigger (Hb values in mmol/L), based on the NIH guidelines, in surgical setting was recommended in the Dutch national Consensus guideline for Blood Transfusion (CBO) [16]. This transfusion trigger policy is based on parameters as Hb level, age and condition of the patient (ASA criteria). In 2010, a Cochrane review of Carless and co-workers reported on seventeen Randomised Controlled Trials (RCTs) including surgical and medical patients, and concluded that a restrictive transfusion policy can reduce the need of receiving a RBC transfusion (further mentioned as *transfusion avoidance*) with 37% (RR 0.63 [95% CI 0.54 to 0.74]; however with a non-significant mean RBC reduction (further mentioned as *RBC sparing*) of 0.75 RBC unit [95% CI 0.20 to 1.3]). However, methodology was poor and significant heterogeneity between studies was present [17]. Only one study (Lotke 1999) reported on elective orthopaedic surgery patients who also donated pre-operatively 2 units of autologous blood (knee replacement surgery, n=152), which resulted in an increased allogeneic transfusion avoidance of 74% (RR 0.26 [95% CI 0.17 to 0.40]).

CURRENT ALTERNATIVES FOR RED BLOOD CELL TRANSFUSIONS

Many alternatives for an allogeneic RBC transfusion are available. Not all interventions are widely applied in the Netherlands. Next to efficacy, important factors that are of influence are costs and user friendliness. Based on recent randomised controlled studies, the efficacy of the different modalities will be discussed, first in general and in more detail for the elective

orthopaedic surgery patients. These alternatives for an allogeneic blood transfusion can be subdivided in two main groups:

A. Non-pharmacotherapeutical interventions:

1. *pre*-operative alternatives: Preoperative Autologous Donation (PAD)
2. *peri*-operative alternatives:
   a. Acute Normovolaemic Haemodilution (ANH), in which one or several units of whole blood is taken just before surgery and at the same time (=isovolaemic) the lost blood volume is replaced by normal saline or colloids. The retained whole blood is then transfused back to the patient during or after surgery;
   b. use of the cell saver, that collects autologous wound blood *during* (and sometimes *after*) surgery. The shed blood is washed, concentrated and then re-infused.
3. *post*-operative alternatives: devices that collect and re-infuse autologous wound blood *after* surgery (non-washed, filtered by several types of devices) by means of a wound-drainage and re-infusion system.

B. Pharmacotherapeutical interventions

This concerns the pre-operative use of Erythropoietin (Epo) and the peri-operative use of anti-fibrinolytics (e.g. aprotinin, tranexamic acid) and fibrin glue. The use of (intravenous) iron as a new modality will be discussed in chapter eight of this thesis.

CURRENT EVIDENCE ON TRANSFUSION ALTERNATIVES IN THE *GENERAL SURGICAL* POPULATION

Ad A Non-pharmacotherapeutical interventions

A Cochrane review by Henry and co-workers (2010) reported on 13 trials (n=1506) and concluded that **PAD** as a single intervention resulted in a significant transfusion avoidance of 68%. However, autologous donors were more likely to undergo transfusion with allogeneic and/or autologous blood (OR 1.24; 95% CI 1.02 to 1.51). The authors concluded, that overall transfusion rates were very high, raising the question of the true benefit of PAD.

In 2004, two systematic reviews reported on the use of **ANH** in elective surgery [18,19]. Carless and co-workers performed a systematic review on several autologous transfusion techniques (PAD, ANH and cell saver). Of 30 trials, ANH resulted in a significant transfusion avoidance of 31%. Mean RBC use was reported in 7 trials resulting in a significant mean RBC sparing of 1.9 units. However, studies were small, and methodology was judged as poor. Also, the blood sparing effect was less when a transfusion protocol (in 60%) was used. [18] Segal and co-workers compared 42 RCTs on ANH, but did not find a significant reduction in transfusion avoidance compared to controls, and a non-significant increase of 11%

compared to another blood conservation method (mostly PAD). They concluded that use of ANH can not be encouraged [19].

The effect of washed autologous salvaged blood (by means of cell saver) or non-washed salvaged blood (by means of post-operative re-infusion systems) on RBC use was investigated In a Cochrane review [20]: 75 randomised studies up to 1 June 2009 were analysed. The authors concluded a significant overall transfusion avoidance of 38% and mean RBC sparing of 0.68 RBC unit.

Table 1. Transfusion avoidance and mean Red Blood Cell (RBC) reduction of transfusion alternatives in the general surgical population

Author (year)	Transfusion alternative	% transfusion avoidance	RR [95% CI]	Mean RBC reduction/patient [95% CI]
Henry (2010) [37]#	PAD	68%	0.32 [0.22 to 0.47]	NA
Carless (2004) [18]#	ANH	31%	0.69 [0.56 to 0.84]	1.9 [1.1 to 2.7]
Segal (2004) [19]	ANH	4% (NS)	0.90-1.01 [NA]	NA
Carless (2006) [20]#	Autologous blood salvage	38%	0.62 [0.55 to 0.70]	0.68 [0.49 to 0.88]
Laupacis (1998) [21]	Epo (cardiac surgery)	75%	0.25 [0.08 to 0.82]	NA

#=Cochrane review
Abbreviations: CI=Confidence Interval; PAD=Preoperative Autologous Donation; ANH=Acute Normovolaemic Haemodilution; NA=Not Available; NS=Not Significant; Epo=Erythropoietin.

Ad B Pharmacotherapeutical interventions

In a meta-analysis by Laupacis and co-workers [21] , Epo resulted in a significant transfusion avoidance (with or without the combination with PAD) with the largest effect (75% avoidance) in cardiac surgery patients (Table 1). A systematic review on the use of anti-fibrinolytics for minimising perioperative allogeneic blood transfusion and on their adverse events was published in 2007 and updated in 2011 by Henry and co-workers, comparing aprotinin, TraneXamic Acid (TXA) and Epsilon AminoCaproic Acid (EACA). [22] That review of over 250 clinical trials reported on the use of anti-fibrinolytic drugs in major surgery and found that these reduced bleeding, as well as the need for transfusions of red blood cells. Consequently, the need for revision surgery because of bleeding (in cardiac surgery) was reduced as well. This update was especially focussed on safety, since the results of the BART study (2008), a study in cardiac surgery patients comparing aprotinin to TXA and to EACA, showed that aprotinin was significantly associated with increased mortality compared to the lysine analogoues TXA and EACA. This resulted in abandoning the use of aprotinin in patients [23]. This finding suggests a potential bias of under-reporting adverse events, since

only 18 of 76 trials of aprotinin reported adverse events. Also, more studies with positive effects were published, resulting in asymmetric funnel plots, suggesting publication bias. The explanation for the increased mortality rates after aprotinin in comparison to lysine analogues might be due to a direct adverse effect by aprotinin or due to a protective effect of the lysine analogues. The lysine analogues TXA and EACA appeared to be safe.

Current evidence on transfusion alternatives in *elective hip-and knee replacement surgery* patients

Ad A Non-pharmacotherapeutical interventions:

Ad 1 Preoperative alternatives

Preoperative autologous donation (PAD)

Five trials reported on orthopaedic surgery patients (n=425) and showed a significant transfusion avoidance of 79%. However, autologous orthopaedic donors were more likely to have an increased overall transfusion rate with allogeneic and/or autologous blood (OR 1.78; 95% CI 0.61 to 5.20). In The Netherlands, PAD is predominantly collected at Sanquin Blood Supply (donor centres). Only one hospital, the Sint Maartenskliniek, a specialised orthopaedic centre in Nijmegen, has implemented this procedure as an in-house procedure within their own hospital setting.

The modality of PAD is relatively expensive and complex and needs a fixed surgery date (the patient needs to visit the centre several times to donate blood). In addition, the likelihood for mistakes by switching of blood products is high [24]. The British guidelines only advise PAD in exceptional cases if the normal donor stock is not sufficient as in cases of rare blood group typing and/or antibodies against public antigens etc [25]. The new CBO Guidelines (Richtlijn Bloedtransfusie) 2011 advises a limited use of PAD due to the complex logistics, the relative high costs, the lack of additional safety and the waste of plasma products (side product of PAD) and recommends for it's use for certain indications, like lack of compatible blood units in case of rare blood groups or antibodies to public or multiple red blood cell antigens and the occurrence of former haemolytic transfusion reactions with unknown cause.

Ad 2a Acute Normovolaemic Haemodilution (ANH)

ANH is not often applied in the Netherlands, possibly due to its logistical difficulties. In the two systematic reviews of Carless and Segal, ANH was investigated in 6 and 13 orthopaedic trials, respectively. Both did not report a significant benefit of ANH on transfusion avoidance. Compared to another blood sparing modality (PAD or TXA), ANH was less effective in orthopaedic patients [19]. As a transfusion alternative, ANH is therefore not recommended in knee-and hip surgery.

Ad 2B en 3

Autologous re-infusion (cell saver and post-operative re-infusion systems)

In a Cochrane review [20] evaluating all randomised studies up to 1 June 2009, in which the effect on RBC use of washed shed blood (by means of cell saver) or non-washed shed blood by means of post-operative re-infusion systems was investigated, 36 studies reported on orthopaedic surgery (6 of which had been conducted in the Netherlands). The authors concluded a significant transfusion avoidance of 54% and a significant mean RBC sparing of 0.82 RBC unit per patient as well. The outcomes were similar using washed or non-washed blood. The authors also concluded that the methodological quality was poor and that the findings could have been influenced by bias. There was a lack of concealment, meaning that allocation of the randomisation was not centralised, but often on location by drawing an opaque envelope. This method can be susceptible to bias, because it can not be ruled out that the envelopes are drawn in the proper order [26].

Table 2. Transfusion avoidance and mean red blood cell (RBC) reduction of transfusion alternatives in the elective knee-and hip replacement surgical population

Author (year)	Transfusion alternative	% transfusion avoidance	RR [95% CI]	Mean RBC reduction / patient [95% CI]
Henry (2010) [37]#	PAD	79%	0.21 [0.11 to 0.43]	NA
Carless (2004) [18]#	ANH	21%	0.79 [0.60 to 1.06]	NA
Segal (2004) [19]	ANH	23% to none	0.77 to 1.06 [0.47 to 1.37]	NA
Carless (2006) [20]#	Autologous blood salvage	54%	0.46[0.37 to 0.57]	0.82 [-0.27 to -1.36]
Laupacis (1998) [21]	Epo with PAD	58%	0.42 [0.28 to 0.62]	NA
idem	Epo only	64%	0.36 [0.24 to 0.56]	0.14 [-0.04 to 0.31]
Fergusson (submitted)	Epo	56%	0.44 [0.31 to 0.64]	0.61 [0.22 to 1.01]
Henry (2011) [22]#	APR vs Co (n=1146)	32%	0.68 [0.5 to 0.89]	NA
idem	TXA vs Co (n=1381)	51%	0.49 [0.39-0.62]	NA
idem	EACA vs Co (n=304)	0%	1.00 [0.93 to 1.08]	NA

#=Cochrane review

Abbreviations: APR=APRotinin; PAD=Preoperative Autologous Donation; ANH=Acute Normovolaemic Haemodilution; NA=Not Available; NS=Not Significant; Epo=Erythropoietin; Co=Control group; TXA=TraneXamic Acid; EACA=Epsilon AminoCaproic Acid

Ad B. Pharmacotherapeutical interventions

Erythropoietin (Epo)

Many randomised studies have been published in which the effect of Epo on RBC use has been investigated. Older studies report on the effect of Epo on the efficacy of donating PAD. In a meta-analysis by Laupacis et al [21] , in orthopaedic surgery, Epo resulted in a significant transfusion avoidance of 58% with or 64% without the combination with PAD (11 trials with PAD and 3 trials without PAD), but Epo did not significantly reduce the mean RBC use. The main reason for this finding was the low mean number of transfusions in the control group (0.46 RBC unit/patient), and a subsequent non-significant decrease of 0.14 RBC unit per patient after use of Epo. This finding was confirmed by a large European randomised study (n=695) (the EEST study) by Weber and colleagues [27], who found a proportion of 12% transfused patients in the Epo group which was significantly lower than the 46% transfused patients of the control group ($p<0.001$), but found a non-significant difference in mean RBC use (1.25 RBC/patiënt [SD 0.51] versus 1.42 RBC/patiënt [SD 0.70], respectively; $p=0.14$). A recent meta-analysis by Fergusson and co-workers performed on elective orthopaedic surgery studies until August 2007 (submitted) resulted in 36 evaluable RCTs of which Epo dose varied (>1800 UI/kg or <1800 UI/kg), as well as time of administration (pre-or postoperatively), use of PAD, route of Epo administration (subcutaneously or intravenously) and use of a transfusion threshold (in 15 trials not reported). Overall, compared to the control group, a significant RBC avoidance of 56% was found as well as a significant RBC sparing of 0.61 RBC unit. The aggregated risks of DVT was 3.5%, and 0.20% of myocardial infarction (MI), 0.29% of stroke, 0.15% of PE and 0.13% of death. It was concluded that Epo was a safe blood sparing modality in orthopaedic surgery, however, Epo exceeds the costs of an allogeneic blood transfusion [28,29].

Anti-fibrinolytics

Since trials on anti-fibrinolytics comprised mostly cardiac surgery patients, which are rather different from orthopaedic surgery patients, outcome on RBC use may be quite different between these study populations. The results of trials on orthopaedic surgery patients are discussed here, and when possible, separately reported:

1. Interventional drug versus control group

Of 108 trials of **aprotinin** compared with a control group, 15 were conducted in orthopaedic surgery with a total of 1146 patients. In these patients, the use of aprotinin resulted in a significant transfusion avoidance of 32%, however studies were heterogeneous ($p<0.001$). Total blood loss was also reduced by around 400 mL (Mean difference -399 mL [-563 to -235 mL]) (n=430; 10 studies), heterogeneity was again significant ($p<0.007$). Compared to controls, the use of aprotinin (pooled data) did not result in increased risk for mortality nor

in an increased risk for myocardial infarction or for thrombotic events (stroke, deep vein thrombosis or pulmonary embolus).

Of 65 reported trials on **TXA** compared with controls, 27 involved orthopaedic surgery (n=1381 patients), where a significant transfusion avoidance of 51% was found (RR 0.49, however heterogeneity between trials was present (p<0.0007). Total blood loss was significantly reduced by 446 mL [95% CI of mean difference 338 to 555 mL], also with heterogeneity between trials (p<0.00001).Compared to controls, the use of TXA (pooled data) did not result in increased mortality risk nor increased risk of myocardial infarction or thrombotic events (stroke, deep vein thrombosis or pulmonary embolus).

Of 16 reported trials of **EACA** compared with controls, four trials involved orthopaedic surgery (n=304 patients) of which the use of EACA did not reduce the need for allogeneic RBC transfusions. Two trials reported a marginally effective reduction in total blood loss of 300 mL [95% CI of mean difference 77 to 523 mL]. Mortality risk, risk of myocardial infarction, and thrombotic events were not increased.

2. Comparison between anti-fibrinolytics

A. Aprotinin versus TXA

Of 21 trials on aprotinin versus TXA that reported data on the number of patients exposed to allogeneic RBC transfusions, only one study [30] was performed in orthopaedic surgery patients (knee replacement surgery; n=36). The study consisted of three groups of 12 patients: controls versus TXA versus aprotinin, respectively) and showed no difference in the transfusion rate between all three groups. No adverse outcomes were reported other than deep venous thromboses (n=2 in TXA group, n=1 in aprotinin group and n=0 in control group).

B. Aprotinin versus EACA

Of 12 reported trials, three (Amar 2003;n=69 spine, Ray 2005; n=45 hip, Urban 2001; n=60 spine) were in orthopaedic surgery patients: no significant transfusion avoidance was found with aprotinin compared to EACA (RR 0.82; 95% CI 0.48 to 1.40) [31-33]. The relative risk, however, was comparable to the risk in cardiac surgery patients, in which a significant transfusion avoidance with aprotinin was found (RR 0.82; 95% CI 0.76 to 0.89). Ray and co-workers performed a study on 45 total hip arthroplasty patients and did not find a difference in blood loss between aprotinin and EACA (each 15 patients per group) or in mean RBC reduction or transfusion avoidance.

Pooled data on adverse events (myocardial infarction, thrombotic events) showed no difference between the study- and control groups. In the single hip surgery trial of Ray and co-workers [32] no thrombotic events as DVT or PE were found. Six cardiac adverse events were reported postoperatively in the intervention groups: two non-ST elevation MI, two atrial fibrillation (AF), and two patients with both MI and AF, however this did not reach statistical significance compared to the control group in these 45 patients (p=0.08).

C. TXA versus EACA

Of 8 reported trials (n=2003), only one orthopaedic study (knee replacement surgery; n=127) compared TXA (n=35) with EACA (n=32) and placebo (n=60) and found no difference in blood loss between TXA and EACA or in transfusion avoidance or mean RBC reduction or in adverse events [34].

Conclusions on the role of anti-fibrinolytics in orthopaedic blood management

Compared to controls, both aprotinin and TXA, but not EACA showed significant transfusion avoidance. Compared to one another, only three orthopaedic trials on knee- and hip replacement surgery, were available: no advantage of aprotinin compared to the lysine analogues could be found regarding blood loss and the need for RBC transfusions. None of these studies reported on mortality and only one study reported more cardiac adverse events [32]. Since data are lacking, and numbers were small, no valid conclusions can be drawn regarding the blood sparing effect or regarding adverse events in orthopaedic surgery due to the low numbers of study patients.

Fibrin glue

Carless and co-workers (2009) concluded in a Cochrane review that especially in orthopaedic surgery, where blood loss is often substantial, fibrin sealants appeared to demonstrate their greatest beneficial effects by significantly reducing blood loss and transfusion avoidance by 32% (RR 0.68; 95% CI 0.51 to 0.89), but large RCTs were lacking [35]. Therefore, the authors support initiating well-conducted large clinical trials on this matter.

SCOPE OF THIS THESIS

As discussed, several transfusion alternatives are available to reduce RBC use in elective orthopaedic surgery. Since the patient population eligible for knee-or hip replacement surgery is exponentially growing, expecting more than 100.000 of these type of surgeries in 2030 in the Netherlands [36], optimising peri-operative blood management is not only highly relevant with respect to patient risk and benefit, but also important in terms of cost effectiveness.

However, we found that insufficient evidence was published to provide a strategy for the optimal use these transfusion alternatives: most studies compared only one transfusion alternative with controls, which is inconsistent with daily practice; or studies were of older date, comparing transfusion alternatives as PAD with ANH, both are not widely applied anymore, especially not in the Netherlands; or studies did not use a transfusion protocol or used a liberal transfusion policy, which both may overestimate the blood sparing effect of the intervention.

To gain insight in the transfusion policy, we first evaluated RBC use during 6 months in the orthopaedic ward in the LUMC in 2000 (January to July). We found that a 68% reduction could be reached if a restrictive transfusion policy would be implemented. This was the reason to start a Randomised Controlled Trial (RCT), to investigate the effect of a restrictive transfusion trigger on mean RBC use and transfusion avoidance. To anticipate on a study to evaluate the use of postoperative autologous wound blood we compared the feasibility and effectiveness of different types of post-operative re-infusion devices, that were available in 2003, and to explore their possible use in a larger trial on the combined use of transfusion alternatives. Finally, we were fully prepared to study the effect of combinations of the mostly applied transfusion alternatives, while using a strict transfusion threshold, currently recommended in blood management protocols. The aim of this thesis is to optimise Patient Blood Management by providing evidence for cost-effective measures in elective orthopaedic surgery.

OUTLINE OF THIS THESIS

Chapter 2 describes the results of a RCT on the effect of a restrictive trigger on RBC sparing. In three hospitals, a restrictive transfusion policy was compared with standard care transfusion policy. A randomised comparison of transfusion triggers in elective orthopaedic surgery using leucocyte-depleted red blood cells was performed. The clinical consequences of this restrictive transfusion policy on post-operative complications and well-being are discussed in **Chapter 3.** Quality of Life and fatigue scores in relation to postoperative haemoglobin levels were analysed in **Chapter 4.**

In **Chapter 5** we investigated the efficacy and feasibility of two types of postoperative drainage and re-infusion systems and compared these to a control group. To evaluate the immuno-modulatory effects of salvaged blood in the post-operative patient, we analysed the effect of autologous salvaged blood re-infusion on the patients' cytokine gene expression profiles compared to the effect of surgery itself (**Chapter 6**).

Chapter 7 reports the combined strategies of Epo and autologous salvaged blood on RBC use compared to a control group under a restrictive transfusion policy (TOMaat study). In **Chapter 8,** future trends and ongoing studies are discussed in order to aim for an optimal and Tailor Made Patient Blood Management Program for elective orthopaedic surgery patients. In the final chapter, **Chapter 9**, an implementation protocol is described to investigate the barriers and facilitators for implementation of the TOMaat study results in daily practice.

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Chapter 2

A randomized comparison of transfusion triggers in elective orthopaedic surgery using leucocyte-depleted red blood cells

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ABSTRACT

Objective: In elective orthopaedic hip- and knee replacement surgery patients, we studied the effect of implementation of a uniform transfusion policy on RBC usage.

Study design and methods: A randomized, controlled study. A new uniform, restrictive transfusion policy was compared with standard care, which varied among the three participating hospitals. Only prestorage leukocyte-depleted RBC(s) were used. Primary end-point was RBC usage, related to length of hospital stay. Secondary end-points were Hb levels, mobilisation delay and postoperative complications.

Results: 603 patients were evaluated. Adherence to the protocol was over 95%. Overall mean RBC usage was 0.78 U/patient in the new policy group and 0.86 U/patients in the standard care policy group (mean difference 0.08; 95% CI [-0.3; 0.2]; p=0.53). In two hospitals the new transfusion policy resulted in a RBC reduction of 30% (0.58U RBC/patient) (p=0.17) and 41% (0.29 U RBC/patient) (p=0.05), respectively. In the third hospital, however, RBC usage increased by 39% (0.31 U RBC/ patient) (p=0.02) with the new policy, due to a more restrictive standard care policy in that hospital. Length of hospital stay was not influenced by either policy.

Conclusions: Implementation of a uniform transfusion protocol for elective lower joint arthroplasty patients is feasible, but does not always lead to a RBC reduction. Length of hospital stay was not affected.

INTRODUCTION

Concerns about transfusion-associated complications have stimulated the use of drugs and devices to reduce peri-operative blood transfusions [1-3]. To value such interventions they must be compared with an appropriate and uniform use of allogeneic RBC transfusions. In itself, one of the tools to accomplish a reduction in allogeneic RBC transfusions is a standardized protocol for the use of a restrictive transfusion trigger. Studies on the use of such a protocol in (orthopaedic hip fracture) surgery report an allogeneic RBC reduction between 40 and 80%, depending on the type of restriction used [4-6]. In elective orthopaedic surgery, a randomized study in which different transfusion trigger protocols are compared has never been performed. Additionally, except for studies in paediatric and neonatal patients, all previous studies that compared different transfusion triggers used non leukocyte-depleted RBC units [4,5,7]. We only used pre-storage leukocyte-depleted (LD) RBC(s) and conducted a randomized controlled trial among elective orthopaedic surgery patients in three Dutch hospitals to investigate the effect of a new, restrictive transfusion protocol compared with the standard care on the magnitude of reduction in RBC transfusions and its effects on length of hospital stay (LOHS), postoperative complications and rehabilitation.

PATIENTS AND METHODS

Outcome measures

Primary objective was to investigate whether or not a reduction of RBC usage was associated with a prolonged hospital stay.

Primary outcome was RBC usage. With the new transfusion policy we aimed at a reduction of 40% in RBC use without increasing hospital stay. Secondary end-points were: postoperative Hb values (g/dL) at day +1, day +4 and day +14, mobilization delay (days) and postoperative complication rate.

Inclusion criteria:

All patients of 18 years and older scheduled for a primary or revision total hip replacement (THR)- or total knee replacement (TKR) surgery of three Dutch participating hospitals were eligible for inclusion.

Exclusion criteria:

Refusal of allogeneic transfusions (e.g. Jehovah's witnesses).

Study design

A randomized open study stratified by hospital, type of surgery and risk group. Eligible patients were informed during the preoperative intake at the orthopaedic outpatient clinic and after obtaining informed consent were randomly assigned to a new transfusion policy (protocol A) or standard care (protocol B). The new policy, which was risk level based and uniform among the three participating hospitals, is described in Table 1, and was meant to include the more restrictive transfusion policy. In this policy, age and co-morbidity were determinants for the used risk levels for transfusion. Three clinical risk groups (low, intermediate and high) were defined: age groups less than 50 years, 50 to 70 years, and older than 70 years or presence of significant co-morbidity (i.e. cardiovascular and pulmonary disease, and/or insulin dependent diabetes). The standard care policies, which varied among hospitals, are described in Table 2, and were supposed to include the more liberal transfusion policies. Randomization took place as follows: all patients were stratified by hospital, type of surgery (primary/revision THR/TKR) and risk group. For each stratum a separate randomization list was created, using blocks of variable length to avoid predictability of the random treatment assignment towards the end of each block. Treatment allocation was random using a uniform distribution for a pregenerated list of sufficient length, based on the maximum expected sample size in each stratum. For each subject to be randomized, a sheet of paper with all relevant stratification and group-allocation information was produced and placed in a sealed opaque envelope. Batches were created according to the stratification factors. After receiving informed consent, the patient was preoperatively allocated by the research nurse to one of the groups by opening the first sealed envelope from the appropriate stratum.

Due to a universal leukocyte-depletion policy in the Netherlands, our data comprises only pre-storage LD- RBC(s). Intra-operative transfusions were guarded by the anaesthesiologist and post-operative transfusions by the orthopaedic surgeon. Both were informed about the treatment assignment in order to avoid protocol violations, but they were not involved in the coordination and evaluation of the study. The chart data were written on the Clinical Research Form and placed in the database by the research nurse, who had access to the medical records in which the study assignment was noted. The study investigators, however, were blinded for the randomization arm. Transfusion trigger deviations were regarded as protocol violations. The following postoperative complications were scored: infections, ICU stay, transfusion reactions (defined by the national hemovigilance association), neuro-psychiatric, cardiovascular, haemorrhagic and drug related complications, and death. Post-operative infections were defined according to the CDC criteria [8]. Wound infections were scored according to Gaine et al [9]. Mobilisation was defined according to the hospital protocols (hospital number 1: mobilisation from day +2 onwards, hospitals number 2 and 3: mobilisation from day +1 onwards) and was recorded by the orthopaedic surgeon on the ward. Postoperative discharge from the hospital was based on physical properties of the

patient: they had to be ambulated with a crutch and had to be able to walk a staircase with ease. Hospital number 2 used a short-stay protocol for the most healthy and mobile patients. Follow up ended at the outpatient clinic 14 days after surgery or (in case of a hospital stay of more than 14 days) at final discharge. All patients provided informed consent, and the trial was conducted according to good clinical practices and the Declaration of Helsinki. The study was approved by the Medical Ethical Committees of the three participating hospitals.

Table 1. Transfusion policies: new, uniform restrictive transfusion policy (Protocol A)

Low risk group (patients younger than 50 years of age)					
Within 4 hours of surgery			After 4 hours of surgery		
If Hb	≥6.4 g/dL:	0 RBC	If Hb	≥6.4 g/dL:	0 RBC
	4.8 - <6.4:	1 RBC		5.6 - <6.4:	1 RBC
	<4.8:	2 RBC(s)		<5.6:	2 RBC(s)
Intermediate risk group (patients from 50 to 70 years of age)					
Within 4 hours of surgery			After 4 hours of surgery		
If Hb	≥7.2 g/dL:	0 RBC	If Hb	If Hb ≥8.1g /dL:	0 RBC
	6.4 - <7.2:	1 RBC		7.2 - <8.1:	1 RBC
	<6.4:	2 RBC(s)		<7.2:	2 RBC(s)
High risk group[a] (see below)					
Within 4 hours of surgery			After 4 hours of surgery		
If Hb	≥8.9 g /dL:	0 RBC	If Hb	If Hb ≥9.7 g /dL:	0 RBC
	8.1 - <8.9	1 RBC		8.9 - <9.7:	1 RBC
	7.2 - <8.1:	2 RBC(s)		8.1 - <8.9:	2 RBC(s)
	<7.2:	3 RBC(s)		<8.1:	3 RBC(s)

Hb values were originally in mmol/L (e.g. 4.0 / 5.0 / 6.0 mmol/L) which is common use in the Netherlands
[a]High risk includes one or more of the following:
(i) any heart rhythm different than sinus rhythm.
(ii) unstable cardiac ischemia (by history or ECG)
(iii) myocardial infarction < 6 months
(iv) heart failure
(v) heart valve disease
(vi) age (from 70 years onwards)
(vii) serious peripheral arterial disease, including large vessel surgery (aortic aneurysm, peripheral vessels).
(viii) cerebral arterial disease (CVA or TIA in history)
(ix) hypertension with left ventricular hypertrophy (LVH) (shown on ECG/ echocardiogram)
(x) serious pulmonary disease, expressed in polyglobulism (emphysema / pulmonary fibrosis)
(xi) insulin dependent diabetes mellitus

Table 2. Transfusion policies: standard care transfusion policies (Protocol B)

Hospital number 1 (University Medical Center):

Peri-operative transfusion policy (day 0):
if Hb between 8.1 and 9.7 g /dL and dependent on blood loss: 1-2 RBC(s).

Post operative transfusion policy (from day 1):
if Hb <9.7 g /dL : 2 RBC(s), independent of age, risk status

Hospital number 2 (general hospital):

Peri-operative transfusion policy (day 0):

I keep Hb >6.4 g/dL in case of age < 60 years and ASA[a] class 1

II keep Hb >8.1 g.dL in case of age ≥ 60 years and ASA[a] class 1, 2, 3

III keep Hb >9.7 g/dL in case of ASA[a] class 4 or serious cardiopulmonary disease

[a]American Society of Anesthesiologists

Post operative transfusion policy (from day 1):

I keep Hb > 9.7 g/dL in case of co-morbidity as: IC / CCU admission, uremia, serious heart-, lung- or vessel disease:

II If no co-morbidity exists, the transfusion trigger is age-dependent:

Age (years)	**Hb (g/dL)**
>70	10.5
50-70	9.7
25-50	8.9
<25	8.1

Hospital number 3 (general hospital):

Peri-operative transfusion policy (day 0):
if Hb <9.7 g/dL and dependent on (expected) blood loss: 2 RBC(s)

Post operative transfusion policy (from day 1):

I Patients with cardiac history:

if Hb <9.7 g/dL: 2 RBC(s)

II Patients without cardiac history if symptomatic (nausea, dizziness, tachycardia, general malaise, paleness):

if Hb 7.2 g/dL – 8.1 g/dL: 2 RBC(s)

III If Hb ≤7.2 g/dL: 2 RBC(s)

Sample size calculation

The initial sample size calculation (power 0.90; alpha 0.05) was based on pilot data of hospital number 1, from which a 40% RBC reduction (in terms of RBC units divided by the total patients in each randomization group) was expected by introducing the new transfusion policy. Since the main statistical analysis is a comparison of group means, the reduction for which the study was powered, was transformed to an absolute reduction from an estimated mean RBC use of 2.6 units (SD 2.4) in one group to a mean RBC use of 1.6 units (SD 2.4) in the

other group. A t-test with adjustment for possibly non-normally distributed data needed 2 groups of 125 patients to achieve 90% power for a treatment effect of 1 RBC unit at a pooled SD of 2.4, while at the same time powering with 90% for equivalence in length of stay with a delta of at most four days between the groups. At the time the study protocol was designed a mean hospital stay of 10 to 12 days was usual, therefore a prolongation of hospital stay of more than four days was not acceptable from a clinical point of view. To adjust for non-evaluable patients, each of 3 hospitals had at least to randomize 100 patients. An interim analysis was performed after the first 125 patients became evaluable. A formal stopping rule was pre-specified to enable the trial to stop for futility as well as efficacy, using a simple Bonferroni correction for multiple testing (alpha=0.025). This pre-specified rule also included the condition of a maximal prolongation of hospital stay of four days in the new policy group, which was expected to include the most restrictive transfusion policy: so if RBC use was significantly lower in the new policy group, but hospital stay increased by more than four days, the new transfusion policy was considered not to be clinically nor economically beneficial. A much lower percentage of patients who were actually transfused (33% in stead of the expected 75%, which was calculated from the pilot study), irrespective of hospital and trial arm, resulted in an adjustment of the group sizes, leading to two groups of 300 evaluable patients.

Statistical analysis

Analysis was performed on an intention-to-treat basis and for the parametric analyses stratified by all stratification factors in the design. Frequencies were described as mean and SD, and in addition median and interquartile range in case of a non-normal distribution. Analysis of laboratory parameters between patients and other numerical end-points was performed with the ANOVA-test for between-group comparisons and by a paired t-test (or a mixed model) for within-patients effects. Differences between the groups in the number of RBC transfusions and the total number of units RBC transfusions given were analysed with the non-parametric Mann-Whitney test. Categorical end-points were tested using the Chi-square test or Fisher's Exact test. LOHS and age were analysed as a continuous variable. In case of heterogeneity between the three hospitals concerning the primary and secondary end-points, subgroup analyses by hospital will be performed.

Regarding the primary end-point, a P-value of less than 0.05 was considered statistically significant. For the analysis of the secondary end-points we used a Bonferroni correction to adjust for multiple testing (significant P-value of less than 0.01). Data were analysed using the SPSS statistical program (version 11.0) for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Patient enrolment and baseline characteristics

From 2001 to 2003, 713 patients were assessed for eligibility of which 619 consecutive patients were included. Sixteen patients were not analysed because of the following reasons: cancellation of surgery in seven, death before surgery in one, consent withdrawn before surgery in six and charts missing in two cases (Figure 1: Flow chart).

Baseline characteristics of the excluded group were comparable with the analysed group (data not shown). The included patients were equally assigned to the randomization groups within each hospital.

Baseline characteristics between the two randomization groups (protocol A and protocol B) were comparable, except for female patients, who were represented more in the new policy group (P=0.01) and for patients with rheumatoid arthritis, who were represented more in the standard care group (P=0.02) (Table 3).

The baseline characteristics between hospitals were comparable, except for hospital number 1, the university medical centre, who included a significantly higher proportion of Rheumatoid Arthritis (RA) patients (31.7% versus 9.2% and 3.3% in the other hospitals, respectively; P<0.001). Also, hospital number 1 included patients with a lower mean age (SD) than the other hospitals (67.1 (11.7) versus 71.1 (9.8) and 70.5 (9.9); p<0.001). This age difference can be explained by the larger RA patient population, who are generally younger when indicated for joint replacement surgery. Co-morbidities such as hypertension, myocardial infarction, heart failure, diabetes, stroke, peripheral arterial disease and arrhythmia were all comparable among hospitals, as well as use of medication (steroids, non-steroidal anti-inflammatory drugs (NSAID's), anticoagulants etc). Autologous re-infusion by cell saver was used in two hospitals in a total of 9 cases (2% of total; twice in hospital number 2 and seven times in hospital number 3).

Primary outcome

RBC usage

Overall RBC usage in the group with the new policy was 0.78 U/patient (SD 1.4) and 0.86 U /patient (SD 1.6) in the standard care group, with an overall mean difference of 0.08 (95% CI of mean difference [-0.3, 0.2]; P=0.53). LOHS was comparable between the groups (mean difference of - 0.6 days (95% CI of mean difference [-1.2, 0.5]; P=0.21)) (Table 4).

Secondary outcome

Results of all categorical, secondary end-points were not significantly different between the new policy and the standard care policy (P>0.05) (Table 4).

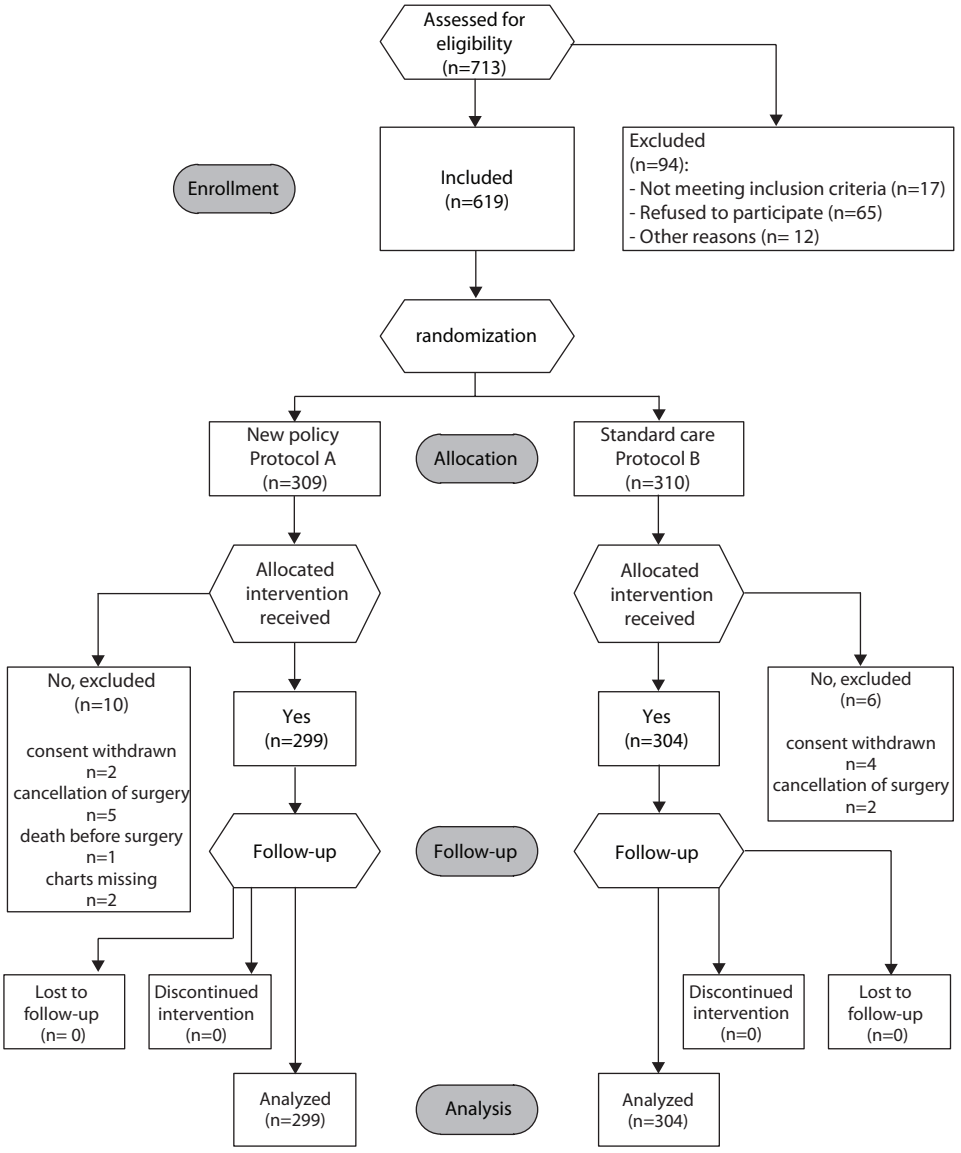

Figure 1. Flowchart

The flowchart shows the progress of all participants in the trial, from the time they are assessed for eligibility until the end of their involvement. In hospital #1 and hospital l#2, the new policy (protocol A) was the most restrictive policy, as expected. In hospital #3 the standard care policy (Protocol B) turned out to be the most restrictive policy.

Table 3. Patient characteristics (by type of policy and of total group)

Parameter Numbers (%) or mean (SD)	New policy (Protocol A)	Standard care (Protocol B)	All patients
Patients			619
Evaluated	299	304	603
Females (%)	215 (71.9)	186 (61.2)[d]	401 (66.5)
Mean age (years)	70.7 (10.2)	70.3 (9.7)	70.4 (9.9)
Mean weight (kg)	78.1 (13.4)	79.0 (13.2)	78.6 (13.2)
Smoking	43 (14.3)	47 (15.5)	90 (15.2)
THR	167 (55.9)	172 (56.6)	339 (56.2)
TKR	111 (37.1)	113 (37.2)	224 (37.1)
Revision THR	18 (6.0)	16 (5.2)	34 (5.6)
Revision TKR	3 (1.0)	3 (1.0)	6 (1.0)
Low risk[a]	14 (4.7)	12 (3.9)	26 (4.3)
Intermediate[b]	80 (26.8)	81 (26.6)	161 (26.7)
High risk[c]	205 (68.6)	211 (69.4)	416 (69.0)
Rheumatoid arthritis	24 (8.0)	43 (14.1)[e]	67 (11.1)
COPD	21 (7.0)	25 (8.3)	46 (7.6)
Mean pre-operative Hb (g/dL)	13.7 (1.4)	13.7 (1.4)	13.7 (1.3)
Mean pre-operative Hct (L/L)	0.41	0.41	0.41

Percentages are within policy group.
[a] low risk: patients younger than 50 years of age without risk factors indicated in Table 1
[b] intermediate risk: patients from 50 to 70 years of age without risk factors indicated in Table 1
[c] high risk: see definition in Table 1.
[d] P=0.01
[e] P=0.02

Subgroup-analysis at individual hospital

Due to heterogeneity of the effects on primary outcome across the three hospitals (P=0.008), we performed a subgroup-analysis by hospital.

Primary outcome

RBC use and the proportion of transfused patients were highest in hospital number 1 (Table 5). In two hospitals (number 1 and 2), the new policy (protocol A) was more restrictive than the standard care, but resulted in a non-significant RBC reduction of 30% in hospital number 1 (P=0.17) and a nearly significant reduction of 41% in hospital number 2 (P=0.05). In the third hospital, however, the standard care was more restrictive, which led to an increase of 39% in RBC usage (P=0.02) and to an increase in the proportion of transfused patients as well (P=0.001). The effect on RBC use differed significantly per hospital (test on interaction; P=0.008). An interaction of RBC use with risk group was found in hospital number 1:

compared to the high risk group, the effect size (this is the difference between the new policy and standard care) was larger in the lowest risk group, namely -4.9 RBC(s) (95% CI [-7.1, -2.7]; P<0.001) and -2.1 RBC(s) (95% CI [-3.2, -1.0]; P<0.001) in the intermediate risk group. In hospital number 2 and 3, no clinical significant interaction with a particular risk group was found. Within each hospital, mean duration of surgery and median blood loss were comparable between randomization groups. Total LOHS differed between hospitals due to the different hospital protocols. Hospital number 2 used a short-stay protocol, which resulted in the shortest LOHS. Due to a non-normal distribution of data, median values are shown, which were comparable within randomization groups. In all hospitals, LOHS was not affected by any transfusion protocol.

Table 4. Results of primary and secondary end-points by randomized group

Parameters	New policy (Protocol A) n=299	Standard care (protocol B) n=304
RBC (units) / patient	0.78 (1.4)	0.86 (1.6)[a]
LOHS (days)	9.6 (5.0)	10.2 (7.4)[b]
Hb day +1 (g/dL)	10.5 (1.6)	10.3 (1.4)
Hb day +4 (g/dL)	10.5 (1.1)	10.5 (1.1)
Hb at discharge (g/dL)	11.4 (1.1)	11.4 (1.3)
Infections	18 (6.0 %)	31 (10.1%)
Cardiovascular complications	34 (11.4%)	23 (7.6%)
Respiratory complications	6 (2.0%)	15 (4.9%)
Neuropsychiatric complications	11 (3.7%)	13 (4.2%)
Hemorrhage	10 (3.3%)	12 (3.9%)
Delayed mobilisation	22 (7.4%)	36 (11.8%)
Mortality	1 (0.3%)	2 (0.7%)
Composite complications	99 (33.1%)	104 (34.2%)

For continuous variables mean (SD) is shown, for categorical variables numbers (percentages) are shown. Percentages are calculated within randomized group (columns)

[a] mean difference 0.08 (95% CI of mean difference: -0.3 to 0.2; P=0.53)

[b] mean difference -0.6 (95% CI of mean difference: -1.2 to 0.5; P= 0.21)

For all categorical complications (infections etc) no difference between groups was found (P-values were all >0.05).

Secondary outcome

Mean post-operative Hb levels were comparable between hospitals and within each hospital between randomization groups. For hospital number 1 mean values were: 9.8 g/dL (SD 1.3) at day +1, 10.3 g/dL (SD 1.1) at day +4 and 10.9 g/dL (SD 1.1) at day +14. For hospital number 2 these values were: 10.8 g/dL (SD 1.6), 10.9 g/dL (SD 1.1) and 11.8 g/dL (SD 1.3),

respectively and for hospital number 3 these values were: 10.5 g/dL (SD 1.3), 10.3 g/dL (SD 1.1) and 11.3 g/dL (SD 1.1), respectively.

Table 5. Subgroup analysis of primary outcome measurements at individual hospital

Primary end-point	New policy (Protocol A)	Standard care (Protocol B)
Median RBC use[a] (25-75% range):		
Hospital #1	0.5 (0.0-2.0)	2.0 (0.0-2.3)
Hospital #2	0.0 (0.0-0.0)	0.0 (0.0-2.0)
Hospital #3	0.0 (0.0-2.0)	0.0 (0.0-0.0)
Proportion transfused patients in %:		
Hospital #1 (n=123)	50.8 (n=61)	54.8 (n=62)
Hospital #2 (n= 206)	20.8 (n=101)	30.8 (n=105)
Hospital #3 (n=274)	38.7 (n=137)	19.7 (n=137)[c]
Median LOHS[b] (25-75% range):		
Hospital #1	10.0 (9.0-13.0)	11.0 (10.0-13.3)
Hospital #2	6.0 (6.0-8.0)	6.0 (6.0-8.0)
Hospital #3	9.0 (8.0-10.0)	9.0 (8.0-10.0)

In hospital#1 and hospital#2, the new policy was the most restrictive policy, as expected. In hospital #3 the standard care policy turned out to be the most restrictive policy.

[a] Mean RBC use (U/patients) (SD): in hospital #1 was 1.34 (2.2) with the new policy and 1.92 (2.4) with standard care. In hospital #2 mean RBC use was 0.42 (1.0) and 0.72 (1.2), respectively and in hospital #3 mean RBC use was 0.80 (1.2), and 0.49 (1.1) (P=0.02), respectively

[b] LOHS must not be prolonged for more than four days in the most restrictive policy group.

[c] P=0.001

In 203 patients (33.7%) a postoperative complication was observed, which was highest in hospital number 1 (83/123=67%). Between transfusion policies, differences were found in composite complications in hospital number 2, which were slightly more represented in the standard care group that had the most liberal policy (n=35 versus n=21 in the new policy group; P=0.04) (Table 6). In hospital number 1, respiratory complications were more observed in the standard care group, that had the most liberal transfusion policy (n=13 versus n=3 in the new policy group; P=0.008). Furthermore, delayed mobilization (i.e. different from the standard ambulation protocol) was reported more frequent in the standard care group of hospital number 3 (n=18 versus n=8 in the new policy group; P=0.04), which contained the most restrictive transfusion policy. Infections occurred in 47 (7.8%) of all patients, which were mostly urinary tract infections (24 cases); 16 patients had wound infections, of which 5 were deep prosthetic infections. The remaining wound infections consisted of mostly superficial wound infections, which resolved uneventful: mild, grade 2 infections (haematoma with or without evident inflammation, but no bacterial growth) and in two cases more severe infections: one grade 3 (bacterial growth with a haematoma, but

Table 6. Subgroup analysis of secondary outcome measurements at individual hospital

Secondary end-points	New policy (Protocol A) n=299	Standard care (Protocol B) n=304
Composite complications[a]		
Hospital #1 (n=123)	43	40
Hospital #2 (n=206)	21	35[b]
Hospital #3 (n=274)	35	29
Infections		
Hospital #1	8	16
Hospital #2	4	9
Hospital #3	6	4
Cardiovascular complications		
Hospital #1	18	10
Hospital #2	8	9
Hospital #3	8	4
Respiratory complications		
Hospital #1	3	13[c]
Hospital #2	1	1
Hospital #3	2	1
Delayed mobilisation		
Hospital #1	12	14
Hospital #2	2	4
Hospital #3	8	18[d]

Stated values are numbers of patients
[a] patients could experience more than one complication
[b] P=0.04
[c] P=0.008 (significant P-value of less than 0.01 (Bonferroni correction for multiple comparisons)).
[d] P=0.04

no evident inflammation) and one grade 4 infection (evident inflammation and bacterial growth). Furthermore, one patient suffered from a pneumonia and in six patients a systemic bacterial infection (n=3) or a localized infection (n=3) was found. One patient had two infections. Respiratory complications were pulmonary embolism in one, pneumonia in one, five cases of transfusion associated cardiac overload (TACO), three cases of respiratory insufficiency due to opiates for pain reduction, bronchospasm in COPD in two and shortness of breath without evident clinical substrate in nine cases. Of all complications, ICU stay of more than 1 day (n=3) and transfusion reactions (n=3) were negligible (<1%). Mortality was found in three cases, one in each hospital, which all occurred in the groups who were transfused with the most liberal policies. The proportion of patients who had a delay in mobilisation differed per hospital, but within hospitals, delay was comparable between randomization groups in hospitals number 1 and 2. In hospital number 3, however, more patients (n=18) were delayed in the group with the most restrictive transfusion policy compared to the patients in the group transfused with the most liberal policy (OR 2.4 (95%

CI [1.0, 5.8]); P=0.008). Nevertheless, total hospital stay in hospital 3 was comparable in both groups. This delay in mobilisation could not be explained by a difference in post-operative Hb level of these patients: mean Hb level at day +1 was 10.2 g/dL with delay and 10.5 g/dL without delay (NS). Other complications as neuropsychiatric and haemorrhage were not different between randomization groups within each hospital.

DISCUSSION

Implementation of a new restrictive transfusion protocol in three different hospitals compared with the standard care did not result in an overall significant reduction of RBC transfusions, however, this study shows that a uniform transfusion policy can be implemented with great reliability, as deviations from the trigger protocol were only 4.5%. By implementing a new presumably restrictive transfusion trigger, we aimed at a reduction in RBC use, but one of the hospitals (number 3) showed an increase instead. This can not be explained by a population difference or a staff compliance difference to the protocol, because patients characteristics (age, gender) were not different from hospital number 2 which was the other general hospital. Hospital 1 and 2 had a liberal standard care transfusion policy. However, hospital 3 turned out to have a different more restrictive standard care policy. Furthermore that policy did not consider age as a risk factor. Thus, their current standard policy resulted in a lower RBC use. As a result, the overall difference in RBC use between the original randomization groups was negligible. Due to heterogeneity between hospitals in primary outcome we performed a subgroup analysis by hospital. Hospital number 1 had a modest RBC reduction, but showed a significant interaction of RBC use with risk group. This is explained by the difference in Hb trigger of age-matched patients between randomization groups in this hospital: the difference was largest in the low risk group (age<50 years): transfusion trigger of 6.4 g/dL with the new protocol and 9.7 g/dL with standard care. In the intermediate group (age 50-70 years) these were 8.1 g/dL and 9.7 g/dL, respectively and 9.7 g/dL in both arms for the high risk group (>70 years). The standard care policy advocated to give two RBC(s) per transfusion against one RBC in the new protocol. In hospital number 2, a high mean age of the patient population (71.1 years) resulted in a large population of high risk patients who were transfused with a trigger of 9.7 g / dL according to the new, restrictive policy (protocol A) compared a trigger of 10.5 g/dL according to standard care (protocol B). Despite this small difference, still an overall reduction of transfused RBC units was found, but not a reduction of the percentage of transfused patients. In none of the three hospitals, the Hb level of the transfusion triggers was identical between randomization arms for age-matched patients, except for high risk patients (age from 70 years onwards). Hospital number 1 had a trigger of 9.7 g/dL in either arm, but differed in number of units transfused (one versus two). The largest difference in

transfusion protocol effect was seen in hospital number 3, due to the exclusion of age in the standard care protocol (protocol B), which resulted in a more restrictive RBC use compared to the new, age-dependent protocol (protocol A).

A reduction of RBC use would not be acceptable at the cost of an increased hospital stay (due to increased morbidity from anaemia). However, the most restrictive transfusion policies were safe and LOHS was not affected by either of the two transfusion policies, although ambulation in hospital number 3 was slightly slowed down in the standard care group that used the most restrictive transfusion policy.

The impact of different transfusion triggers on postoperative complications (e.g. infection rate) in elective orthopaedic surgery patients has not been previously reported. Allogeneic RBC transfusions were found to be associated with a higher post-operative infection rate compared to non- transfused patients [10-15], but these studies were observational and/or retrospective and performed with non leukocyte-reduced RBC(s). Subgroup-analysis by hospital showed that in hospital number 1, respiratory complications were significantly higher in the group with the most liberal transfusion policy. This association should be further studied in future trials.

Of the total of respiratory complications, in 14 of 21 cases the respiratory complications might be explained by the RBC transfusion itself: 5 cases were classified as transfusion related (TACO), whereas in the 9 cases of unclassified hypoxemia, a subclinical TACO or a transfusion related acute lung injury (TRALI) might have been present [16]. The occurrence of post-operative infections was not significantly different between randomization groups. Age appeared no risk factor for post-operative complications, as shown by data of hospital number 3, who had a standard care policy that was more restricted and not age-dependent, but was not associated with an increase in post-operative complications, although mobilisation was significantly delayed in this group. Other factors, such as pre-existent cardiovascular disease may play a role, however the current study was not powered to identify such an effect. In the FOCUS trial [17], an ongoing randomized, multi-centre study on elderly hip fracture patients (from 50 years of age onwards), patients with cardiovascular disease or cardiovascular risk factors are studied to investigate the impact of a restrictive transfusion trigger in this specific patient population with functional outcome as the primary end-point.

This study has some limitations. First, our data can not be extrapolated to other hospitals in general, as the hospital's standard care transfusion policy turned out to be very different between hospitals and therefore, it is unlikely that these three hospitals do represent the overall transfusion policy in the Netherlands. Second, our study was not powered to evaluate mortality or cardiovascular outcomes. Third, it is possible that our assessment of secondary end-points was biased since the trial and classification of outcomes was not blinded. However, the secondary end-points were scored by use of pre-defined objective criteria by the orthopaedic residents not performing the surgery. Fourth, to take RBC usage

as a primary end-point can be seen as a limitation, because transfusion less blood is the intended intervention of a restrictive policy. However, to use another well-accepted primary outcome such as mortality or post-operative complications is difficult, because of the low prevalence of such end-points in this study population. Although it seemed logically that a new, restrictive policy would always result in a RBC reduction, this was not the case in one hospital.

And finally, there is a limitation concerning the un-transfused patients, which is a general problem concerning all transfusion medicine trials: due to the early time of randomization, prior to surgery, the majority of patients included in the study did not meet any of the criteria for transfusion. In our study, this concerns a large part (45 to 80%) of the randomized patients. Ideally a patient should only be randomized when a transfusion is inevitable, but in practice this is very difficult to perform. However, by comparing the randomized patients according to the intention-to-treat principle, both groups remain balanced in terms of the levels of all known confounding factors [18].

In conclusion, implementation of a new, intentionally restrictive transfusion protocol in elective hip and knee replacement surgery is feasible and safe without lengthening the hospital stay. Whether a more restrictive transfusion policy is associated with less post-operative complications should be investigated in further studies which are powered to find this effect.

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Chapter 3

The impact of a restrictive transfusion trigger on post-operative complication rate and well-being in elective orthopaedic surgery: a post-hoc analysis of a randomised study

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ABSTRACT

Background: Peri-operative red blood cell transfusions have been associated with post-operative complications in patients undergoing elective orthopaedic hip- or knee replacement surgery.

Materials and methods: A post-hoc analysis of data extracted from a randomised study on transfusion triggers using pre-storage leukocyte-depleted red blood cells. Patients who received the most restrictive transfusion policy ("restrictive group") were compared with patients who received the most liberal policy ("liberal group"). Endpoints were red blood cell use, hospital stay, haemoglobin levels, postoperative complications and quality of life.

Results: Of 603 patients, 26.4% patients in the restrictive group and 39.1% in the liberal group were transfused (p=0.001). In the restrictive group, fewer postoperative infections (5.4 % versus 10.2 %; p=0.03) and respiratory complications (1.7% versus 4.9%; p=0.03) were observed, whereas hospital stay, cardiovascular complications and mortality rate were not different in both groups. Quality of life scores were not associated with type of transfusion policy, the number of red blood cell transfusions or the transfusion status.

Conclusion: A restrictive transfusion protocol was not associated with worse outcome and showed a trend towards fewer postoperative infections and respiratory complications compared to a liberal transfusion policy. Well-being was not associated with transfusion policy or with red blood cell transfusions.

INTRODUCTION

Reports on the role of allogeneic red blood cell (RBC) transfusions, whether or not leukocyte reduced, on post-operative infection rate in orthopaedic surgery are inconsistent [1-6]. Since many of these studies were observational or retrospective, selection bias at patient inclusion may have occurred. We recently reported the intention-to-treat results of a randomised study among elective orthopaedic surgery patients, that compared a new uniform, intentionally restrictive, transfusion policy with the standard hospital policy, with RBC use as primary endpoint [7]. No differences in RBC use between the randomised arms were observed because in one of the three participating hospitals, the new uniform study trigger for blood transfusion turned out to be less restrictive than their standard trigger, which resulted in an increased RBC use with this new transfusion policy. As well, no significant differences in post-operative complications between the original randomisation groups was present. In the current post-hoc analysis we investigated the effect of the most restrictive transfusion policy in all three participating hospitals, by pooling the patients who were randomised to the most restrictive trigger to a restrictive policy group and the patients who were randomised to the most liberal transfusion policy to a liberal policy group, thereby fully respecting the randomised nature of the data.

A second aim of the study was to evaluate the effect of the transfusion policy (restrictive or liberal) and of RBC transfusions on postoperative functional well being by measuring quality of life (QoL) scores. Previously, we were unable to show any correlation between QoL scores and Hb levels in the early postoperative period in this cohort, but, as suggested by Wallis, Hb and transfusions should be disentangled and thus separately analysed in QoL evaluations [8,9].

METHODS

Establishing groups for the post-hoc analysis

In the original study, within each participating hospital, patients were randomized to either protocol A (new policy) or protocol B (standard policy) [7]. The new transfusion trigger, was risk level based (depending on age and co-morbidity) and uniform among the three participating hospitals (Appendix). The new protocol (A) was more restrictive than the standard policy (B) for two hospitals and the patients randomized to A were labeled "restrictive " and to B "liberal ". In the third hospital, patients randomized to protocol A actually received more RBC transfusions and this group was now labeled "liberal" and the standard policy (protocol B) "restrictive". Only pre-storage leukocyte-depleted (LD-) RBC(s) were used.

Outcome measures

The original primary outcome variable was the number of transfused RBCs. Postoperative complications and QoL were secondary outcome measures and prospectively scored. Complications were categorised in: infections, respiratory complications (pneumonia excluded), neuro-psychiatric, cardiovascular and haemorrhagic complications, mobilisation delay, and mortality. Post-operative infections were pre-defined according to CDC criteria [10]. All wounds were prospectively scored for possible wound infection at postoperative day 5 according to Gaine and coworkers [11]. Cases of unclassified hypoxaemia were further investigated by detailed chart review to investigate their relationship with RBC transfusions (e.g Transfusion Associated Circulatory Overload or Transfusion Related Acute Lung Injury) . QoL questionnaires were scored preoperatively (time point T1) and at postoperative days +4 (time point T2) and +14 (time point T3), using the Functional Status Index (FSI), measuring functionality in daily living; a Visual Analogue Scale (VAS) for fatigue; and the Functional Assessment of Cancer Therapy-Anaemia (FACT-An) subscale, measuring fatigue and other anaemia-related symptoms. All scores ranged from 1 to 100, with lower scores indicating better functioning. Follow up ended at the outpatient clinic 14 days after surgery or at final discharge (if hospital stay was longer than 14 days). Details of the original study and overall results have been reported previously [7,9].

Analysis and statistical methods

Continuous data are summarized as mean and SD, or median and inter-quartile (IQ) range in case of a non-normal distribution. A comparison of laboratory parameters and other numerical endpoints (like hospital stay and age) between groups was performed by a Student t-test in case of normal distributions and by the non-parametric counterpart (Mann-Whitney) in case of non-normal distributions (the number of patients receiving RBC transfusions and the total number of units RBC administered). In case of categorical endpoints, comparisons were made on proportions using the Chi-square statistic or Fisher's Exact test. A common (pooled) Odds Ratio was computed as an overall effect measure among the three hospitals since all tests for heterogeneity were non-significant ($p>0.10$). RBC use of both groups was compared to verify that the restrictive group indeed used fewer RBCs (significant p-value of less than 0.05). Pearson correlation coefficients [+ 95% CI] were calculated between FSI, Fact-Anemia and VAS scores and number of RBC transfusions for time points T1, T2 and T3. If $r \geq 0.20$, scores at T2 and T3 were corrected for preoperative scores of FSI, Fact-Anemia and VAS, and for peri- and post-operative variables (duration of surgery, surgical blood loss and post-operative complications) as possible confounders. Student's t-tests were used to compare the QoL scores with dichotomous variables (transfusion status: yes/no or type of transfusion policy: restrictive/liberal). In case of a significant difference between means ($p<0.05$), regression analysis was performed to further evaluate the association of FSI, Fact Anemia and VAS scores, correcting for possible confounders.

For the analysis of the post-operative endpoints, we used a Bonferroni correction to adjust for multiple testing of seven variables (infections, respiratory complications (pneumonia excluded), neuro-psychiatric, cardiovascular and haemorrhagic complications, mobilisation delay and mortality) (significant p - value of less than 0.01). Data were analysed using the SPSS statistical program (version 15.0) for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Of 603 included patients, 299 were assigned to the restrictive group and 304 to the liberal group. The baseline characteristics (age, sex, type of surgery, co-morbidities, use of medication, pre-operative haemoglobin (Hb) level) were balanced in both groups except for history of chronic obstructive pulmonary disease (COPD), of which 32 (10.7%) patients were in the restrictive group versus 14 (4.6%) in the liberal group (p=0.005) (Table 1).

Table 1. Patient baseline characteristics

Parameter **Numbers (%) or mean (SD)**	**Restrictive group**[a] **n=299**	**Liberal group**[b] **n=304**	**P-value**
Females	190 (63.5)	211 (69.4)	0.13
Mean age (years)	70.2 (10.3)	70.7 (9.6)	
Mean weight (kg)	79.5 (13.4)	77.7 (13.1)	
Smoking	43 (14.4)	47 (15.5)	
Total hip replacement (THR)	166 (55.5)	173 (56.9)	
Total knee replacement (TKR)	113 (37.8)	111 (36.5)	
Revision THR	16 (5.4)	18 (5.9)	
Revision TKR	4 (1.3)	2 (0.7)	
Low risk[c]	12 (4.0)	14 (4.6)	
Intermediate risk[d]	81 (27.1)	80 (26.3)	
High risk[e]	206 (68.9)	210 (69.1)	
Rheumatoid arthritis	27 (9.0)	40 (13.2)	0.11
Chronic Obstructive Pulmonary Disorder	32 (10.7)	14 (4.6)	0.005
Mean pre-operative Hb (g/dL)	13.8 (1.4)	13.5 (1.3)	0.02
Mean pre-operative Hct (L/L)	0.41 (0.04)	0.40 (0.04)	

Percentages are within policy group.
[a] restrictive group: patients assigned to the most restrictive transfusion policy.
[b] liberal group: patients assigned to the most liberal transfusion policy.
[c] low risk: patients younger than 50 years of age without risk factors.
[d] intermediate risk: patients from 50 to 70 years of age without risk factors.
[e] High risk includes one or more of the following risk factors:
any heart rhythm different than sinus rhythm, unstable cardiac ischaemia (by history or ECG), myocardial infarction less than 6 months, heart failure, heart valve disease, age from 70 years onwards, serious peripheral arterial disease, including large vessel surgery (aortic aneurysm, peripheral vessels), cerebral arterial disease (CVA or TIA in history), hypertension with left ventricular hypertrophy (LVH) (shown on ECG/ echocardiogram), serious pulmonary disease, expressed in polyglobulinaemia (emphysema / pulmonary fibrosis), insulin dependent diabetes mellitus.

Clinical endpoints

The proportion of transfused patients was smaller (26.4%) in the restrictive group compared to the liberal group (39.1%, p=0.001), as was the mean RBC use per patient (p=0.003) (Table 2). No difference in hospital stay (p=0.27) was noted between the groups. Mean duration of surgery and median blood loss were comparable between groups (data not shown).

Table 2. RBC use and post-operative clinical endpoints by assigned transfusion policy group

Clinical endpoints Numbers (%) or mean (SD)	**Restrictive group[a] n=299**	**Liberal group[b] n=304**	**P**	**common OR[c] (95% CI)**
N / proportion transfused patients (in %)	79 / 26.4	119 / 39.1	0.001	
RBC use (U/patients)	0.64 (1.4)	1.00 (1.6)[d]	0.003	
LOHS (days)	9.6 (5.1)	10.2 (7.4)[e]	0.27	
Hb day +1 (g/dL) (SD)	10.6 (1.6)	10.3 (1.4)	0.02	
Hb day +4 (g/dL) (SD)	10.5 (1.2)	10.5 (1.2)	0.99	
Hb at discharge (g/dL) (SD)	11.4 (1.3)	11.4 (1.2)	0.99	
Infections	16 (5.4)	31 (10.2)	0.03	2.0 (1.1-3.8)
Cardiovascular complications	30 (10.0)	27 (8.9)	0.63	0.9 (0.5-1.5)
Respiratory complications	5 (1.7)	15 (4.9)	0.03	3.1 (1.1-8.5)
Neuro-psychiatric complications	12 (4.0)	12 (3.9)	0.98	1.0 (0.4-2.2)
Haemorrhage	10 (3.3)	12 (3.9)	0.68	1.2 (0.5-2.9)
Delayed mobilisation	32 (10.7)	26 (8.6)	0.37	1.3 (0.7-2.2)
Mortality	0 (0)	3 (1.0)	0.25	
Composite complications[f]	93 (31.1)	110 (36.2)	0.18	1.3 (0.9-1.9)

[a] restrictive group: patients assigned to the most restrictive transfusion polic.y
[b] liberal group: patients assigned to the most liberal transfusion policy.
[c] to estimate complication risk, a common odds ratio (OR) is calculated.
[d] 95% CI of difference [0.12, 0.60].
[e] 95% CI of difference [-1.6, 0.4].
[f] patients could experience more than one complication
Median RBC use (IQ range) was 0.0 (0-2.0) in the liberal policy group and 0.0 (0-1.0) in the restrictive policy group

Infections occurred in 47 (7.8 %) of all patients, of which 16 patients were in the restrictive group (5.4%) and 31 patients in the liberal group (10.2%, p=0.03). Pooled risk estimates were calculated for post-operative complications, which resulted in an elevated risk of infections (common OR=2.0, p=0.03) and respiratory complications (common OR=3.1, p=0.03) in the liberal group, however both were not significant after correction for multiple testing (significance level p<0.01). Other post-operative endpoints were also not different between the groups.

Table 3 shows that infections (mainly urine tract infections and wound infections) and respiratory complications occurred more often in transfused patients, respectively 66% (31 of 47) and 70% (14 of 20) of patients developing these complications had been transfused. Of the patients who developed infections, median RBC use in the restrictive group was 0.5 units (IQ range 0-2.0) and 2.0 in the liberal group (IQ range 1.0-3.0). Two patients had already been treated for a pre-existent infection (jaw and urine tract infection, one in each group).

Table 3. Infections and respiratory complications by assigned transfusion policy group in relation to RBC use (yes or no)

Clinical endpoints **Numbers of patients (n)**	**Restrictive group (n=299)** **RBC use:** **79 yes/ 220 no**	**Liberal group (n=304)** **RBC use:** **119 yes/185 no**	**P-value**
Infections (total number n=47)	**16(8/8)**	**31(23/8)**	**0.03**
Urine tract infection (UTI) (n=24)	8 (4/4)	16 (12/4)	
Wound infection (n=16)	6 (2 /4)	10 (9/1)	
Of which deep prosthetic infection (n=6)	3 (1/2)	3 (3/0)	
Pneumonia (n=1)	0	1 (0/1)	
Systemic bacterial infection (n=3)	1[a] (0/1)	2 (1/1)	
Other (localized) (n=3)	1 (1/0)	2 (1/1)	
Of which pre-existent infection (n=2)	1 (1/0) Jaw	1 (1/0) UTI	
Respiratory complications (total number n=20)	**5 (3/2)**	**15 (11/4)**	**0.03**
TACO (n=5)	2 (2/0)	3 (3/0)	
Bronchospasm in COPD (n=2)	1 (0/1)	1 (0/1)	
Respiratory insufficiency due to opiates (n=3)	0	3 (2/1)	
Pulmonary embolism (n=1)	0	1 (0/1)	
Unclassified hypoxaemia (n=9)	2 (1[b]/1)	7 (6[c]/1)	

Abbreviations: UTI= Urine Tract Infection; TACO=Transfusion Associated Circulatory Overload; COPD=Chronic Obstructive Pulmonary Disease

[a] this patient also had a deep prosthetic wound infection

[b] this case was possibly transfusion related (TACO)

[c] one case was possibly transfusion related (TRALI)

Patients with respiratory complications received a median RBC use of 1.0 unit in the restrictive group (IQ range 0-3.5) and of 2.0 in the liberal group (IQ range 2-2.75). Patients with postoperative infections or respiratory complications had significantly longer hospital stays compared to compared to patients without these complications: median hospital stay 12.0 [9.0-12.0] and 13.0 [10-17] days with infections and respiratory complications respectively, compared to 9.0 [7-10] in patients without these complications ($p<0.001$) (not shown).

Of five patients, the respiratory complications were related to a RBC transfusion and diagnosed as Transfusion Associated Circulatory Overload (TACO). Of nine patients with unclassified hypoxaemia, seven had received RBC transfusions. Detailed chart review of these seven patients further revealed two possible transfusion related cases. One 73-year old male with a history of CABG, PTCA and hypertension, who had knee surgery and one postoperative RBC transfusion, following a low Hb value of 9.2 g/dL, after which the patient developed cardiac failure (possible TACO) that responded well to diuretics (with compatible chest X-ray). The other was a 76-year old male patient with a history of CABG who received two RBC transfusions for a postoperative Hb value of 9.1 g/dL, with transient hypoxaemia, which needed oxygen support and resolved uneventfully (possible TACO or TRALI, although chest X-ray was not taken). A third 34-year old patient with a history of Still's disease, had knee surgery followed by massive postoperative blood loss of 2 litre by drains, with dyspnoea and tachypnoea and a postoperative Hb of 9.1 g/dL. After the patient received 4 RBC transfusions to compensate for the blood loss, he recovered completely. Of the remaining four transfused patients with unclassified hypoxaemia a relationship with RBC transfusion could not be found, mainly due to lack of chart information. However, in all patients hypoxaemia was mild and all patients completely recovered without additional mechanical ventilation.

QoL and fatigue scores were not associated with the type of transfusion policy (non-significant differences in mean scores between transfusion policy groups or number of RBC transfusions, except for FSI scores measuring daily activity which showed a significant, but weak correlation $r=0.36$ ($p<0.001$) with the number of RBC transfusions at time-point T2 (4 days postoperatively). However, this association disappeared after correction for the possible confounders pre-operative FSI score (T1), duration of surgery, surgical blood loss and post-operative complications, which lowered r to 0.08 ($p=0.085$). The transfusion status (being transfused or not) was also significantly associated with the FSI score at T2, with better scores if not transfused, but also with the VAS scores at T2, the FSI score at T3 and the Fact-Fatigue score at T2 (all had significant differences in mean scores between transfused and non-transfused groups with p-values <0.001). After correction for the four possible confounders, in all cases significance was lost.

DISCUSSION

In this post-hoc analysis, we compared a restrictive transfusion policy with a liberal policy and evaluated the clinical impact on post-operative complications and well-being. The restrictive transfusion policy resulted in an absolute reduction of 0.36 RBC unit per patient and a 31% relative risk reduction of proportion of transfused patients (13% absolute decrease from 39% to 26%). This finding is in line with the findings of Carless and co-workers, who

performed a meta-analysis of 17 randomised studies on transfusion triggers in a variety of patient groups including orthopaedic surgery and found an average relative risk reduction of 37% [12]. Although we found only an absolute reduction of 5% (from 36% to 31%) in the composite postoperative complication rates, the liberal group had more often infections and respiratory complications. The majority of postoperative infections and respiratory complications occurred in transfused patients. Estimated risks for these complications were respectively doubled and tripled in the groups assigned to the liberal transfusion trigger. However, if corrected for multiple testing ($p<0.01$), the significance between the groups was lost. A decreased infection rate with a restrictive transfusion policy was also found by Carless and co-workers, who analysed four randomised studies that reported infection rate, with a pooled risk ratio of 0.76 (95% CI 0.60 to 0.76). Two of those studies used leukoreduced RBCs [12].

The finding of an increased respiratory complication rate with a liberal transfusion policy has not been reported earlier. Postoperative pulmonary morbidity has been associated with RBC transfusions in cardiac surgery [13]. In our dataset, detailed chart review of seven transfused cases with unclassified hypoxaemia revealed a possible transfusion-related complication in two, and in a third a complete recovery thanks to the transfusions. Due to lack of information (no chest x-rays) of the remaining four cases, we could not rule out a sub-clinical transfusion associated circulatory overload (TACO) or transfusion related acute lung injury (TRALI) as a possible underlying cause [14]. Despite the use of leukocyte-depleted RBCs in both groups, the patients assigned to a restrictive transfusion policy had a lower incidence of postoperative infections and respiratory complications compared to the patients assigned to a more liberal transfusion policy, which phenomenon we hypothesize to be a consequence of the transfusion policy, with no consequences on well-being. These data strongly suggest that use of a restrictive transfusion policy is important in blood management programs, and even should be the first step in implementation, aiming for improved patient outcome.

In order to evaluate whether RBC transfusions contributed to well being by separating the effects of transfusion from the effects of the need for transfusion, we correlated QoL scores to RBC use. The number of RBC transfusions and the transfusion status was, after correction for possible confounders, not associated with QoL and fatigue scores at any time-point. We may therefore conclude that the number of RBC transfusions or the transfusion status was not related to well being and functioning in the direct postoperative period. We previously showed no effect of anaemia on QoL in this cohort.

This study has some limitations. First, the study was not powered to evaluate post-operative complications, since the prevalence of these complications is low, nor was the study powered to evaluate the relationship between RBC use and postoperative functioning and well being. Second, by reassigning the randomised groups to a "liberal" and "restrictive" group, the validity of the current "post-hoc" analysis might be disputed. However, since

the allocation was still randomised, the inference and p-values are completely valid as if it were a randomised allocation from the start. Therefore, the results from this study provide a higher level of evidence than data from prospective observational studies. Third, since postoperative anaemia was only moderate in our studied patients, we cannot extrapolate our findings to patients with more severe anaemia.

In conclusion, a restrictive transfusion policy was not associated with a higher complication rate, moreover, this policy might even result in less infections and respiratory complications, with no consequences on well-being. QoL scores were not associated to the number of RBC transfusions or to the transfusion status, suggesting that these were not of influence on well being and functioning in the direct postoperative period in moderately anaemic patients.

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APPENDIX

Transfusion policies from the original study protocol

Hb values were originally in mmol/L (e.g. 4.0 / 5.0 / 6.0 mmol/L) which is common use in the Netherlands

Protocol A: New, uniform transfusion policy (all participating hospitals)

Low risk group (patients younger than 50 years of age)					
Within 4 hours of surgery			After 4 hours of surgery		
If Hb	≥6.4 g/dL:	0 RBC	If Hb	≥6.4 g/dL:	0 RBC
	4.8 - <6.4 :	1 RBC		5.6 - <6.4:	1 RBC
	<4.8:	2 RBC(s)		<5.6:	2 RBC(s)
Intermediate risk group (patients from 50 to 70 years of age)					
Within 4 hours of surgery			After 4 hours of surgery		
If Hb	≥7.2 g/dL:	0 RBC	If Hb ≥	8.1g /dL:	0 RBC
	6.4 - <7.2:	1 RBC		7.2 - <8.1:	1 RBC
	<6.4:	2 RBC(s)		<7.2:	2 RBC(s)
High risk group[a] (see below)					
Within 4 hours of surgery			After 4 hours of surgery		
If Hb	≥8.9 g /dL:	0 RBC	If Hb	≥9.7 g /dL:	0 RBC
	8.1 - <8.9 :	1 RBC		8.9 - <9.7:	1 RBC
	7.2 - <8.1 :	2 RBC(s)		8.1 - <8.9:	2 RBC(s)
	<7.2:	3 RBC(s)		<8.1:	3 RBC(s)

[a]High risk includes one or more of the following:
- any heart rhythm different than sinus rhythm.
- unstable cardiac ischemia (by history or ECG)
- myocardial infarction less than 6 months
- heart failure
- heart valve disease
- age (from 70 years onwards).
- serious peripheral arterial disease, including large vessel surgery (aortic aneurysm, peripheral vessels).
- cerebral arterial disease (CVA or TIA in history)
- hypertension with left ventricular hypertrophy (LVH) (shown on ECG/ echocardiogram)
- serious pulmonary disease, expressed in polyglobulism (emphysema / pulmonary fibrosis).
- insulin dependent diabetes mellitus.

Protocol B: Standard care transfusion policies

***Hospital number 1 (University Medical Center):**

- Peri-operative transfusion policy (day 0):

  if Hb between 8.1 and 9.7 g /dL and dependent on blood loss: 1-2 RBC(s).

- Post operative transfusion policy (from day 1) :

  if Hb <9.7 g /dL : 2 RBC(s), independent of age, risk status

***Hospital number 2 (general hospital):**

- Peri-operative transfusion policy (day 0):

  I keep Hb >6.4 g/dL in case of age < 60 years and ASA[a] class 1

  II keep Hb >8.1 g.dL in case of age ≥ 60 years and ASA[a] class 1, 2, 3

  III keep Hb >9.7 g/dL in case of ASA[a] class 4 or serious cardiopulmonary disease

[a]American Society of Anesthesiologists

- Post operative transfusion policy (from day 1):

  I keep Hb >9.7 g/dL in case of co-morbidity as: IC / CCU admission, uremia, serious heart-, lung- or vessel disease:

  II If no co-morbidity exists, the transfusion trigger is age-dependent:

Age (years)	**Hb (g/dL)**
>70	10.5
50-70	9.7
25-50	8.9
<25	8.1

***Hospital number 3 (general hospital):**

- Peri-operative transfusion policy (day 0):

  if Hb <9.7 g/dL and dependent on (expected) blood loss: 2 RBC(s)

- Post operative transfusion policy (from day 1):

  I Patients with cardiac history:

  - if Hb <9.7 g/dL: 2 RBC(s)

  II Patients without cardiac history if symptomatic (nausea, dizziness, tachycardia, general malaise, paleness):

  - if Hb 7.2 g/dL – 8.1 g/dL: 2 RBC(s)

  III If Hb ≤7.2 g/dL 2 RBC(s)

Chapter 4

Postoperative anaemia after joint replacement surgery is not related to quality of life during the first two weeks postoperatively

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ABSTRACT

Background: Lower limb joint replacement surgery provides a considerable improvement in quality of life (QoL), but is associated with peroperative blood loss, and with anemia in the direct postoperative period. General acceptance of low transfusion thresholds and shorter postoperative hospital stays will result in patients leaving hospital with low hemoglobin (Hb) levels. To evaluate the role of QoL scores as a possible alternative for Hb values to serve as a further indicator for RBC transfusion, we performed a secondary analysis of a previously conducted randomized clinical trial (RCT) to compare QoL and fatigue scores with simultaneously measured pre- and postoperative Hb levels, in total hip and knee arthroplasty patients.

Methods: QoL measurement was measured preoperatively and twice up to 14 days postoperatively using the Functional Status Index (FSI), the Visual Analogue Score (VAS)-Fatigue score, and the Functional Assessment of Cancer Therapy Anemia (FACT-Anemia) subscale. Pearson correlation coefficients between (change in) FSI, VAS-Fatigue and FACT-Anemia subscale scores and (change in) Hb levels were calculated. Additionally, partial correlations were calculated and linear regression analysis was performed, correcting for possible confounding variables.

Results: 603 patients were evaluated. All patients scored worse postoperatively, but none of the scores correlated with Hb values, neither after correcting for confounding factors. Even more, the changes between preoperative and postoperative Hb levels were not correlated with changes in fatigue scores.

Conclusions: In hip- and knee-prosthesis surgery no correlation existed between postoperative Hb levels or acute postoperative decline in Hb values and QoL scores (FSI, VAS-Fatigue or FACT-Anemia).

Lower limb joint replacement (hip- and knee-) surgery provides a considerable improvement in quality of life (QoL), but is associated with peroperative blood loss and with anemia in the direct post-operative period [1-3]. General acceptance now exists for low transfusion thresholds. These latter, combined with an early postoperative discharge of patients from the hospital, will result in patients discharged with low hemoglobin (Hb) levels, which may compromise revalidation. To date, two studies are available on the effects of anemia on QoL, especially on fatigue, in the post-operative period after total hip and knee replacement surgery [4,5]. However, these studies comprised less than 100 patients each, studied different postoperative intervals and Hb values were not always measured simultaneously with the questionnaires. We investigated the relationship between QoL and fatigue scores and Hb levels, in order to see whether these scores could serve as a further indicator for RBC transfusion. We used data from a large prospective, randomized study that compared a liberal to a restrictive transfusion trigger with red blood cell use as a primary endpoint [6].

MATERIALS AND METHODS

Study design

Secondary analysis of a previously conducted prospective, randomized study that compared two different transfusion policies with the mean use of allogeneic leucocyte-depleted red blood cell (RBC) units as primary end point. In the original study, QoL measurement was pre-defined as secondary endpoint. The transfusion policies differed from a minimal threshold of 7.0 g/dL to a maximal threshold of 10 to 10.5 g/dL. The study included patients of 18 years and older scheduled for a primary or revision total hip replacement or total knee replacement surgery at either of three Dutch participating hospitals (Leiden University Medical Center, Leiden; HAGA hospital, The Hague; and Reinier de Graaf Hospital, Delft), during a three year period (2001-2003). Refusal of allogeneic transfusions (e.g. Jehovah's witnesses) was the only exclusion criterion.

Procedures

QoL scores were measured pre-operatively and at post-operative days +4 and + 14 using:

1. the Functional Status Index (FSI), which is a reliable and valid functional assessment instrument in rheumatoid arthritis (RA) and in patients after hip fracture (scale 0-4: with lower scores indicating better functioning, 0=not relevant) (Appendix 1) [7, 8]. Total scores were recalculated to range from 0 to 100. It should be noted that in literature FSI scores are reported as the higher, the better. For better comparison we reversed the scores in opposite direction to ensure that all scores were pointed at the same direction.
2. the VAS fatigue score (scale 1-10: lower scores indicate better functioning and well-being). For reasons of comparison, we recalculated the scores to range from 0 to 100.
3. the Functional Assessment of Cancer Therapy Anemia (FACT-Anemia) subscale (with 13 fatigue (F) items and 7 non-fatigue (NF) items), which was validated for the association with Hb levels in cancer patients (scale 0-4: lower scores indicate better functioning and well-being) (Appendix 2) [9]. Total scores were recalculated to range from 0 to 100 for FACT-F and FACT-NF items. The FACT-Anemia scale was used specifically for its fatigue items, although its validity and reliability is not known in an orthopaedic population.

Patients completed the first set of questionnaires preoperatively (T1). On postoperative days +4 (during hospitalisation) and +14 (in the outpatient setting) the patient completed the second (T2) and third set (T3) of questionnaires. Hb levels were measured at the same moment as the QoL scores were taken. Follow up ended at the outpatient clinic 14 days after surgery or after discharge of the patient (in case of a prolonged hospital stay of more than 14 days) (T3).

Defining clinically minimal important difference (MID)

To evaluate responsiveness, MID was defined using anchor-based approaches, when available [10].

Anchor-based methods assess which changes on the measurement instrument correspond with a minimal important change defined on the anchor. An external criterion, generally a global change or transition question, is used to operationalize a relevant or an important change. The advantage is that the concept of 'minimal importance' is explicitly defined and incorporated in these methods.

For FACT-Fatigue and VAS fatigue scores, MID was defined as a difference in score of 3.0 (score range 0-42) and 10 (score range 0-100), respectively [11,12]. As FACT-Fatigue scores were recalculated to a range from 0 to 100, MID was recalculated to a difference of 7.1. Concerning the FSI, anchor-based estimates were not available. Therefore we used a difference of 0.5 SD for MID at all time points, based on distribution-based methods [13]. All validation studies for FSI had only been performed among rheumatoid arthritis patients and not always in a surgical setting, whereas for Fact-Fatigue these were all performed among cancer patients.

Statistical analysis

Variables were described as mean and SD, or median and inter-quartile (IQ) range in case of a non-normal distribution. Pearson correlation coefficients (+ 95% confidence interval [CI]) were calculated for time points T1, T2 and T3, between FSI, Fact-Anemia, VAS scores and Hb values as well as between changes in scores and Hb levels from preoperative to postoperative values obtained at T2 and T3. Subgroup analysis was performed to compare scores between groups concerning age, gender, type of surgery, preoperatively anemia, and comorbidity (cardiovascular, respiratory, RA, diabetes), randomization group and numbers of RBC transfusions. Partial correlation coefficients were calculated, individually controlling for the variables mentioned. Hb levels were additionally analyzed as categorized variables (using tertiles: low, intermediate and high Hb groups), and mean QoL scores were compared between the highest and lowest group using Student's t-tests. By linear regression analysis, the correlation between Hb and QoL scores was evaluated after adjusting for the same variables mentioned. Pre-operative anemia was based on WHO criteria [14]. P values of less than 0.05 were considered statistically significant.

RESULTS

Baseline characteristics

Of 619 included consecutive patients, 16 patients could not be analyzed because of the following reasons: cancellation of surgery in seven, death before surgery in one, consent

withdrawn before surgery in six and charts missing in two cases. Baseline characteristics of the excluded group were comparable with those of the analyzed group (data not shown). In Table 1, characteristics of the remaining 603 included patients are shown. During surgery, 78 patients (13%) received general anaesthesia and 525 (87%) patients received a loco-regional technique (spinal with or without epidural anaesthesia). Median total blood loss was 400 mL (IQ range 0-600 mL). Overall mean RBC usage was 0.78 U/patient in the new uniform policy group and 0.86 U/patient in the standard care policy group (mean difference 0.08; 95% CI, -0.3 to 0.2; p=0.53).

QoL Outcomes

All 603 patients completed the first set (T1) of questionnaires (pre-operatively), 92% (n=554) completed the second set (T2) (4 days after surgery) and 81% (n=487) the third set (T3). Ten percent of patients (n=59) had a hospital stay of more than 14 days, with a maximum of 100 days (median 18 days, IQ range 16-21 days). In Table 2, mean Hb levels and mean scores of all questionnaires are shown for all time points which show that at postoperative days 4 and 14 patients deteriorated in all scores except the FACT-NF score. Patients with RA scored worse compared to the group without RA at all time points for VAS-Fatigue and FACT-Fatigue, but mainly preoperatively (mean difference -12.4 [95% CI, -20.3 to -4.6], p=0.02 and -10.2 [95% CI, -15.0 to -5.4], p<0.001, respectively). Also the FSI scores were worse, but only preoperatively (T1) and postoperatively at T2 (mean difference at T1 of -9.3 [95% CI, -17.3 to -1.4], p=0.002 and at T2 of -9.3 [95% CI, -15.7 to -2.8], p=0.005, respectively).

At each time point, the three fatigue questionnaires correlated with variable extent: correlations were weak between FSI and VAS fatigue (r=0.29, [95% CI, 0.20 to 0.37], p<0.001) and Fact-F (r=0.39, [95% CI, 0.32 to 0.46], p<0.001), whereas a better correlation was found between VAS fatigue and Fact-F (r=0.70, [95% CI, 0.65 to 0.74],p<0.001). Within each type of questionnaire, pre-operative scores were weakly correlated with postoperative scores: for FSI, at T2 (r=0.41, [95% CI, 0.32 to 0.48], p<0.001) and at T3 (r=0.25, [95% CI, 0.16 to 0.34], p<0.001); for VAS, at T2 (r=0.43, [95% CI 0.34 to 0.51], p<0.001) and at T3 (r=0.47, [95% CI, 0.37 to 0,56], p< 0.001); for FACT-F at T2 (r=0.40, [95% CI, 0.32 to 0.47], p<0.001) and at T3 (r=0.41, [95% CI, 0.33 to 0.49], p<0.001) and for FACT-NF at T2 (r=0.44, [95% CI, 0.37 to 0.51], p<0.001) and at T3 (r=0.40, [95% CI, 0.32 to 0.48], p<0.001).

We found significant differences between the FSI and Fact-F scores of preoperatively anaemic patients (mean ± SD Hb level of 11.6 ± 0.8 g/dL) compared to pre-operatively nonanaemic patients (mean Hb (SD) of 14.0 (1.1) g/dL) at several time-points (not shown). However, these differences were small and less than the defined clinically MID.

Table 1. Baseline characteristics of 603 patients undergoing total hip or knee replacement surgery

	All subjects
Demographics	
Age, years	70.4 (9.9)
Male/Female	202/401
Weight, kilograms	78.6 (13.3)
Type of surgery	
Total hip replacement	339 (56.2)
Total knee replacement	224 (37.1)
Revision total hip replacement	34 (5.6)
Revision Total knee replacement	6 (1.0)
Underlying diseases	
Rheumatoid arthritis	67 (11.1)
COPD	46 (7.6)
Hypertension	269 (44.7)
Myocardial infarction	27 (4.5)
Cardiac failure	58 (9.6)
CVA/TIA	32 (5.3)
Peripheral vascular disease	34 (5.6)
Arythmia	50 (8.3)
Laboratory findings	
Pre-operative hemoglobin, g/dL	13.7 (1.3)
Pre-operative hematocrit, L/L	0.41 (0.04)
Pre-operative anemia	98 (16.3)

Continuous data are presented as mean (SD) and categorical data as number (%).
COPD=chronic obstructive pulmonary disease; CVA/TIA=cerebrovascular accident/transient ischemic attack.

Table 2. Mean hemoglobin levels and score values* of the three questionnaires at the three measured time points (T1=preoperatively, T2= four days postoperatively, T3=14 days postoperatively / at discharge)[a]

Mean (SD) scores of:	T1	T2	T3
Hemoglobin (g/dL)	13.7 (1.3)	10.5 (1.1)	11.4 (1.2)
FSI	14.6 (17.0)	47.5 (23.0)	31.4 (19.0)
VAS	31.0 (27.5)	38.9 (28.6)	32.4 (27.0)
Fact-F	26.9 (18.4)	34.9 (19.7)	29.3 (18.5)
median (IQR)	23 (11-40)	32 (20-48)	25 (14-41)
Fact-NF	10.5 (12.5)	11.8 (13.9)	8.5 (12.7)
median (IQR)	6 (0-19)	6 (0-19)	6 (0-13)

*Range of scores of FSI, VAS and Fact-Anemia (both Fact-F and Fact-NF) questionnaires from 0 to 100.
[a] Lower scores indicate better functioning.

Table 3. Correlations (r) of Hb values with FSI, VAS, FACT-F, and FACT-NF scores on several time points (T1=preoperatively, T2= four days postoperatively, T3=14 days postoperatively / at discharge)

Correlations r of Hb values with:	T1 [95% CI]; p	T2 [95% CI]; p	T3 [95% CI]; p
FSI	0.22 [0.14, 0.30]; p<0.001	0.21 [0.13, 0.29]; p<0.001	0.22 [0.13, 0.30]; p<0.001
VAS	-0.04 [-0.12, 0.04]; p=0.33	-0.04 [-0.12, 0.04]; p=0.34	-0.02 [-0.11, 0.07]; p=0.66
FACT-F	-0.11 [-0.19, -0.03]; p=0.007	-0.10 [-0.18, -0.02]; p=0.02	-0.05 [-0.14, 0.04]; p=0.27
FACT-NF	-0.05 [-0.13, 0.03]; p=0.22	-0.05 [-0.13, 0.03]; p=0.24	+0.03 [-0.06, 0.12]; p=0.51

Hb levels and QoL scores

Mean ± SD postoperative Hb values on days +4 and +14/discharge were 10.5 ± 1.1 and 11.4 ± 1.2 g/dL, respectively. Hb values were not correlated with VAS-scores and weakly correlated with FSI-, and FACT-Anemia scores at several time points (Figure 1A-L and Table 3). Although some correlations were significant, these were very weak, as the magnitude (expressed by R^2) did not exceed 0.04 (0.22 times 0.22), so at most 4% of the total variability in any of the four scores was explained at any time point by Hb. When Hb levels were categorized into three equal subgroups, significantly different scores on the FSI and Fact-F were seen between the lowest (mean Hb level, 12.2 g/dL) and highest Hb subgroup (mean Hb level, 15.1 g/dL) at T1, and on the FSI, Fact-F and VAS at T2 (mean Hb levels, respectively, 9.3 and 11.8 g/dL), but not at T3 (mean Hb levels, respectively, 10.1 and 12.9 g/dL). Again, these differences were small and less than the clinically MID. Fact-NF scores showed no differences at any time points. No correlation of greater than 0.30 between Hb and fatigue scores was found, neither after individually controlling for clinical and demographic characteristics by partial correlation analysis nor after simultaneous correction for these variables in linear multivariable regression analysis. The randomization group, which included a more or less restrictive transfusion policy, and the number of RBC transfusions were not of any influence on the outcome of QoL and fatigue scores either.

Change in scores compared to (change in) Hb levels

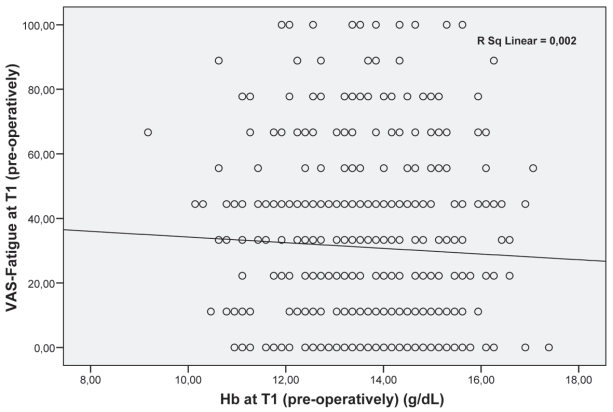
We compared changes in scores between the preoperative and postoperative follow-up moments T2 and T3 with the absolute Hb values at the same endpoints in accordance with Conlon et al, for the elderly population (aged from 65 years onwards, n=455), and found no

correlation of any significance. This was also the case when we compared the change scores with the change in Hb values (delta Hb; Table 4A). For the total study population, the results were also not different (Table 4B).

Table 4. Correlations between (delta) Hb level and changes of FSI, VAS and FACT scores at T1 compared to T2 (T2-T1) and T3 (T3-T1) including 95% CI and p-values

Change of	Hb (T2)	Delta Hb (T2-T1)	Hb (T3)	Delta Hb (T3-T1)
A. Population aged 65 years and older (n=455)				
FSI	0.08 [-0.03, 0.19]; p=0.17	0.11 [0.002,0.22]; p=0.05	0.08 [-0.04, 0.19]; p=0.16	0.16 [0.05, 0.28]; p=0.006
VAS	-0.03 [-0.16, 0.10]; p=0.68	0.05 [-0.08, 0.18]; p=0.46	0.11 [-0.04, 0.25]; p=0.14	0.15 [0.005, 0.30]; p=0.04
FACT-F	-0.09 [-0.19, 0.01]; p=0.07	-0.07 [-0.17, 0.03]; p=0.16	0.08 [-0.04, 0.19]; p=0.18	0.06 [-0.06, 0.17]; p=0.29
FACT-NF	-0.07 [-0.17, 0.03]; p=0.17	-0.05 [-0.15, 0.05]; p=0.34	0.02 [-0.10, 0.14]; p=0.68	0.06 [-0.06, 0.18]; p=0.30
B. Total study population				
FSI	0.08 [-0.01, 0.17]; p=0.08	0.06 [-0.03, 0.15]; p=0.22	0.05 [-0.05, 0.15]; p=0.28	0.16 [0.06, 0.25]; p=0.002
VAS	-0.03 [-0.14, 0.08]; p=0.56	0.03 [-0.08, 0.14]; p=0.57	0.06 [-0.06, 0.18]; p=0.30	0.08 [-0.04, 0.20]; p=0.20
FACT-F	-0.09 [-0.18, 0.002]; p=0.04	-0.05 [-0.14, 0.04]; p=0.27	0.05 [-0.05, 0.15]; p=0.32	0.05 [-0.05, 0.15]; p=0.30
FACT-NF	-0.10 [-0.19, -0.01]; p=0.03	-0.07 [-0.16, 0.02]; p=0.13	-0.001 [-0.10, 0.10]; p=0.99	0.02 [-0.08, 0.12]; p=0.73

A



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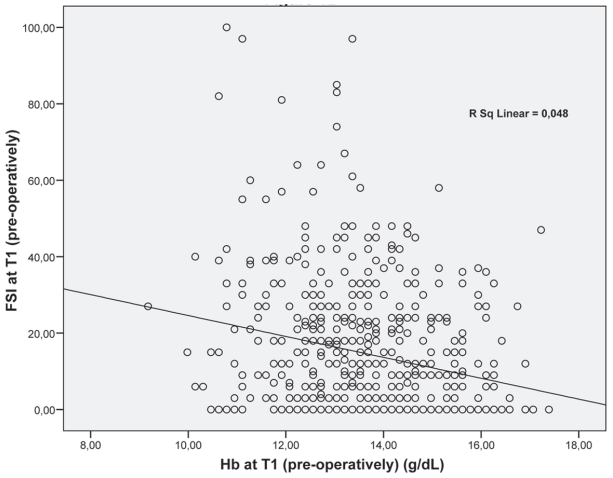

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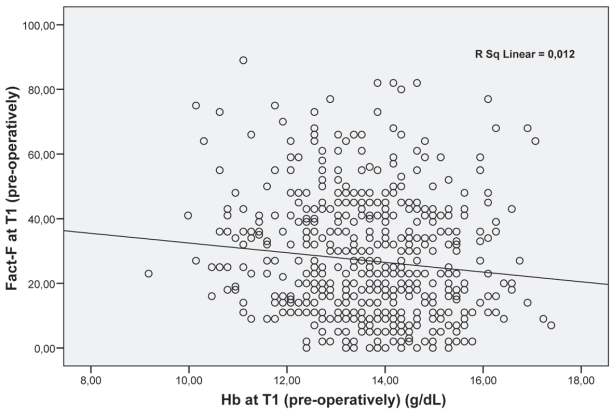
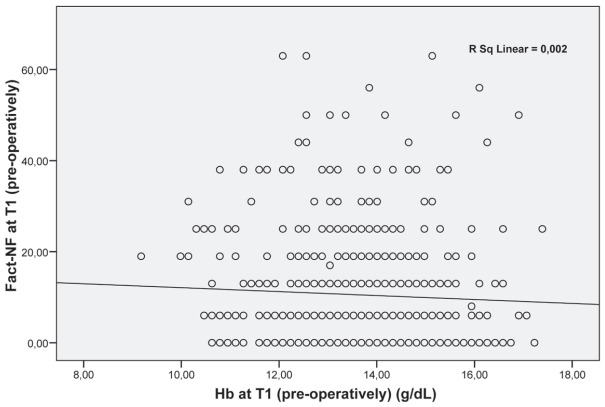


Figure 1. Correlation of scores with Hb levels, measured pre-and postoperatively (A-L). Scatter plots of VAS-Fatigue, FSI, Fact-F and Fact-NF in relation to the Hb values at T1 (preoperatively; A-D), at T2 (4 days post-operatively; E-H) and at T3 (14 days post-operatively; I-L). Linear regression lines with associating R^2 values were added.

D



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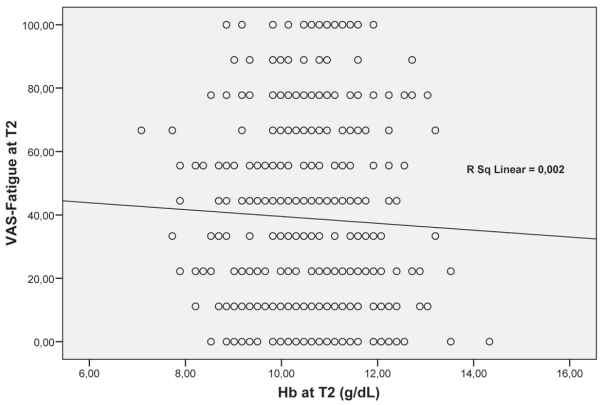

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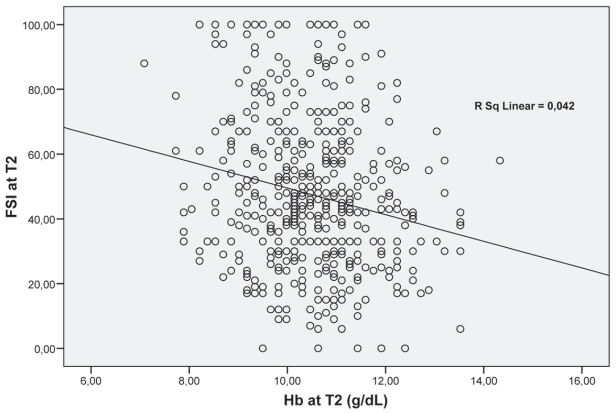

Figure 1. *Continued*

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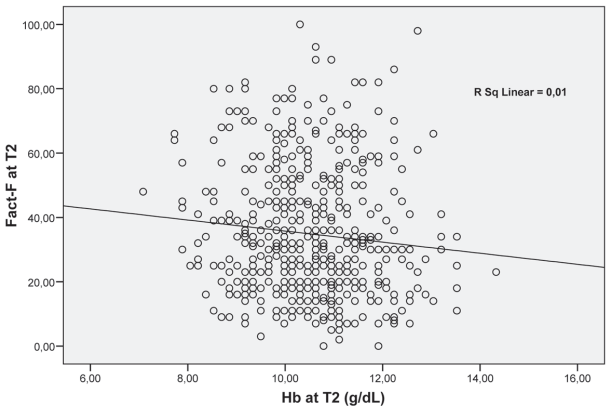

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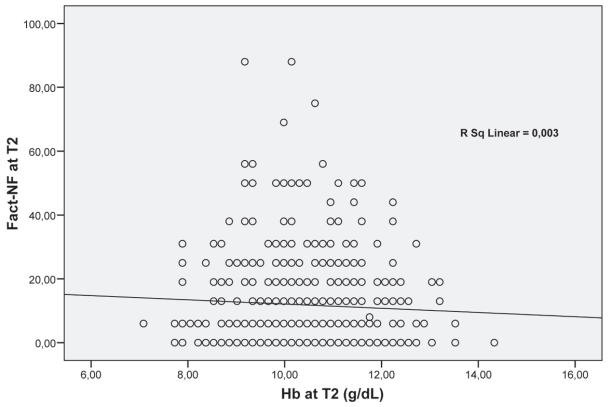

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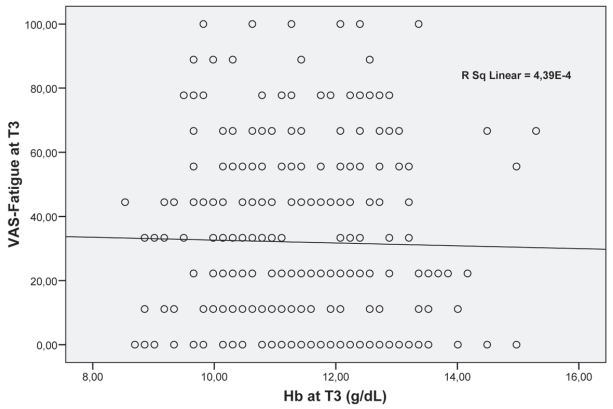

Figure 1. *Continued*

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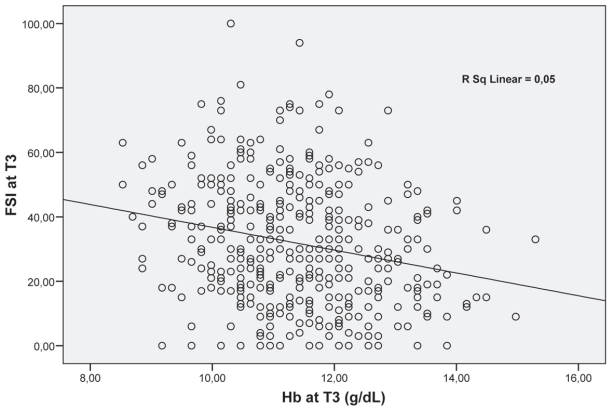

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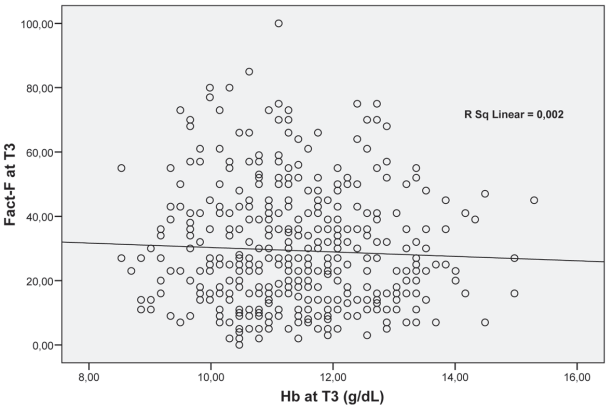

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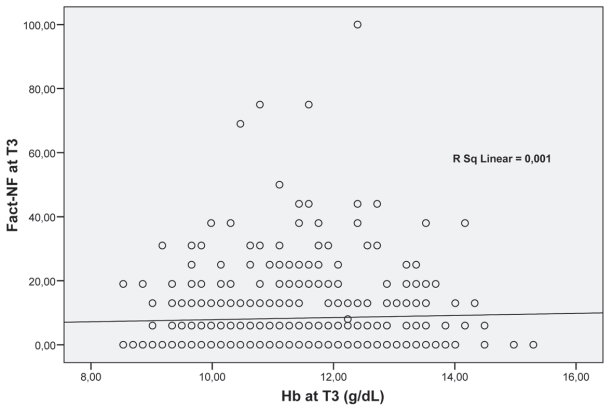

Figure 1. *Continued*

DISCUSSION

This study was based on a large orthopaedic population of more than 600 patients, in which Hb levels and QoL- and fatigue scores were measured at simultaneous time points. In this elective surgery population, symptoms of fatigue were not correlated with Hb levels at any time-point up to 14 days after surgery. All scores deteriorated in this early postoperative period. Some correlations with Hb level were significant, but these were very weak, and at most 4% of the total variability with a maximum of 9% in any of the four scores could be explained at any time point by Hb levels. After controlling for clinical and demographic factors, the association between Hb and QoL scores remained weak. Not even the randomisation group and the number of RBC transfusions influenced this outcome. Scores of anaemic patients were slightly worse at all time points compared to non-anemic patients, as well as scores of the lowest Hb group compared to the highest group, but the differences did not meet the definition of clinically important difference.

The findings are in agreement with the findings of Wallis and coworkers, who compared the SF-36 questionnaire and an in-house linear analogue QoL scale with Hb levels up to 8 weeks after total hip surgery in 30 patients and found no correlation [4]. Conlon et al [5] found a linear correlation in the change of FACT-Anemia and SF-36 scores, taken pre-operatively and 2 months after total hip arthroplasty, and the absolute Hb values taken pre-operatively and 8 days post-operatively in 87 patients. Improvement in overall SF-36 score was 8.6 points for every Hb increase of 1 g/dL on day 8, and improvement in FACT-Anemia score was 2.9 points for a similar Hb increase, indicating that patients with a lower Hb at discharge at day 8 consistently reported less improvement in QoL and fatigue than those discharged with a higher Hb level. Our data could not confirm this.

Both the studies performed by Wallis et al and Conlon et al differed in design from ours by obtaining questionnaires at different and later postoperative time points and -more importantly- without a simultaneous Hb measurement. Because our follow up period ended at two weeks postoperatively, it is possible, that FACT-Anemia scores correlate with Hb values at later time points. We did not include a more prolonged follow-up, since the basic goal of the original study was to evaluate the effect of a restricted perioperative transfusion trigger on RBC use and to investigate direct postoperative effects such as hospital stay, delay of mobilisation and, in this report, quality of life, fatigue and function with the purpose to find a further indicator for RBC transfusion.

The FACT-Anemia questionnaire was originally developed for cancer patients and seems less applicable to lower joint surgery patients, who generally suffer a more acute anemia due to intra- and postoperative blood loss as compared to anemia due to a chronic disease (e.g. cancer, use of chemotherapy).

In the immediate postoperative period, the three used questionnaires, showed intercorrelations between 11 and 70%, suggesting that these scoring systems measure

a similar construct with respect to QoL and fatigue. As the FACT-NF subscale was not responsive, it seems not informative in this context.

Although the FSI is a reliable and valid instrument to measure functional outcome in orthopaedic patients, this functional scale did not correlate with postoperative acute Hb level decrease or absolute Hb levels. Other factors in the postoperative period apparently overrule the inconvenience of anemia or a decrease in Hb level.

In hip fracture patients, Carson and coworkers investigated postoperative recovery in relation to Hb levels and found no difference in recovery between a discharge Hb of 8 to 10 g/dL and more than 10 g/dL [15]. Halm et al used the Functional Independence Motor (FIM) mobility score to measure functional mobility within 60 days follow up after hip fracture surgery, also found no association with pre- or post-operative Hb levels [16]. These findings as well as our study show that the used QoL and fatigue questionnaires, are not suitable as a monitor for acute postoperative anemia, at least not in the range of Hb levels observed in this study.

In conclusion, we found that Hb levels do not correlate with the FSI, VAS and FACT-Anemia scores in the immediate postoperative period after lower limb joint replacement surgery, which is associated with a more acute decrease in Hb level and which is different from the setting of chronic anemia for which some questionnaires were developed. However, this does not preclude an effect after a longer postoperative interval or in patients with lower absolute Hb levels.

Acknowledgements

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APPENDIX 1

The Functional Status Index (FSI) has 11 questions. Each question is scored from 0 to 4 with lower scores (except for 0) indicating better functioning.

0= this question is not relevant
1= no help needed
2= uses a device
3= needs human assistance
4= not possible due to health reasons

1. walking 10 feet
2. getting into and out of bed
3. putting socks and shoes on
4. getting on and off the toilet
5. rising from an armless chair
6. getting in and out of a bath/shower
7. taking a bath or shower
8. walking a block
9. .getting into a car
10. putting on pants
11. climbing five stairs

APPENDIX 2

The FACT-Anemia questionnaire comprises of fatigue (F) items and non-fatigue (NF) items. Each item is scored from 0 to 4, with lower scores indicating better functioning and well-being.

0=not at all
1=a little
2=more than a little
3=very much
4=very strongly

I feel fatigued (F)
I feel weak all over (F)
I feel listless ("washed out") (F)
I feel tired (is skipped because of VAS fatigue score asked separately)
I have trouble starting things because I am tired (F)
I have trouble finishing things because I am tired (F)
I have energy (F)
I have trouble walking (NF)
I am able to do my usual things (F)
I need to sleep during the day (F)
I feel light-headed (dizzy) (NF)
I get headaches (NF)
I have been short of breath (NF)
I have pain in my chest (NF)
I am too tired to eat (F)
I am motivated to do my usual activities (NF)
I need help doing my usual activities (F)
I am frustrated by being too tired to do the things I want to do (F)
I have limited my social activities because I am tired (F).

Chapter 5

Efficacy, safety and user-friendliness of two devices for postoperative autologous shed red blood cell re-infusion in elective orthopaedic surgery patients: a randomized pilot-study

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ABSTRACT

To determine the safety, efficacy and user-friendliness of two different postoperative autologous blood re-infusion systems an open, randomized, controlled study was performed.

Eligible consecutive primary and revision total hip and knee replacement patients were randomized for one of the two systems or for a control group in which shed blood was not re-infused. The nursing staff scored user friendliness. Patients were monitored after re-infusion. In all three patient groups a restrictive transfusion trigger was used.

Sixty-nine of 70 randomized patients were evaluated. Ease of use, efficacy and safety of both re-infusion systems were comparable. There was no difference in allogeneic blood use between the groups. Thirty per cent of the patients re-infused with autologous blood developed a mainly mild, febrile transfusion reaction. No other adverse reactions were seen. Signs of coagulopathy after re-infusion were not found. In multivariate analysis autologous re-infusion was an independent factor associated with a shorter hospital stay. Both postoperative autologous blood re-infusion systems were of equal efficacy and safety. The contribution of autologous wound blood re-infusion to reduce allogeneic transfusions must be investigated in a larger study.

After total knee or hip arthroplasty a total blood loss of 750 ml or more is common [14]. Postoperative re-infusion of autologous shed wound blood would be a possibility to save the use of allogeneic red blood cell (RBC) transfusions [12,16,17]. Preliminary studies also suggest a reduction in postoperative (wound) infections using autologous wound blood transfusions [8,12,18]. Several re-infusion devices (of which the collected blood is leukocyte reduced, washed or "unprocessed") are available, but limited comparison between systems is available [3,10,11]. For the design of a large randomized study, investigating the value of several blood management interventions in orthopaedic surgery, we wanted to make a valid decision between commercially available postoperative autologous blood re-infusion systems. In an open randomized, controlled study in primary and revision total hip replacement (THR) and total knee replacement (TKR) patients, two different postoperative re-infusion systems currently used in the Netherlands were compared with respect to efficacy, safety and user friendliness.

PATIENTS, MATERIALS AND METHODS

In 2003, patients of 18 years and older who were scheduled for a primary or revision THR or TKR at the Leiden University Medical Centre (LUMC) were included for this study. All patients were of American Society of Anaesthesiologists (ASA) 2 or 3 category. Exclusion criteria were: sickle cell anaemia, cancer, bacterially contaminated wounds and participation in other blood management studies. Patients were randomized to three groups: A, a control group with a standard closed suction wound drainage system not intended for re-infusion; B, a re-infusion system, using continuous suction at a vacuum pressure of 120 mm Hg and just prior to re-infusion a double shielded 40 micron filter (Pall Lipiguard VS filter) entrapping lipids larger than 10 micron and 2 log of leukocytes (DONOR™ system, Van Straten Medical, Nieuwegein, The Netherlands); C, a re-infusion system, which uses intermittent suction pressure by a manually expandable bag at a maximum pressure of 90 mm Hg and three filters: a 200 mm filter, a secondary 80 mm filter and prior to re-infusion a third 40 mm filter (Bellovac A.B.T.®, Astra Tech, Zoetermeer, The Netherlands). The companies of the drainage devices were allowed to train the nursing staff on theoretical aspects in two sessions and practical aspects at bedside in 4 sessions. Thereafter the companies were available for advice if needed.

The study was approved by the local Medical Ethical Committee and written informed consent was given by all patients. A randomisation list was generated by a statistical software package: preoperatively, the patient was randomly assigned to one of the three groups by opening a sealed envelope with the randomisation number. General anaesthesia or loco-regional anaesthesia was chosen based on each patients' requirements. An ischaemic tourniquet was applied to all TKR patients, whereas THR patients were operated

in the lateral position by a direct lateral approach to the hip. During the study a restrictive transfusion trigger according to the Dutch guidelines was used (CBO consensus guidelines, 2004). No intra-operative blood saving measurements were performed. A sterile disposable drainage system set was intra-operatively inserted, just before suturing the wound, to drain wound blood. One Redon catheter was placed intra-articular. Blood collection began after skin closure (THR) or 15 minutes after tourniquet deflation (TKR).

Outcome measures

Outcome measures were efficacy, safety and user-friendliness of the wound drainage devices. User-friendliness was scored by the nursing staff by the following questions, ranked on a scale from 1 to 5 (1= very difficult, 5=very easy): frequency of handling the same system (first time or more than once), extent of burden to the patient and the nurse, occurrence and chance of wrong use and user-friendliness in daily practice. Efficacy and safety were measured by blood loss, units of allogeneic RBCs transfused, amount of autologous blood re-infused, transfusion reactions, postoperative haemoglobin (Hb) level, delay in mobilisation beyond the routine schedule at day 3 and length of hospital stay (LOHS) (defined as the interval between the day of surgery until the day of discharge from the hospital). Infections were scored according to CDC-criteria [9], wound infections were scored according to Gaine et al (2000). Transfusion reactions were scored according to the Dutch hemovigilance criteria, including mild reactions such as a temperature rise ≥1°C. Criteria for discharge were according to the hospital protocol. A serious adverse event was defined as a transfusion reaction of the third or fourth degree (life threatening or death). Antibiotics and anticoagulant therapy were given according to the standard hospital protocols. Total amounts of shed blood were measured within and after 24 hours for group A, and within and after 6 hours for groups B and C, respectively, until the drain was removed.

Laboratory analysis

Venous blood samples were taken pre-operatively and on day 1 after surgery for Hb (g dL^{-1}), haematocrit (Hct), White Blood Cell (WBC) count (x10^9 L^{-1}), Thrombocyte (Tr) count (x10^9 L^{-1}) and LDH (U L^{-1}). Levels of interleukin (IL)-6, IL-10 and IL-12 cytokines were measured by ELISA with a detection range of 1 to 50.000 pg mL^{-1} (Sanquin diagnostics, Amsterdam, the Netherlands). Percentage antitrombin activity was measured by chromogenic assay (Coamatic Antithrombin, Chromogenix–Instrumentation Laboratory SpA, Milan, Italy) on an automated coagulation analyser (STA-R®, Diagnostica Stago, Asnières sur Seine, France), fibrinogen (g L^{-1}) was measured according to the Clauss method on an automated coagulation analyser (Electra 1800C, Medical Laboratory Automation, Inc., Pleasantville, NY), and D-dimers (ng mL^{-1}) were determined by an automated immuno-analyser (VIDAS®, bioMérieux, Breda, the Netherlands) with a maximal measurable value of 50.000 ng mL^{-1}. In case of re-infusion of autologous shed blood (group B and C patients), additional venous

blood samples were taken immediately after re-infusion was completed. At discharge, patients' Hb and Hct were measured.

Shed blood samples (up to a maximum of 150 ml) were taken immediately after start of drainage (T0), pre-filter (T1) and post-filter just before re-infusion (max. 6 hours after surgery) (T2). These samples were analysed for Hb, WBC count, Tr, Free Hb (mg dL^{-1}) and LDH. Plasma and serum were stored at -80°C for coagulation factor and cytokine measurements. In addition, samples were taken for bacterial culture.

Statistical analysis

The study size was not based on statistical power calculations, but on descriptive comparisons. To allow for detection of large differences between the systems, each randomisation group consisted of minimal 20 patients. Statistical analysis was performed in SPSS for Windows 11.0. Frequencies were described as mean and standard deviation (SD), or median and range in case of a nonparametric distribution. Analysis of laboratory parameters between patients and other numeric endpoints was performed with the ANOVA-test and analysis within the patients with a paired t-test. Differences between the groups in the number of RBC transfusions and the total number of units RBC transfusions given were analysed with the non-parametric Mann-Whitney test. Categorical endpoints were tested using the Chi-square test or Fisher's Exact test. LOHS was analysed as a continuous variable and dichotomized (< eight days and ≥ eight days). Age was analysed as a continuous variable and categorized into four groups (≤40, 41-65, 66-75, >75 years). End points were analysed univariate, and when relevant, to correct for confounding factors, multivariate analysis with backward conditional regression was performed. P values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics are shown in Table 1. Of 70 patients included, one patient was not operated, leaving 69 evaluable patients. 42 (61%) patients had a primary THR, 20 (29%) a primary TKR, four (6%) a revision THR and three (4%) a revision TKR. THR patients were underrepresented and TKR over-represented in group A as compared to the wound blood re-infusion groups B and C (p=0.06). Diagnosis (osteo-arthritis and rheumatoid arthritis), co-morbidity (arterial disease, pulmonary disease, diabetes) and use of medication were comparable in the three groups.

Concerning intra-operative parameters (Table 2), groups were comparable for surgery duration and blood loss. Four patients received intra-operative blood transfusions (total of seven units RBC's) because of large blood loss of more than 1500 mL. No transfusion reactions were seen.

Table 1. Baseline characteristics

	ABC (n=69)	A[a] (n=22)	B[a] (n=23)	C[a] (n=24)
Baseline parameters				
Female (%)	62	68	61	58
Age (years) mean ± SD	60 ± 16.0	58 ± 14.3	66 ± 15.6	58 ± 17.2
Weight (kg) mean ± SD	79 ± 14.9	85 ± 21.9	77 ± 13.1	76 ± 17.1
Type of surgery				
THR primary/ revision (n)[b]	42/4	10/1	15/2	17/1
TKR primary/ revision (n)[b]	20/3	8/3	6/0	6/0
Pre-operative lab				
Hb (g dL^{-1}) mean ± SD	13.7 ± 1.61[c]	13.7 ± 1.61	13.7 ± 1.77[c]	13.7 ± 1.61
Hct (L L^{-1}) mean ± SD	0.40 ± 0.04[c]	0.40 ± 0.04	0.40 ± 0.04[c]	0.40 ± 0.04
WBC (x10^9 L^{-1}) mean ± SD	7.8 ± 2.4[c]	8.2 ± 3.3	8.4 ± 2.0[c]	7.0 ± 1.2
Tr (x10^9 L^{-1}) mean ± SD	274 ± 84[d]	291 ± 100[c]	281 ± 76[c]	252 ± 75

[a] A, control group; B, DONOR™ group; C, Bellovac A.B.T.® group
[b] difference in type of surgery (n) of re-infusion groups compared to control group: P=0.06
[c] one missing value
[d] two missing values

Questionnaires to score the user-friendliness of the drainage systems, were completed in 28 of 30 re-infused cases (response rate of 93%). Outcome of both devices was comparable with a learning effect after handling the same device for more than one time (Figure 1).

Of 47 patients randomized for the use of a shed blood re-infusion system, 30 (64%) were actually re-infused (mean 401 ± 170 mL): 11 of 12 (92%) of the TKR patients (mean 591 ± 322 mL) and 19 of 35 (54%) of THR patients (mean 290 ± 170 mL) (Table 2). Of 17 patients not re-infused, in 11 cases the collected volume was too low (<100 mL), four patients dropped out from the study due to venous access problems and in two cases accidentally a control drainage system was placed. No serious adverse events after re-infusion were observed. Six patients had mild reactions (transient fever and/or shivers) after re-infusion.

Of the total of 71 units RBCs transfused, 64 (90%) were given to 32 patients postoperatively from day +1 onwards post surgery. The main reason for postoperative transfusions was a low Hb value (in 92%). The mean Hb trigger for RBC transfusions was 8.4 g dL^{-1} (SD 1.45).

Table 2. Intra- and postoperative parameters

	n	ABC	n	A[a]	n	B[a]	n	C[a]
Intra operative parameters								
Duration of surgery (min) mean ± SD	69	146 ± 44	22	156 ± 48	23	137 ± 42	24	145 ± 44
THR	46	138 ± 40	11	143 ± 36	17	128 ± 37	18	145 ± 45
TKR	23	161 ± 49	11	169 ± 55	6	163 ± 47	6	146 ± 43
Blood loss (mL) median (range)	69	450 (0-2400)	22	313 (0-1625)	22	500 (0-2400)	23	485 (0-1700)
THR	44	600 (0-2400)	11	600 (250-1625)	16	563 (0-2400)	17	750 (200-1700)
TKR	23	0 (0-835)	11	0 (0-835)	6	0 (0-500)	6	0 (0-250)
Postoperative parameters								
LOHS (days) mean ± SD		8.2 ± 3.3		9.0 ± 2.8		7.8 ± 4.0		7.9 ± 3.1
THR		7.7 ± 3.6		8.9 ± 3.4		7.4 ± 4.3		7.4 ± 2.8
TKR		9.2 ± 2.7		9.1 ± 2.3		9.0 ± 2.4		9.5 ± 3.8
Total drain production (mL) median (range)								
THR		445 (45-1350)		538 (65-1245)		440 (100-1350)		430 (45-1230)
TKR		963 (350-1745)		885 (455-1710)		630 (350-1150)		1443 (960-1745)
Shed blood re-infusion (mL)[b]								
mean ± SD	30	64%			15	329 ± 274	15	472 ± 265
THR	19	54%			9	260.0 ± 192.0	10	317.5 ± 151.5
TKR	11	92%			6	433.3 ± 359.2	5	781.0 ± 126.3
Re-infusion reactions	30	6				4		2
Intra- and postoperative allogeneic RBC transfusions								
Number of units (% of patients)	32	(46%)	10	(45%)	13	(57%)	9	(38%)
THR	20	(43%)	4	(36%)	10	(59%)	6	(33%)
TKR	12	(52%)	6	(55%)	3	(50%)	3	(50%)
RBC/transfused patient ± SD		2.2 ± 0.8		1.9 ± 0.7		2.4 ± 1.0		2.3 ± 0.7
THR		2.3 ± 0.6		2.5 ± 0.6		2.2 ± 0.6		2.2 ± 0.4
TKR		2.2 ± 1.2		1.5 ± 0.5		3.0 ± 0.7		2.7± 1.2
Transfusion reactions	2		1	shivers	1	shivers		
Hb values (g dL^{-1}) day+1 mean ± SD		10.3 ± 1.45		10.5 ± 1.45		10.0 ± 1.45		10.3 ± 1.61
at discharge mean ± SD		10.8 ± 1.29		10.6 ± 1.13		10.6 ± 1.13		10.9 ± 1.45

[a] A, control group; B, DONOR™ group; C, Bellovac A.B.T.® group
[b] this concerns only groups B and C

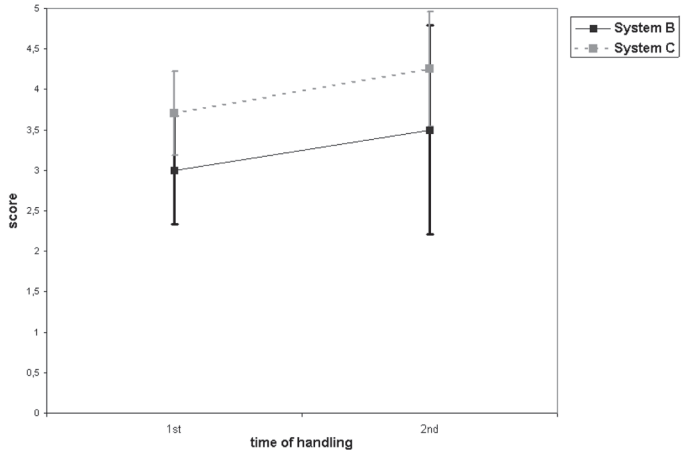

Figure 1. Scores of questionnaire: user-friendliness (n=28).

The figure shows the mean scores of system B (DONOR™) at first time of handling (n=10)and at second time of handling (n=4) and of system C (Bellovac A.B.T.®) at first time of handling (n=6) and at second (or more) time of handling (n=8). One nurse handled system C four times. Scores: 1=very difficult; 2= difficult; 3=neutral; 4=easy; 5=very easy.

Neither the percentage of patients transfused, nor the transfusion index [mean units of RBCs transfused per patient (1.0 ± 1.2 for both THR and TKR)] differed among the three groups. Six patients could not be mobilized on the third day after surgery: two control patients, three of group B and one patient of group C were delayed. Overall, the infection rate was 13 % (9/69) and was not different between groups: urinary tract infections (n=3), superficial wound infections (n=2), one deep wound infection resulting in prosthesis revision surgery, localized infections elsewhere (n=2) and bacteraemia (n=1).

Mean LOHS was slightly shorter in the THR re-infusion groups B and C and significantly less patients had a LOHS longer than 8 days as compared to the control group. In multivariate analysis, re-infusion group, age group, type of surgery and gender were entered, of which re-infusion group and age group remained independent variables to LOHS (p=0.02; corrected odds ratio=0.230 for re-infusion group and p=0.002; corrected odds ratio=2.775 for age group).

Laboratory analysis

Venous blood samples

Postoperative Hb values and Hb at discharge were similar in the groups (Table 2). In both re-infusion groups B and C, the D-dimer values on day 1 postoperatively were high (Table

3), resulting from high D-dimer values (>50.000 ng mL^{-1}) in the re-infused shed blood, and other coagulation values were comparable. In all groups, analysis of paired samples within the whole study group showed a significant decrease of antithrombin levels at day +1 after surgery compared with the pre-operative samples [103.2 to 83.6 % ($p<0.001$)]. In the re-infused patients this decrease was present directly after re-infusion [101.0 to 81.6 % ($p<0.001$); n=22] and remained stable up to the first postoperative day. Fibrinogen levels in the re-infused patients were also significantly decreased just after re-infusion: from 4,62 to 3,35 g L^{-1} ($p<0.001$) (n=22) and increased between time of re-infusion and day+1 (n=23): from 3,37 to 4,70 g L^{-1} ($p<0.001$). Fibrinogen levels at day+1 of the re-infused patients and the control patients were not significantly different.

Table 3. Coagulation parameters of venous blood samples

Group	Moment of sampling	Antithrombin (%) Mean (SD)	D-dimers (ng mL^{-1}) Median (range)	Fibrinogen (g L^{-1}) Mean (SD)
A[a]	Day –1	104.2 (9.7)	645 (225-9578)	4.33 (1.1)
	Day +1	85.9 (11.2)	1719 (695-15736)	4.67 (1.1)
B[a]	Day –1	103.3 (14.0)	995 (225-6247)	4.69 (1.4)
	Directly after re-infusion	80.8 (10.1)	39101 (5750-50000)	3.57 (0.8)
	Day +1	82.8 (13.6)	6172 (691-19224)	5.15 (1.1)
C[a]	Day –1	102.7 (11.5)	528 (233-6251)	4.19 (0.9)
	Directly after re-infusion	80.3 (20.6)	42437 (15730-50000)	3.01 (1.1)
	Day +1	82.2 (11.6)	7113 (601-17894)	4.63 (0.8)

[a] A: control group, B: DONOR™ group, C: Bellovac-A.B.T.® group

IL-12 levels in venous blood were not different between re-infused and control patients (median 38 pg mL^{-1} (range 5-615) on day-1 and 27 pg mL^{-1} (range 5-559) on day +1). Pre-operative values correlated with postoperative values (Figure 2). Measurable pre-operative IL-6 values (i.e. >1 pg mL^{-1}), were present in 54% of patients (n=34) with a median of 188 pg mL^{-1} and a wide range of 5-8758. After re-infusion, the median value (n=24) increased to 648 pg mL^{-1} (range 89-3512) and dropped to 361 pg mL^{-1} (range 48-4419) on day+1 after surgery (n=51) without a difference between groups. IL-10 levels were below detection level in 92% pre-operatively, in 72% directly after re-infusion and in 79% on day +1. The detectable values ranged from 4 to 126 pg mL^{-1}.

Patients' LDH after re-infusion was slightly elevated (446 ± 85 U L^{-1}).

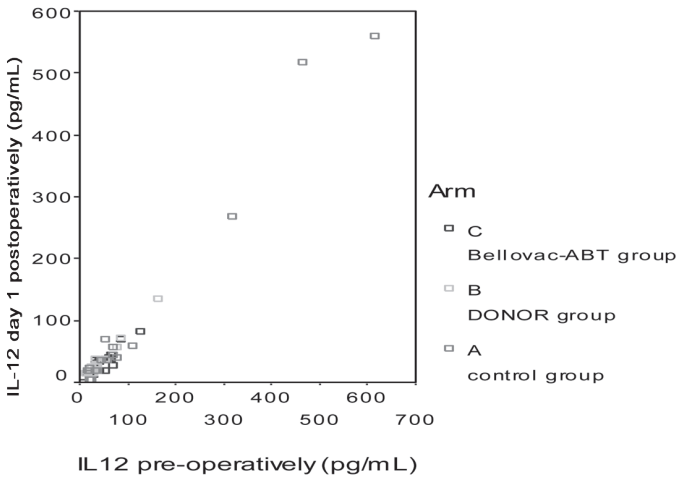

Figure 2. IL-12 levels of venous blood samples (pre- and postoperatively).

Shed blood samples

Whole-blood volume, Hb, Hct, platelets and haemolysis parameters of shed blood samples were comparable between both systems before (T1) and after filtering-just before re-infusion (T2) (Table 4), except for leukocyte count, as the Lipiguard filter used in drainage system B had a modest leucocyte depleting effect: from 5.6 x 10^9 L^{-1} (T1) to 1.9 x 10^9 L^{-1} (T2). IL-6 levels exceeded the maximal measurable value of 50.000 pg mL^{-1} in 4 of 37 (11%) wound blood samples at T0, in 20 of 33 (61%) at T1 (after 4-6 h of collecting) and in 21 of 35 (60%) prior to re-infusion at T2. IL-10 levels could not be detected in 6-14% at all time points. The range in the other samples was 4-157 pg mL^{-1}. Mean IL-12 levels (± SD) are shown in Table 4.

No bacterial contamination was found. There was no relation with transfusion reactions and cytokine levels in the re-infused shed blood. In one case, high haemolysis parameters were found in association with a febrile transfusion reaction (LDH and free Hb levels of 9890 U L^{-1} and 239 mmol L^{-1}, respectively). LDH of the patient directly after re-infusion was 610 U L^{-1}, but decreased to a nearly normal value on the next day (454 U L^{-1}).

Table 4. Mean shed blood values (± SD) just before re-infusion (T2).

Groups B and C shed blood values	
Hb (g dL^{-1})	8.4 ± 1.61
Thrombocytes (x10^9 L^{-1})	40 ± 22
Free Hb (mg dL^{-1})	184 ± 111.2
LDH (U L^{-1})	2511 ± 1853
Antithrombin (%)	39 ± 12.0
IL-12 (pg mL^{-1})	48 ± 35.0

DISCUSSION

Both postoperative autologous blood re-infusion systems turned out to be user-friendly and feasible to use. For both drainage systems a learning effect was present. The use of drainage systems was more effective for TKR patients, who were re-infused in 92% of the cases, compared to 54% of THR patients. Clearly, in TKR patients more blood is drained post-operatively. The overall relatively low percentage of re-infusion (60% and 63% for systems B and C respectively) was partly due to the use of 150 mL shed blood for study analysis and could increase to 75%. Approximately 50-100 mL shed blood remained in the system even after full re-infusion. In this pilot study, no reduction of allogeneic RBC transfusions was found, as was reported by others in THR patients [13]. However, our study was not powered for this conclusion.

No serious adverse events were seen. In one case, a reaction occurred upon fairly haemolytic blood. In literature, transient fever reactions during auto-transfusion have been related with surgery using cement [19]. In our study, however, in only two of these six patients bone cement (Palacos, Biomet Merck Inc., Warsaw, IN, USA) was used. Although the percentage of (30%; 6 of 20) mild febrile transfusion reactions was higher than previously reported [2,6,7], this may be due to the inclusion of all mild febrile reactions (≥1°C rise in temperature). A slower pace of re-infusion might prevent such transfusion reactions. However, there was no relation with transfusion reactions and cytokine levels in the shed blood or in the patient immediately after re-infusion, despite the extreme high levels of IL-6 in the shed blood. Preoperatively, IL-6 was undetectable in 46% of patients, but increased in all cases post-surgery. IL-12 values were between 0-600 pg mL^{-1} and not affected by surgery, autologous re-infusion or allogeneic transfusions (Figure 2). Although previous reports found an increased level of IL-10 after surgery and allogeneic transfusions might lead to deviation towards a T helper 2 type cytokine pattern, associated with an immune-suppressed state and even with LOHS, in our small pilot study we could not confirm this.

Despite IL-10 was present in shed blood, this was not recovered in the circulation after re-infusion.

A mean rise in temperature of 0,5°C immediately after re-infusion was found. Fever upon re-infusion has been attributed to high pro-inflammatory cytokine levels in re-infused blood [2]. The control group showed a similar pattern of temperature rise at this interval after surgery. It has been suggested that this is rather a response to the surgical procedure itself [11].

There were no signs of triggering of diffuse intra-vascular coagulation after shed blood re-infusion. Antithrombin activity levels decreased after surgery by 20% and remained stable in the patient after re-infusion up to the first postoperative day. Postoperative fibrinogen levels at day +1 in the re-infused patients were not significantly different compared to the control group. In all patient groups, fibrinogen levels also decreased significantly after surgery, probably due to a dilution factor (fluid infusion) and consumption during surgery, and increased to a slightly higher level on day +1 due to an acute phase response. Fibrinogen levels were not measured in shed blood, because these values are extremely low [6,10]. D-dimer levels in shed blood were very high due to coagulation activation and fibrinolysis in the wound area, and were passively infused in the patient in case of re-infusion which resulted in significant elevated levels in the patient directly after re-infusion up to day 1. All these data suggest that re-infusion of shed blood does not induce coagulopathy.

Despite the fact that no difference in the allogeneic RBC transfusion rate was found, in the control group the LOHS was one day longer than in the re-infusion groups B and C. In multivariate analysis, re-infusion group remained an independent variable to LOHS. This finding, however, should be interpreted with caution, because our study was not powered for this conclusion. A reduction in LOHS was also seen by Newman et al (1997) and Shulman et al (2002) who both found a reduction of two days, but this was associated with a reduction in allogeneic RBC's. It has been postulated that the pro-inflammatory cytokines in shed blood activate natural killer (NK) cells [5]. Whether and how such a stimulation of NK cells might have an effect on LOHS is unknown. We observed no difference in postoperative infections, but our study was not powered for this purpose and served as a pilot study for a large randomized controlled trial (International Standard Randomized Controlled Trial Number 96327523).

To conclude, the use of a postoperative autologous shed-blood re-infusion system is user-friendly and easily implemented in a blood management protocol. Efficacy is greatest in TKR patients. Concerning safety, no serious adverse events were seen. Although there was no blood saving, LOHS was slightly shorter in the re-infusion groups compared to the control group. Larger sufficiently powered studies are necessary to evaluate presumed reduction in allogeneic transfusions, postoperative infections and LOHS by the use of re-infusion of shed wound blood. As no difference in user friendliness and efficacy between

the two autologous shed blood re-infusion systems was found, the choice for a particular device can be based on economical aspects.

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Chapter 6

Salvaged blood and cytokine gene expression after hip surgery

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Submitted

ABSTRACT

Background: In orthopaedic surgery, re-infusion of autologous salvaged blood can be used as an alternative for allogeneic blood transfusion. In addition, autologous salvaged blood might stimulate an immune response reducing infections in the postoperative period.

Questions/purposes: We investigated whether re-infusion of washed or unwashed salvaged blood resulted in different cytokine gene expression (GE) profiles, indicative for immunomodulation, in patients after hip replacement surgery compared to the effect of the surgery itself.

Methods: Observational study of patients participating in a clinical study on transfusion alternatives. From 11 patients, whole blood samples before and 24 hours after surgery were collected for GE array analysis of 114 cytokines, followed by reverse transcriptase RealTime-PCR for selected genes in 56 patients, of which 19 received washed and 13 received unwashed autologous blood and 24 were control patients.

Results: After surgery, IL-8, TNFsf10 and TNFsf13B showed most frequent up- and down-regulations and were selected for PCR analysis. Overall, post-surgical inter-patient variations were large and exceeded the differences observed with or without autologous blood reinfusion. Unwashed salvaged blood re-infusion was associated with slightly more up-regulation of TNFsf13B GE compared to controls or patients who received washed salvaged blood.

Conclusion: Both autologous washed or unwashed salvaged blood re-infusion after hip surgery was associated with minor systemic changes in cytokine GE. The results suggest a more pro-inflammatory response after unwashed as compared to washed salvaged blood.

Clinical relevance: Salvaged blood re-infusion adds minor immuno-modulatory alterations compared to the hip replacement surgery itself. Unwashed blood may be somewhat more pro-inflammatory.

Allogeneic blood transfusions (ABT) in orthopaedic surgery have been associated with an increased risk for post-operative infections [7]. In order to diminish the use of ABT, various blood saving strategies are employed. In particular in orthopaedic surgery, post operative re-infusion of salvaged blood is an often used strategy [12,20,31]. Preliminary studies found a decrease of post-operative infections using salvaged blood as compared to patients transfused with allogeneic blood [31]. This protection against postoperative infections has been attributed to the stimulation of natural killer (NK) precursor cells by re-infusion of salvaged blood in contrast to the suppression of NK cells, which may occur after joint surgery and ABT [18].

Salvaged blood contains cytokines and activated leukocytes (neutrophils and macrophages), which are attracted by the local exudate at the surgical site. As compared to peripheral blood values prior to surgery, several pro- and anti-inflammatory cytokine genes become upregulated in neutrophils present in salvaged blood during the maximally 6 hours allowed between collection and re-infusion [2,5,6,11,13,19,21,28]. Before re-infusion, the collected salvaged blood can be washed to eliminate these cytokines, or filtered to remove debris and (activated) leukocytes, but not cytokines [1,2]. Filtration however, may activate the complement system [13,19], while washing of shed blood was reported to increase tumor necrosis factor-α (TNF-α) [6].

Re-infusion of filtered or washed salvaged blood results in a, mostly transient, increase of some cytokine levels in vivo (e.g. IL-6), with slight differences between the two modalities [1,5,18,21,28,32].

Additionally, after re-infusion of salvaged blood transient elevations in plasma cytokine levels have been measured, which may be associated with fever. Approximately 30% of patients [28] experience mild febrile transfusion reactions after re-infusion, although after orthopaedic surgery without re-infusion of salvaged blood a rise in temperature has also been observed [14]. It is however not elucidated whether these passively administered factors have an effect on the host immune reactivity. We investigated a possible effect on the recipient immune status 24 hours after hip surgery and autologous salvaged blood re-infusion. We compared whether re-infusion of salvaged blood had resulted in different cytokine gene expression profiles compared to controls and secondly whether washed or unwashed salvaged blood re-infusion resulted in different cytokine gene expression patterns.

MATERIAL AND METHODS

An observational study in elective orthopaedic hip replacement (THR) surgery patients, selected from a randomised controlled trial (RCT; Current Controlled Trials number, ISRCTN 96327523; Netherlands Trial Number, NTR303) evaluating various blood management

strategies in orthopaedic surgery. In this study, patients were randomised for either no autologous re-infusion (control group), or an intra- and post-operative blood salvage system that washes and concentrates the collected red blood cells before return to the patient (OrthoPAT®, Haemonetics, Breda, Netherlands), or a post-operative re-infusion system that filters unwashed salvaged blood (DONOR™ system, Van Straten Medical, Nieuwegein, the Netherlands). Both devices are FDA-approved. The ethics committee approved the protocol and the amendments, and all patients provided written informed consent before enrolment. Details of the study and overall results are reported separately [29].

From September 2006 until February 2008 pre and post operative whole blood samples from 56 patients undergoing elective THR surgery at the Leiden University Medical Center, Leiden, The Netherlands, were collected for RNA analysis. Exclusion criteria were revision surgery, rheumatoid arthritis and erythropoietin use. Initially, a pilot study of 11 patients was performed in order to select genes for additional reverse transcriptase RealTime-PCR (RT-PCR), which was based on gene expression changes after surgery. IL-8 data of the pilot study were used to calculate the necessary sample sizes (power of 90 and alpha of 0.05) to detect a difference in post-operative gene expression between cell saver recipients (washed salvaged blood) and controls (hypothesis 1), between unwashed drain reinfusion patients and controls (hypothesis 2) and between cell saver (washed) and unwashed drain reinfusion patients (hypothesis 3). These comparisons resulted in group sizes of 22 control patients, 58 unwashed wound drain reinfusion recipients and 10 cell saver recipients.

Some samples were invalid for analysis, leaving a total group of 24 control patients (i.e. with a regular wound drain), 13 drain re-infusion patients, and 19 cell saver patients. Thus the sample size was adequate to evaluate hypothesis 1, but sample sizes were inadequate for hypotheses 2 and 3.

Patient whole blood samples were taken before and 24 hours after surgery. Two samples of 500 mL were stabilised as soon as possible with RNALater (Applied Biosystems/Ambion, Austin, TX USA) and stored at -40˚C. RNA extraction was performed using the RiboPure™-blood kit (Applied Biosystems/Ambion, Austin, TX USA) according to standard protocol including the optional DNase treatment. RNA quantity was determined with the NanoDrop 1000 photospectrometer (NanoDrop products, Wilmington, DE, USA). Additionally, good RNA integrity was verified for a random selection of samples with the Bioanalyzer (Agilent Technologies, Santa Clara, CA USA). Preceding experiments had shown that the quality and quantity of isolated RNA are not influenced by storage time up to 72 hours at 2-6˚C before RNA stabilisation (data not shown).

Gene expression analysis was performed with the commercially available cytokine array (OHS-021, SA Biosciences™, Frederick, MD USA). This array contains 114 hybridisation spots for cytokines. Amplification and labelling of RNA was performed according to manufacturer's protocol of the TrueAMP 2.0 kit (SA Biosciences™, Frederick, MD USA). The cRNA was hybridised for 18 hours upon the array. The spots on the arrays were visualized

with a ChemiDoc XRS system (Bio-Rad Laboratories, Hercules, CA USA). Quantification of spot intensities was conducted using the GEArray Analysis Suite (SA Biosciences™, Frederick MD USA). Spot intensities were corrected for inter-array variation by subtracting the average value of spot intensities from all individual values per array. Gene expression values were expressed as ratios that compared the post surgery sample to the pre surgery sample.

The two-step reverse transcriptase Real-Time PCR assays were developed and executed as described earlier [27]. All assays had a taqman® probe with FAM dye and a minor groove binding non-fluorescent quencher (Applied Biosystems Inc., Foster City, CA USA).

For all patient samples, cDNA was synthesized from the extracted RNA with the SuperScript® II Reverse Transcriptase, using random primers (Invitrogen Corporation, Carlsbad, CA USA). The 7500 Fast RT-PCR instrument (Applied Biosystems Inc., Foster City, CA USA) was used on standard modus and standard program with the Gene Expression master mix (Applied Biosystems Inc., Foster City, CA USA). Each sample was run in quadruplo. A measurement was considered an outlier if it had a value which deviated more than 0.4 threshold cycle (Ct) from the average of the data points and was then discarded. If a second outlier was detected, the entire sample was discarded, and repeated in a new run. In each run a reference sample, consisting of pooled cDNA of 6 healthy adult blood donors, was taken along in 4-fold. All RT-PCR output was corrected for the reference sample from the same run. Relative quantification (RQ) was calculated as post/pre operative gene expression ratio within each patient.

All data were analysed statistically with SPSS version 15.0 for windows (SPSS Inc., Chicago, IL USA). The fold change in gene expression after surgery was compared between the control, cell saver and drain groups using Mann-Whitney U tests.

Changes in RT-PCR gene expression were correlated with clinical variables, including volume of re-infusion and infection rate. For this purpose, Spearman correlations were performed. We considered a p-value <0.05 statistically significant. Data are expressed as medians with interquartile ranges (IQR).

RESULTS

Patient characteristics showed no differences between the three study groups (24 control patients, 19 washed cell saver and 13 unwashed drain patients), except for a larger amount of blood loss in the drain group compared to the other groups (Table 1). Although cell saver recipients received smaller volumes of re-infused blood than those of unwashed drain blood, the amount of re-infused RBCs was comparable because of the more concentrated haemoglobin levels in the washed cell saver blood compared to unwashed salvaged blood.

Table 1. Patient demographics

	Control (N=24)	Washed salvaged blood (N=19)	Unwashed salvaged blood (N=13)
Age, median (IQR), years	65 (50-75)	70 (67-78)	64 (51-73)
Gender, N, Male	10	6	7
Weight, median (IQR), kg	76 (68-84)	73 (63-82)	78 (73-90)
Corticosteroids, N	1	1	0
NSAIDs, N	5	5	3
CRP, median (IQR), mg/L	3 (3-6)	3 (3-8)	5 (3-8)
BSE, median (IQR), mm/h	10 (6-30)	8 (5-29)	9 (5-14)
Surgery			
Duration of surgery, median (IQR), minutes	120 (105-150)	120 (105-133)	135 (110-150)
Type prothesis, cemented	11	15	7
Total blood loss during surgery, median (IQR), mL	443 (305-673)	235 (120-400)	370 (175-725)
Post-surgery			
Total blood loss post surgery, median (IQR), mL	270 (235-450)	265 (155-630)	780 (460-910)
autologous re-infusion, median (IQR), mL	N.A.	115 (70-200)	340 (200-500)
Hemoglobin, day +1 median (IQR), mmol/L	6.4 (6.0-6.8)	6.5 (6.1-7.0)	6.3 (5.8-7.7)
Leukocytes, day+1 median (IQR), 10^9/L	8.2 (7.4-9.2)	7.9 (6.8-9.2)	9.2 (8.4-10.4)
Temperature, day +1, median (IQR), °C	38.0 (37.7-38.2)	37.9 (37.5-38.0)	37.8 (37.1-38-5)
Hospital stay, median (IQR), days	7 (6-9)	8 (7-10)	7 (6-10)
Complications up to day 14			
Infections, N	0	1	0
Wound leakage, N	2	2	1
Other complications, N[a]	4	2	0

None of the patients has had previous CVA. None of the patients received antifibrinolytic agents. NSAIDs were stopped 3-10 days prior to surgery. None of the patients died. N.A. not applicable.
[a] Two control and two cell saver patients had cardio-vascular events. One hip dislocation and one pulmonary embolism occurred in the control group.

In the pilot group of 11 patients, 44 out of 114 cytokine genes were expressed above the detection limit on at least one of the arrays (Figure 1A). Seventeen of these showed at least a 2-fold change after surgery in minimally one patient (Figure1B; Table 2) of which nine genes were up-regulated, 3 showed down-regulations and 5 genes exhibited up-regulations in some, and down-regulations in other patients. The most striking responses were seen in the pro-inflammatory cytokines IL-8, TNF superfamily 13B (TNF sf13B) and inTNF sf10: IL-8 showed up-regulation in 3, and was down-regulated in 3 other patients. Control patients showed only minor changes in IL-8 gene expression, while in patients receiving unwashed

salvaged blood the gene was up- and in most patients receiving washed salvaged blood down-regulated. TNF super family 13B (TNFsf13B) was more than 2-fold up-regulated after surgery in 5 of 11 patients, and TNFsf10 was up-regulated in 3 and down-regulated in 1 patient. The remaining 14 genes showed only changes in a single patient (n=10) or showed a mixed response (up- and down-regulation in different patients) and were not further addressed. RT-PCR in the pilot patients confirmed the array results and IL-8, TNFsf10 and TNFsf13B were subsequently studied in extended patient groups by RT-PCR.

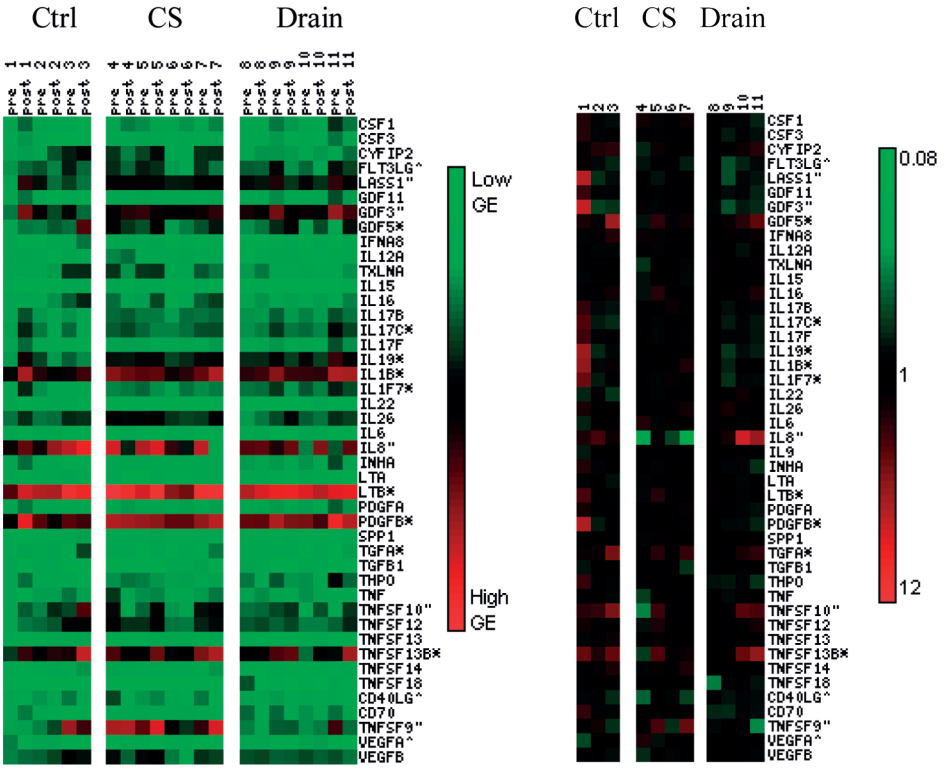

Figure 1.

Ctrl=control group; CS=washed salvaged blood by cell saver; Drain=unwashed salvaged blood by drain reinfusion.

A: Gene expression (GE) of all genes detectable on at least one array. Red presents high GE, green is low GE.

B: ΔGE of all detectable genes. Both post-RBC samples are compared to the pre sample of each patient. Green is a down-regulation, black indicates no major changes and red represents up-regulation.

* Genes which exhibit at least a 2-fold up-regulation after surgery in at least one patient.

^ Genes exhibiting at least a 2-fold down-regulation after surgery in at least 1 patient.

"Genes exhibiting at least 2-fold up-regulation and a 2-fold down-regulation after surgery in at least one patient each.

Figures were constructed with matrix2png 1.0.7 [24].

Table 2. Gene expression (GE) fold changes after surgery of the pilot group on the arrays

	Control			Washed salvaged blood				Unwashed salvaged blood			
CD40-L	0.81	0.87	0.60	**0.39**	1.1	0.78	0.51	0.70	0.98	0.81	0.74
FLT3-L	1.3	0.75	0.64	0.70	0.92	0.87	0.65	**0.45**	0.97	0.64	0.76
GDF-3	**5.3**	**0.50**	0.57	1.2	0.81	1.2	1.2	**0.44**	0.79	0.67	0.61
GDF-5	1.5	1.2	**3.9**	0.70	1.7	1.2	1.4	1.3	1.2	1.6	**2.4**
IL-1β	**3.6**	0.75	1.3	0.87	1.0	1.1	1.5	0.65	1.1	0.96	0.95
IL1-F7	**2.7**	0.67	0.86	1.1	0.80	1.3	1.1	0.57	1.0	0.78	0.76
IL-8	1.6	**2.0**	1.4	**0.19**	1.3	**0.50**	**0.08**	1.5	1.1	**5.7**	**3.7**
IL-17C	**2.2**	0.67	0.61	1.1	0.84	1.1	1.0	0.68	1.1	0.85	0.69
IL-19	**3.8**	0.61	0.77	0.98	0.99	1.2	1.1	0.55	1.0	0.73	0.65
LASS1	**4.7**	0.58	0.73	0.96	0.70	1.0	1.0	0.45	0.93	0.77	0.70
LT-β	**2.0**	0.95	0.85	0.78	1.6	0.87	1.0	1.0	1.2	0.82	1.1
PDGF-β	**4.4**	0.67	0.87	0.91	0.95	1.0	1.3	0.77	0.98	0.76	0.65
TGF-α	1.3	1.3	**2.9**	0.88	1.6	1.1	1.8	1.1	0.97	1.5	1.7
TNFsf9	1.0	1.4	0.58	1.1	**2.1**	0.59	2.6	1.0	0.76	1.1	**0.29**
TNFsf10	1.6	1.7	**3.1**	**0.30**	1.9	0.89	1.1	1.2	1.1	**2.3**	**2.1**
TNFsf13B	**2.6**	1.5	**2.5**	0.53	**2.2**	1.3	1.3	1.1	1.0	**2.6**	**3.5**
VEGF- α	**0.48**	1.0	0.97	1.5	0.72	0.92	0.97	0.98	1.1	1.0	0.88

All GE values are ratios of GE after surgery with or without autologous re-infusion in comparison with GE before surgery. Each column represents a patient. More than two-fold changes are presented in bold.

In the extended population, however, IL-8 gene expression after surgery showed large inter-individual differences of more than 2 logs, mostly down-regulations (Table 3; Figure 2), in all three groups of control patients, cell saver and drain recipients. In most patients TNFsf10 and TNFsf13B gene expression levels were elevated after surgery. These increases were higher in the patients receiving unwashed salvaged blood than in control patients and in patients receiving washed salvaged blood, which was significant for TNFsf13B gene expression between drain and cell saver patients (Table 3; Figure 2).

None of the relevant clinical variables clearly correlated with gene expression of IL-8, TNFsf10 and TNFsf13B (Table 4), including volume drainage blood re-infused, neither within nor between the washed cell saver and unwashed drain groups.

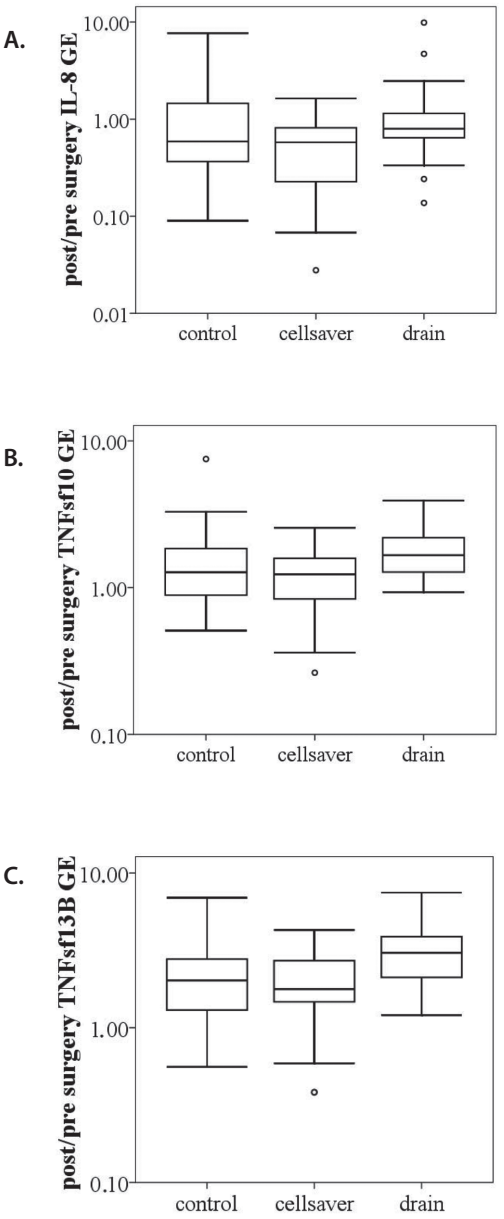

Figure 2. Boxplots showing median fold changes in gene expression (GE) measured with RT-PCR. A: IL-8, B: TNFsf10, C: TNFsf13B.

Table 3. Fold changes in gene expression (GE) for 3 selected cytokines with RT-PCR in the total group

	Control	washed salvaged blood	unwashed salvaged blood	Ctrl vs CS p-value	Ctrl vs drain p-value	CS vs drain p-value
N	24	19	13			
Post/pre IL8 median (IQR)	0.59 (0.36-1.62)	0.57 (0.16-0.85)	0.79 (0.48-1.80)	0.3	0.4	0.081
Post/pre TNFsf10 median (IQR)	1.27 (0.86-1.87)	1.23 (0.81-1.60)	1.66 (1.12-2.42)	0.8	0.086	0.081
Post/pre TNFsf13B median (IQR)	2.02 (1.28-2.83)	1.78 (1.44-2.75)	3.05 (1.75-4.22)	0.6	0.14	0.033

All GE values are ratios of GE after surgery with or without autologous re-infusion in comparison with GE before surgery. CS: cell saver etc.

Table 4. Associations of gene expression (GE) with clinical variables

		IL-8		TNFsf10		TNFsf13B	
	N	correlation coefficient	p-value	correlation coefficient	p-value	Correlation coefficient	p-value
Age	56	-0.238	0.078	-0.121	0.4	-0.052	0.7
Gender	56	-0.221	0.10	-0.228	0.091	-0.21	0.12
before surgery							
NSAIDs	56	-0.132	0.3	-0.198	0.14	-0.035	0.8
during surgery							
Duration of surgery	52	0.225	0.11	-0.115	0.4	-0.179	0.2
Blood loss during surgery	56	0.062	0.6	0.003	1.0	0.104	0.4
after surgery							
Blood loss after surgery	51	0.212	0.13	0.044	0.8	0.054	0.7
Reinfusion volume	56	0.056	0.7	0.149	0. 3	0.190	0.16
Hemoglobin	56	0.141	0.3	-0.052	0.7	-0.065	0.6
Leukocytes	55	-0.012	0.9	0.273	0.044	0.272	0.045
Temperature	48	0.018	0.9	0.272	0.064	0.345	0.018

Spearman correlations were performed.

DISCUSSION

Autologous salvaged blood re-infusion is a widely used alternative for allogeneic blood transfusions and preliminary reports claim a reduced incidence of post-operative infections through beneficial immunomodulation. Although salvaged blood contains cytokines and activated cells which are re-infused into the patient, an effect on the recipient immune system is however less documented. We investigated alterations in patient cytokine gene expression profiles in relation to autologous salvaged blood product re-infusion and secondly whether washing of salvaged blood made a difference. For initial selection of relevant cytokines we performed a pilot study investigating 114 cytokine genes on expression array in 11 patients; only 3 cytokines came up as candidate altered gene expressions. These were subsequently evaluated by PCR in an extended cohort of 56 patients.

Our study had some limitations. Despite homogeneity of the population, we still detected major inter patient variation in gene expression, for which we were unable to find a possible explanation. Also, not enough patients were available to comply with the sample sizes required to detect statistically significant differences in gene expression ratio's between control and drain patients. However, as gene expression ratio's, analysed by PCR, were smaller than observed on the array results, not the sample size, but the smaller differences in gene expression ratio's explain the non-significant results. As only one of the control patients had received allogeneic RBC transfusions during or within 24 hours after sampling, we were not able to compare the effect of autologous re-infusion with the effect of allogeneic RBCs on cytokine gene expression. By only measuring before and 24 hours after surgery, we might have missed a peak in gene expression [3,4,16], although a previous study that investigated the surgery effect on cytokine gene expression showed changes only after 24 hours, and not at 6 hours after surgery [21].

We found in the pilot study patients that IL-8, TNFsf10 and TNFsf13B showed most frequently up-or down-regulations. However in the larger patient population, increases of IL-8, TNFsf10 and -13B gene expression after surgery were less pronounced (Table 3). Surgery by itself had major effects on the gene expression profiles and additional effects of salvaged blood re-infusion were minor, although small additional up-regulation remained present after re-infused unwashed drain blood.

Despite re-infusion of inflammatory cytokines in drain blood and leukocytes with up-regulated cytokine genes in cell saver blood, the re-infusion of this salvaged blood showed, after 24 hours, only minor systemic changes in cytokine gene expression in excess of the surgical procedure itself. Although these minor up-regulations of IL-8, TNFsf10 and TNFsf13B involve genes which are among other effects, all associated with NK cell activation [10,15,18,30], the most potent NK-cell activating cytokines (IL-2, -12, -15, -18 and interferon-α) did not show changes on the array, although their activation may have occurred before or after the time point of 24 hours post-surgery [33].

The main function of IL-8 is neutrophil chemoattraction, but it also stimulates neutrophil and T-cell activity [23]. Control patients did not show changes in IL-8 gene expression, while patients receiving unwashed salvaged blood often showed up-regulation and patients receiving washed salvaged blood mostly showed down-regulation of this gene. TNFsf10 encodes for TNF-related apoptosis inducing ligand (TRAIL), has a function in apoptosis induction and plays a role linking the adaptive and innate immune system [25], TNFsf13B is associated with proliferation of B-cells [6], also referred to as B-cell activating factor (BAFF) [26]. The BAFF-Receptor induces cell signalling through the IKKβ and NF-κB pathways, promoting transcription of IL-8, TNFsf13B and CD40L [17].

The large inter-patient variation in gene expression, was not explained by (type of) salvaged blood transfusion, or associated to other clinical variables including postoperative anaemia, leukocytosis, fever, blood loss and volume of re-infused salvaged blood. Absence of an association between IL-8 gene expression and re-infused volume, has been previously published [9]. Rather, other inter-individual (genetic) or unknown environmental factors may play a role.

We found only one other study that evaluated cytokine gene expression in neutrophils after hip arthroplasty [11]. This study compared the neutrophils in salvaged blood to those in pre-surgical and post-surgical circulating blood in the same patient without re-infusion of the salvaged blood. The authors found that other cytokines, such as interleukin-1 receptor antagonist (IL1RA), interleukin-18 receptor 1 (IL18R1), macrophage migration inhibitory factor (MIF), and macrophage inflammatory protein 3alpha (CCL20) were upregulated both in the patient and in salvaged blood, whereas interleukin-8 receptor beta (IL8RB/CXCR2) was consistently downregulated. Our findings, reflecting the total leukocyte population, did not only report systemic gene expression changes after surgery, but also compared gene expression changes after re-infusion of washed or unwashed salvaged blood with the presurgical situation and control surgery patients. We found a stronger up-regulation of TNFsf13B after receiving autologous unwashed blood compared to controls or washed salvaged blood. The differences in complications can not be related to either the washed or unwashed re-infusion of blood, since it is a post-hoc analysis with inadequate power for detecting a relevant difference between the groups.

In conclusion, inter-patient variations of unidentified cause showed more changes in cytokine gene expression after hip surgery than re-infusion of shed salvaged blood. Unwashed filtered drain blood showed more up-regulation of the pro-inflammatory cytokines IL-8, TNFsf10 and -13B compared to washed salvaged blood or controls.

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Chapter 7

A randomised controlled trial on erythropoietin and blood salvage as transfusion alternatives in orthopaedic surgery using a restrictive transfusion policy

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Submitted

ABSTRACT

Objective: To investigate the combined and separate use of erythropoietin (Epo), cell saver and/or postoperative drain re-infusion devices (DRAIN) as red blood cell (RBC) sparing alternatives.

Design: A multi-centre randomised, controlled trial.

Setting: Four hospitals in the Netherlands using a restrictive transfusion policy.

Participants: 2442 elective knee- and hip-arthroplasty patients aged 18 years and older.

Interventions: Primary stratification by preoperative haemoglobin (Hb) level: stratum I, Hb 10 to 13 g/dL (low Hb), randomised for Epo or no Epo; stratum II, Hb above 13 g/dL (normal Hb), ineligible for Epo. Both strata were also randomised for cell saver, DRAIN or no blood salvage device.

Main outcome measure: Number of RBC transfusions.

Results: Mean RBC use was 0.3 (SD 1.2) units / patient (n=2442) and 11.6% were transfused. Transfusion protocol adherence was above 95%. In Intention-To-Treat analysis, Epo resulted in a significant 50% reduction in transfused patients (OR 0.5, 95% CI 0.35 to 0.75) and a 29% mean RBC reduction (ratio 0.71, 95% CI 0.42 to 1.13). Additional costs due to Epo were estimated at €785 per patient (95% CI 262 to 1309), i.e. €7300 per avoided transfusion (95% CI 1900 to 24000). In both strata, autologous blood re-infusion did not result in RBC reduction and increased costs by €378 per patient (95% CI 161 to 595). Because of significant heterogeneity of treatment effects, primary (n=2258) and revision (n=184) surgery patients were analysed separately. In stratum I the primary surgery group had a 55 % reduction in transfused patients (OR 0.45, 95% CI 0.28 to 0.69) and a 55 % mean RBC reduction (ratio 0.45, 95% CI 0.29 to 0.72) by Epo, whereas autologous blood re-infusion by cell saver or DRAIN did not result in a significant RBC reduction in either strata. No conclusions can be drawn for revision surgery patients.

Conclusions: Even with a restrictive transfusion trigger, Epo contributed significantly as a transfusion alternative for RBC use in knee- and hip-arthroplasty patients with a low Hb, but at unacceptably high costs per avoided transfusion. Possibly due to the restrictive transfusion policy, autologous blood salvage devices were not effective in RBC reduction and consequently only increased costs.

Trial registration: www.controlled-trials.com, number ISRCTN 96327523; Dutch Trial Register NTR303

INTRODUCTION

To achieve optimal blood management, the use of alternatives for red blood cell (RBC) transfusions in orthopaedic surgery is widely accepted. However, the effect on RBC reduction may vary considerably (from 20 to 80%) and is related to the use of a transfusion threshold [1-8]. As transfusion policies have recently become more restrictive, it is questionable whether the currently accepted transfusion alternatives can still effectively reduce RBC use. Over the years, the use of pre-operative au0tologous donation (PAD) has declined due to logistical problems and wastage [9,10]. On the other hand, the use of Erythropoietin (Epo) and peri-operative autologous blood salvage have become increasingly popular worldwide including the Netherlands [11]. In randomised controlled studies of elective hip and knee surgery patients, Epo resulted in a significant reduction in mean RBC use (referred to as "blood-sparing") and a significant reduction in the proportion of transfused patients (referred to as "transfusion-avoiding") for up to 75%, while using a restrictive transfusion threshold of 8 g/dL. These studies also showed that the optimal benefit from Epo can be reached in patients with preoperative Hb levels between 10 to 13 g/dL in order to decrease RBC use [12,7,13].

Using a cell saver intra-operatively, up to 70% of the shed blood can be recovered in orthopaedic surgery [14], which may significantly reduce RBC use [8]. Post-operative re-infusion of autologous shed blood may also result in allogeneic RBC reduction, although these study results are not reported consistently [1-4;15-19]. The evidence for RBC reduction by autologous salvaged blood re-infusion is mostly based on small and/or underpowered studies often not applying a restrictive transfusion threshold. Moreover, evidence is lacking on the effect of combined use of transfusion alternatives. To address this issue we performed a multi-centre study with adequate power (90%), to investigate whether the use of Epo, the intra- and postoperative use of cell saver or the use of a postoperative drainage and re-infusion device (DRAIN) as transfusion alternatives, resulted in allogeneic RBC reduction in patients undergoing elective knee- or hip-replacement surgery while applying a restrictive transfusion policy. Additionally, we compared cost-effectiveness of the use of Epo, cell saver and DRAIN.

METHODS

Patients

Patients were enrolled between May 1st, 2004 and October 1st, 2008 from four hospitals in the Netherlands with study closure after completed follow up on Oct 1st, 2009. The ethics committee at each institution approved the protocol and the amendments, and all patients provided written informed consent before enrolment. The study was undertaken

in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines and local laws and regulations. Eligible patients were aged 18 years and older, being scheduled for a primary or revision hip or knee replacement. All patients received six weeks of postoperative anti-thrombotic prophylaxis with subcutaneous Low-Molecular Weight Heparin (LMWH) starting the day before surgery. Anti-platelet agents (NSAIDs, clopidogrel, acetyl salicylic acid) were discontinued 3 to 10 days before surgery according to the hospital protocol. Oral anticoagulants (acenocoumarol, phenprocoumon) were discontinued with monitoring of INR values, which was required to be 1.8 or lower before surgery.

Patients were excluded if they had: untreated hypertension (diastolic blood pressure >95 mm Hg); a serious disorder of the coronary, peripheral and/or carotid arteries; a recent myocardial infarction or CVA (within 6 months); sickle cell anaemia; a malignancy in the surgical area; a contra-indication for anticoagulation prophylaxis; a known allergy to Epo; an infected wound bed; a revision of an infected prosthesis which was being treated with local antibiotics (e.g. gentamycin bone cement beads); difficulty understanding the Dutch language (unable to give informed consent); or were pregnant or refused homologous blood transfusions.

Study design

We designed a double randomised, multi-centre trial in which the randomisation was stratified for hospital, type of surgery (primary/revision as well as hip/knee), and the preoperative haemoglobin (Hb) level in order to have a balanced randomisation. Double randomisation included randomisation for Epo and randomisation for autologous blood re-infusion by cell saver or DRAIN. By selecting this design, the three transfusion alternatives can be investigated in a combined setting as well as separately, and was intended to resemble daily practice in an optimal way. Randomisation took place in one run for all possible combinations using a computer generated allocation table, but is here described sequentially. Patients were first stratified according to the pre-operative Hb level: stratum I (low Hb) = Hb between 10 and 13 g/dL. These patients were randomised for Epo or no Epo. Stratum II (normal Hb) = Hb of 13 g/dL and higher was not eligible for Epo but continued as a separate non-Epo treatment group. Since knee replacement procedures were performed using a pneumatic tourniquet, which was deflated after wound closure, intra-operative use of cell saver was not applicable due to negligible intra-operative blood loss, and consequently knee replacement surgery patients were excluded from randomisation for cell saver. All patients in both strata were randomised for two (knee surgery) or three (hip surgery) treatment modalities: 1) an intra-and postoperative autologous re-infusion device (cell saver) that washed, filtered and re-infused the autologous shed blood (only in hip surgery), 2) a postoperative autologous re-infusion drainage system (DRAIN) that filtered and re-infused autologous unwashed shed blood (both knee and hip surgery) and 3) no blood salvage device, although a low vacuum wound drain was placed but the collected

blood discarded. The randomisation resulted in the following combinations of modalities: cell saver+DRAIN- (only hip surgery); cell saver-DRAIN+; cell saver-DRAIN- (this group represents the control group). Hence the entire trial consists of nine different treatment modalities: six in stratum I: 1) Epo+cell saver+DRAIN-; 2) Epo+cell saver-DRAIN+; 3) Epo+cell saver-DRAIN-; 4) Epo-cell saver+DRAIN-; 5) Epo-cell saver-DRAIN+; 6) Epo-cell saver-DRAIN- (=control group) and three in stratum II: 7) Epo-cell saver+DRAIN-; 8) Epo-cell saver-DRAIN+; 9) Epo-cell saver-DRAIN- (=control group). For each stratum a separate randomisation list was created, using blocks of random length to avoid predictability of the random treatment assignment towards the end of each block. All patients were transfused according to a restrictive transfusion policy as advised in the Dutch transfusion guidelines (see below) [20]. Preoperative anaemia was defined according to the WHO criteria [21] (for males: Hb <13 g/dL and for females: Hb <12 g/dL). Participating hospitals were free to choose the type of Epo (i.e. alpha-Epo or beta-Epo) and the post-operative drainage system, but were obligated to use the same type throughout the study. The type of cell saver was uniform for all patients.

The transfusion protocol considered age and normal or high risk patients as triggers for transfusion. High risk included: incapability to enlarge cardiac output to compensate for anaemia, serious pulmonary disease or symptomatic cerebro-vascular disease. The following transfusion thresholds were used: Hb=6.4 g/dL (=4.0 mmol/L) for age <60 years and normal risk; Hb=8.1 g/dL (=5.0 mmol/L) for age ≥60 years and normal risk; Hb=9.7 g/dL (=6.0 mmol/L) in case of high risk. Hb values were derived from mmol/L which is the standard unit to denote Hb values in the Netherlands. The protocol included a single-unit transfusion policy (RBC units transfused one by one to reach a target Hb level above the defined Hb thresholds). A check for transfusion protocol adherence was included in the CRF by verifying the Hb, age and cardiovascular history (for risk estimation) of the patient for every transfusion event. The RBC units were prepared from whole blood donations. After centrifugation, followed by plasma- and buffycoat depletion, SAG-M (Saline, Adenine, Glucose, Mannitol) was added, resulting in a RBC product with a Ht between 0.50 and 0.65 L/L (40-54 g Hb) and a total volume of 270-290 mL. A universal pre-storage leukocyte depletion policy was applied, resulting in a leukocyte concentration of less than 1 x 10E6 per unit.

Treatment allocation was random using a uniform distribution and created a pre-generated list of sufficient length, based on the maximum expected sample size in each stratum. For each subject to be randomised, a sheet of paper with all relevant stratification and group-allocation information was produced and placed in a sealed opaque envelope. Batches were created according to the stratification factors. After receiving informed consent, the patient was preoperatively allocated by the research nurse to one of the groups by opening the first sealed envelope from the appropriate stratum. The exact moment of opening the envelope and its associated sequence number was verified against a centrally

stored randomisation list to check for selection bias. Hip surgery patients who were randomised for cell saver were automatically assigned to postoperative autologous blood re-infusion, as the used cell saver collected autologous blood intra- and postoperatively.

In order to avoid protocol violations, clinical-site staff members, clinicians, and patients were aware of study group assignments. The study investigators were blinded. The chart data were written on the Case Report Form (CRF) by the research nurses. All written information was transferred from the paper CRF to the secure on-line web based data management system (ProMISe) of the department of Medical Statistics & BioInformatics in Leiden. A built-in quality management system checked for irregularities, inconsistencies and coding errors and clarification was asked for whenever necessary.

The primary outcome measure was the number of allogeneic RBC transfusions. By comparing the mean RBC use we quantified the "blood-sparing" effect, and by comparing the proportion of transfused patients we quantified the "transfusion-avoiding" effect. Secondary outcomes (not all reported in this manuscript) were length of hospital stay (days), peri- and post-operative complications up to three months after surgery, transfusion reactions, rehabilitation time, quality of life and costs. All primary and secondary endpoints were scored until 3 months after surgery.

Procedures

A fixed weekly dose of 40.000 IU was given to patients randomised for Epo with simultaneous prescription of ferrofumarate 200 mg TID (=195 mg Fe^{2+} a day) during three weeks before surgery. A total of four Epo doses were administered by subcutaneous injection on days -21, -14, -7 and on the day of the operation (day 0), respectively. Hb levels were determined before administration of the fourth dose. If the Hb level exceeded the value of 15 g/dL, the final Epo dose was withheld. The Epo preparations were Neorecormon® (erythropoietin-beta, Roche Nederland BV, Woerden, Netherlands) (three hospitals) or Eprex® (erythropoietin-alpha, Janssen-Cilag BV, Tilburg, Netherlands) (one hospital). A protocol violation was scored if patient did not receive Epo therapy at all after being randomised for Epo. If at least one dose was given this was not regarded as violation and patients were included in the analysis as treated (AT) as having received Epo.

The OrthoPAT® cell saver (Haemonetics, Breda, Netherlands) was used for both intra- and post-operative collection and re-infusion of autologous blood. The collected shed blood was washed, centrifuged and concentrated to a hematocrit of 60-80% before being returned to the patient. Only hip surgery patients were randomised for the use of the cell saver. A protocol violation was scored if the cell saver was assigned but not used. When the cell saver device was truly used, the patient was included in the cell saver group in the AT-analysis whether or not autologous blood had been given to the patient.

Two different DRAIN devices were used: Bellovac-ABT® (Astra-Tech, Zoetermeer, the Netherlands) (two hospitals) and DONOR™ system (Van Straten Medical, Nieuwegein, The

Netherlands) (two hospitals). These systems differ slightly in filtration and vacuum pressure: the DONOR™ system uses a continuous suction at a vacuum pressure of 150 mm Hg and just prior to re-infusion a double shielded 40 micron filter (Pall Lipiguard VS filter) entrapping lipids larger than 10 micron and 2 log of leukocytes. The Bellovac-ABT® system uses intermittent suction pressure by a manually expandable bag at a maximum pressure of 90 mm Hg and three filters: a 200 micron filter, a secondary 80 micron filter and prior to re-infusion a third 40 micron filter. In a feasibility and efficacy study, we found both systems to be comparable [22]. A protocol violation was scored if the device was assigned but not used. When the DRAIN device was truly used, the patient was included in the DRAIN group in the AT- analysis whether or not autologous blood had been returned.

Intra-operative transfusions were prescribed by the anaesthesiologist and post-operative transfusions by the orthopaedic surgeon. Transfusion protocol violations and randomisation violations were recorded.

Serious Adverse Events (SAEs) were defined as events that occurred within one month after surgery, and were labelled as death, life threatening events, (prolongation of) hospitalization and/or events resulting in persistent disability, and categorised into prosthesis related (dislocation, wound infection or deep prosthetic infection, fractures or limitation in movement), thrombo-embolic (deep venous thrombosis diagnosed by ultrasound, pulmonary emboli, stroke or transient ischemic attack, myocardial infarction, cardiovascular other than myocardial infarction, allergic, infection/sepsis (not prosthesis related), malignancy and other events. All SAEs that were reported to the central coordinator during the three month-follow up, were scored.

Statistical methods

The study was designed to have statistical power of 90% with a type I error of 5% (two-sided test) to detect a difference of 75% in mean RBC use by Epo (the alternative to null-hypothesis 1) and a difference of 30% in mean RBC use by autologous blood re-infusion by either cell saver or DRAIN (alternative to null-hypothesis 2). The study design allowed to investigate the Epo versus no Epo effect (comparison 1), the combined autologous versus no autologous effect (comparison 2) and the cell saver versus DRAIN effect (comparison 3) (eFigure 1 and sample size calculation online material only). Various scenario's of literature based estimates of standard deviations were included as well as the possibility of severe treatment and stratum interactions. This required an inclusion of 2250 patients for analysis on an Intention-To-Treat (ITT) basis and included protection against a worst case scenario of high standard deviations and heterogeneity of treatment effects. Assuming a study dropout rate of 10%, our goal was to have 2500 patients eligible for randomisation. An interim analysis was carried out by an independent Data Safety Monitoring Committee (DSMC) at the half way mark (958 inclusions) using an alpha of 2.5% (instead of 5%). As pre-defined stopping criteria were not reached, neither for futility nor for efficacy, the DSMC advised to continue the study until its pre-specified number of patients was obtained.

Conforming to ICH-9 guidelines, the primary analyses were performed both as ITT and As Treated (AT). In case of the Epo (yes/no) covariate, AT is defined as the actual administration of at least one dose of Epo; in case of cell saver or DRAIN it is defined as the actual use of the device whether or not autologous blood had been re-infused to the patient.

Variables were described by frequencies, by mean and SD, and by median and inter-quartile range (IQR) in case of a non-normal distribution. Although RBC use can be severely non-normally distributed, we also report means (and standard deviations), since the power and sample size calculation was based on assumptions of these means. Ratio's (dividing the mean RBC values of two randomised groups to be compared) and 95% confidence intervals (CI) were reported to calculate the proportional reduction between the groups. Confidence intervals were obtained via bootstrapping methods for these highly non-normally distributed ratio's (software package R, using the standard package "boot"). For additional non-parametric testing we used the Mann-Whitney test. When comparing the proportion of patients receiving RBC transfusions, a Mantel-Haenszel procedure was applied, using the main risk factors and stratification variables as strata. This led to an overall, adjusted common Odds Ratio (OR) as a comparison of the probability of "receiving at least one RBC unit" between the randomisation arms. A linear mixed model was used for the primary outcome (RBC use) as a function of the interventions, the stratification factors (hip versus knee and primary versus revision surgery) and their interactions with the intervention. In case of significant interaction, the calculations were based on separate subpopulations (stratified by the interacting term) as pre-specified in the protocol. In case of non-significant interactions, the stratification factor was retained in the model as a main term for adjustment. The stratification factor "centre" was included as a random effect. Even though we were fully aware of the non-normal distribution of the RBC use among the various strata and intervention groups, we reported confidence intervals based on the mixed models for comparison with other literature; therefore significance of differences in this model needs to be interpreted with caution. Each analysis of intervention effect in this additive framework is accompanied by a robust estimate of the treatment effect as a ratio and its associated confidence interval.

After data checking the database was frozen. The conversion process transferred the data to a number of SPSS (version 17.0 for Windows (SPSS Inc, Chicago, IL, USA)) system files which were used for all analyses. The same files were used within the R software to obtain estimates and robust bootstrapped confidence intervals. The SPSS files were read using the library "foreign". A p- value of less than 0.05 was considered statistically significant.

Economic evaluation

Costs were estimated from a hospital perspective, with a three-months time horizon. Health care was valued at the 2011 price level, using market prices for Epo, cell saver and DRAIN (€1293 for four doses [23], €160, and €61, respectively) and using standard prices for

allogeneic RBC products, ICU care, and non-ICU care (€207 per unit, €2249 and €471 per day, respectively) [24]. The total price per unit of RBC use was estimated at four times the product price (i.e. €829 per unit), according to the paper of Shander and co-workers [25]. Average costs were compared according to intention-to-treat, using non-parametric bootstrapping (programmed in Stata/IC 11.0 for Windows). If a strategy resulted in transfusion avoidance but with higher costs, a cost-effectiveness analysis was performed comparing the difference in the proportion of transfused patients to the difference in costs. Confidence intervals for the cost-effectiveness ratio were calculated using net benefit analysis [26].

RESULTS

From May 2004 to October 2008, 3165 patients were screened for eligibility of which 586 patients were not enrolled (Figure 1). After completion of the study in October 2009, 2579 patients had been randomised, of which 2442 (95%) were evaluated. Of the 137 not evaluated patients, for the majority (82%) surgery was cancelled or performed elsewhere, six of whom had received at least one Epo dose. Baseline characteristics are shown in table 1. Mean preoperative Hb at first outpatient visit was 13.8 g/dL (SD 1.3) and mean Ht 0.42 L/L(SD 0.04). Sixty percent were hip procedures and 40% were knee procedures. Seventy percent was female. Revision surgery occurred in 7.5% (n=184), equally divided among the groups. 683 (28%) patients were eligible for Epo. In Table 2, peri-operative characteristics are shown. The median volumes of re-infused blood were 100 mL for cell saver [IQR 50-200 mL] with mean Ht: 0.70 [SD 0.11] and 350 mL for DRAIN [IQR 200-500 mL] with mean Ht:0.34 [SD 0.17]. Postoperative Hb values on day+1 were comparable in the groups with or without autologous blood re-infusion by cell saver or DRAIN. Revision surgery patients differed significantly for intra-operative blood loss, mean duration of surgery and total blood loss ($p<0.05$), but not for the mean and median re-infused volumes.

Primary endpoint

No heterogeneity was found among the four participating hospitals with respect to the effect-size in any comparison of the primary endpoint. Of 2442 evaluated patients, mean RBC use was 0.32 units (U) / patient [SD 1.2] and median use was 0 U/patient [range 0-27]. 11.6% (n=284) of patients received in total 775 RBC transfusions (median 2U [IQR 2-2]). The majority of patients (n=246) were transfused postoperatively up to 14 days after surgery (median of 2 U [IQR 2-2]). The median RBC units used and proportion transfused patients are outlined in Table 2. Overall, revision surgery patients were significantly transfused more often (19.6%) than primary surgery patients (11%) with more RBC transfusions. In addition, hip surgery patients were significantly more often transfused (15%) than knee surgery patients (6.6%), with more transfusions as well. Due to significant interaction between

primary or revision surgery and the allocated treatments (Epo and cell saver and DRAIN; p<0.001), we analysed these patient groups separately (2258 primary and 184 revision surgery).

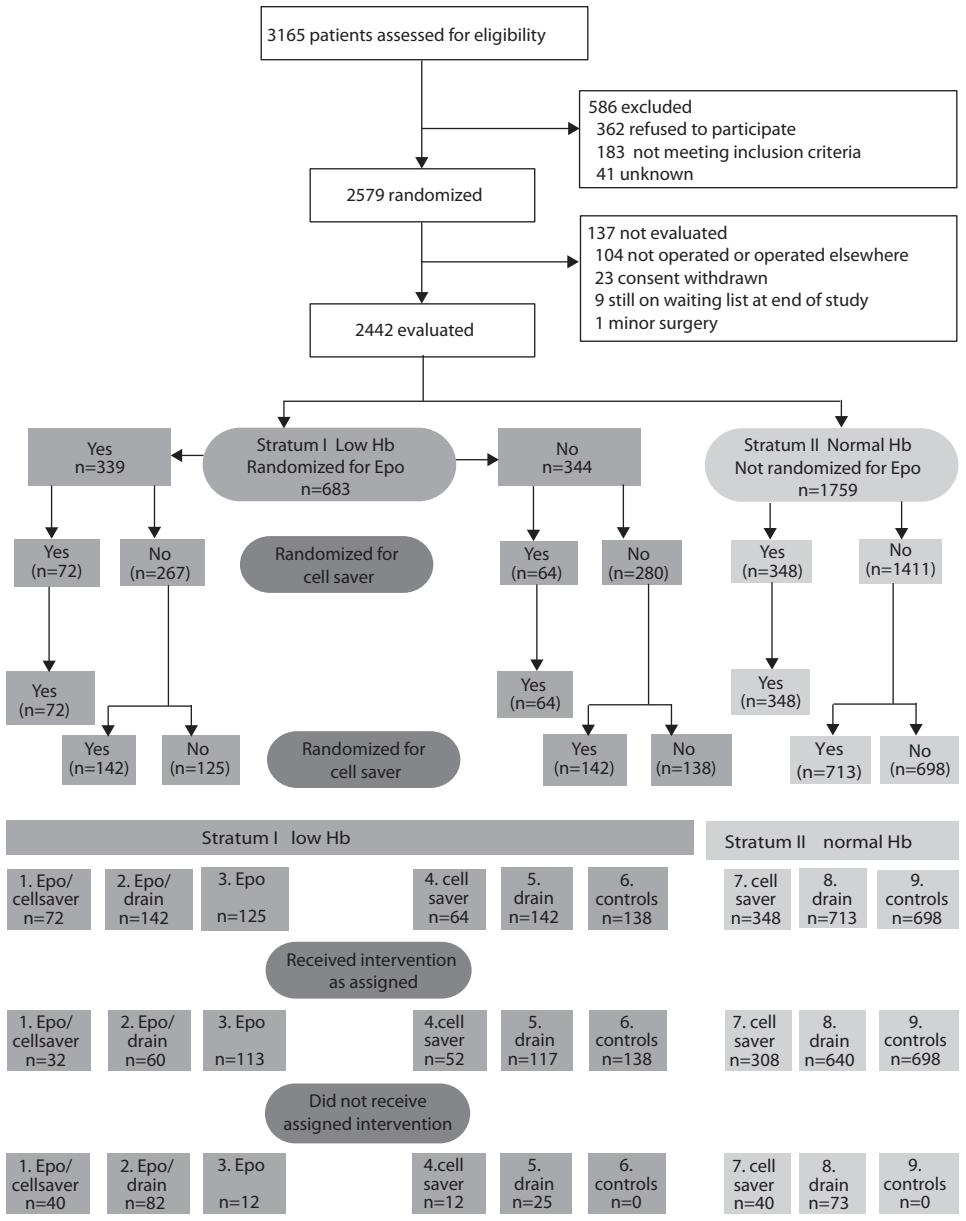

Figure 1. Patient flow diagram

Table 1. Baseline characteristics of the study population

Patient variables	All patients	STRATUM I (LOW HB)						STRATUM II (NORMAL HB)		
	Numbers (%) or mean (SD)	1. Epo/CS	2. Epo/DR	3. Epo	4. CS	5. DR	6.controls	7. CS	8. DR	9. controls
Evaluated	2442	72	142	125	64	142	138	348	713	698
Hip surgery	1464 (60%)[a]	72 (100)	78 (55)	64 (51)	64 (100)	72 (51)	77 (56)	348 (100)	347 (49)	342 (49)
Knee surgery	978 (40%)[b]	0 (0)	64 (45)	61 (49)	0 (0)	70 (49)	61 (44)	0 (0)	366 (51)	356 (51)
Primary hip	1332 (55%)	64 (89)	65 (46)	56 (45)	56 (87)	64 (45)	67 (49)	322 (93)	321 (45)	319 (46)
Primary knee	924 (38%)	0 (0)	61 (43)	56 (45)	0 (0)	64 (45)	60 (43)	0 (0)	344 (48)	339 (49)
Females (%)	1699 (70%)	63 (88)	121 (85)	113 (90)	55 (86)	122 (86)	121(88)	224 (66)	470 (66)	410 (59)
Mean age in years	69 (11)	70 (14)	70 (12)	71 (12)	70 (13)	72 (13)	70 (11)	68 (11)	69 (10)	68 (10)
Mean pre-operative Hb (g/dL) (outpatients; n=2426)	13.8 (1.3)	12.5 (1.2)	12.5 (1.2)	12.5 (1.2)	12.2 (0.9)	12.3 (0.9)	12.6 (0.8)	14.2 (1.0)	14.2 (0.9)	14.5 (1.0)
Pre-operative anaemia	210 (8.6%)	24 (33)	45 (32)	36 (29)	23 (36)	41 (29)	26 (19)	5[e] (1.4)	5[e] (0.7)	5[e] (0.7)
High risk[d]	92 (4%)	3 (4)	9 (6)	4 (3)	4 (6)	4 (3)	5 (4)	15 (4)	25 (4)	23 (3)
Co-morbidities:										
Cardiovascular	1225 (50%)	21 (29)	75 (53)	54 (43)	36 (56)	75 (53)	68 (49)	157 (45)	381 (53)	358 (51)
COPD	197 (8%)	7 (10)	16 (11)	10 (8)	6 (9)	8 (6)	16 (12)	26 (8)	55 (8)	53 (8)
Rheumatoid arthritis	287 (12%)	12 (17)	32 (23)	25 (20)	14 (22)	33 (23)	26 (19)	20 (6)	65 (9)	60 (9)
Diabetes	286 (12%)	4 (6)	29 (2)	20 (16)	10 (16)	16 (11)	25 (18)	33 (10)	83 (12)	66 (10)
Epo eligible	683 (28%)									

Abbreviations: Hb, haemoglobin; SD, standard deviation; Epo, erythropoietin; CS, cell saver; DR, postoperative drain re-infusion; COPD, chronic obstructive pulmonary disease.
For continuous variables mean (SD) is shown, for categorical variables numbers (percentages) are shown. Percentages are calculated within randomised group (columns). Pre-operative anaemia includes a Hb value <12 g/dL for women and a Hb value of <13 g/dL for men (WHO standards)
[a] 1 THP bilateral; [b] 14 TKP bilateral; [c] one TKP bilateral; [d] high risk denotes incapability to enlarge cardiac output to compensate for anaemia, serious pulmonary disease or symptomatic cerebrovascular disease; [e] wrongly randomised to normal Hb stratum

Table 2. Peri-operative patient characteristics by randomised group and transfused RBC units (means and proportion of patients transfused) by primary and revision surgery

Numbers (%) or mean (SD) or median (IQR)	STRATUM I (Low Hb) Randomisation groups 1- 6						STRATUM II (Normal Hb) Randomisation groups 7-9		
Intention-To-Treat analysis (numbers) Total patients n=2442	**1. Epo/CS N=72**	**2. Epo/drain N=142**	**3. Epo N=125**	**4. CS N=64**	**5. Drain N=142**	**6. Control N=138**	**7. CS N=348**	**8. Drain N=713**	**9. Control N=698**
Mean surgery duration (minutes) primary/ revisions	99 (55) 96/128	102 (45) 98/129	98 (44) 94/132	107 (86) 98/166	100 (46) 98/123	102 (40) 100/124	96 (40) 96/108	99 (43) 96/140	97 (40) 96/115
% of cemented prosthesis	34	47	47	39	47	47	25	43	43
Median blood loss (mL) during surgery (IQR)	300 (150-550)	200 (0-500)	200 (0-500)	300 (150-500)	240 (0-450)	200 (0-500)	325 (200-500) 375 (200-500)	200 (0-400)	200 (0-400)
Primary	300 (175-500)	200 (0-400)	200 (0-475)	300 (150-470)	200 (0-400)	160 (0-450)	300 (225-575)	200 (0-400)	200 (0-400)
Revisions	200 (135-660)	500 (200-925)	500 (0-880)	500 (200-1600)	500 (115-600)	500 (200-575)		350 (0-825)	275 (0-500)
Median total blood loss (mL) (IQR)	525 (350-900)	700 (350-1050)	650 (400-1000)	550 (300-800)	700 (400-1050)	650 (400-950)	550 (300-850)	700 (400-1000)	700 (400-1000)
Primary	525 (400-860)	690 (330-1050)	650 (400-1000)	500 (275-800)	665 (390-1015)	660 (350-980)	545 (310-850)	680 (400-990)	700 (400-1000)
Revisions	470 (250-1460)	900 (525-1570)	560 (300-915)	890 (470-1880)	860 (500-1200)	550 (400-870)	600 (410-1030)	820 (550-1375)	635 (360-975)
Median re-infused volume (mL) (IQR)	200 (100-415)	320 (200-500)	NA	100 (50-175)	320 (190-500)	NA	100 (40-200)	350 (150-500)	NA
g/dL Hb day+1 (SD)	10.3 (1.8)	10.5 (1.5)	10.8 (1.5)	9.5 (1.0)	9.5 (1.1)	9.5 (1.3)	10.8 (1.3)	11.1 (1.3)	11.0 (1.3)
Median units RBCs (IQR)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-2)	0 (0-1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Primary	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-2)	0 (0-1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Revisions	0 (0-1.5)	0 (0-2.8)	0 (0-0)	0 (0-1.5)	0 (0-2)	0 (0-2)	0 (0-0)	0 (0-0)	0 (0-0)
Proportion transfused (%) primary / revisions	21 20/25	18 15/44	10 9/15	30 30/25	28 28/29	23 23/27	7.7 7/15	7.7 7/17	8.3 8/10

Abbreviations: RBC=red blood cell; SD=standard deviation; IQR=inter-quartile range; Hb=haemoglobin;Epo=erythropoietin; CS=cell saver; Drain=postoperative drain re-infusion; mL=millilitre; NA, not applicable.

For continuous variables mean (SD) is shown, and median (IQ range) in case of a non- normal distribution. For categorical variables numbers (percentages) are shown. Percentages are calculated within randomised group (columns). Day+1 denotes one day postoperatively.

In Table 3 the intention-to-treat analysis of the effects of Epo and autologous blood re-infusion (combined cell saver and DRAIN effect) on mean RBC units used (mean difference and calculated ratio's with 95% CI) and proportion transfused patients (with OR and 95% CI) are outlined. To investigate the overall Epo effect in stratum I, regardless of the use of autologous blood, pooled estimates were calculated comparing the Epo+ and Epo- groups (a test for heterogeneity was not significant). The separate cell saver and DRAIN effect showed no difference (eTable 1).

In the low Hb stratum (stratum I) autologous blood re-infusion neither resulted in a decrease of mean RBC use nor in a decrease in proportion of transfused patients in either the total or primary surgery subgroup. Among those randomised to receive Epo, autologous blood use by cell saver or DRAIN even resulted in an increase in both mean RBC use (ratio 0.45, 95% CI 0.28 to 0.69; p<0.01) and the proportion of transfused patients (adjusted OR 2.2, 95% CI 1.1 to 4.4; p=0.02) compared to those without blood sparing devices. This effect mainly occurred in the revision surgery patients. The pooled Epo effect on RBC use in the total, primary and revision, group, showed a transfusion avoidance in 50% of patients (adjusted OR 0.50, 95% CI 0.35 to 0.75; p<0.001) from 26% to 16% (10% absolute difference), independent of assignment to autologous blood re-infusion and a non-significant 29% mean RBC reduction from 0.71 to 0.50 U/patient (ratio 0.71, 95% CI 0.42 to 1.13; p=0.15). Among the primary surgery patients, Epo was effective in both blood sparing (55% mean RBC reduction; ratio 0.45, 95% CI 0.28 to 0.69; p<0.01); and transfusion avoidance (55% reduction in transfused patients; adjusted OR 0.45, 95% CI 0.29 to 0.72; p<0.001) from 26% to 14% (12% absolute difference).

In the normal Hb stratum (II) of the total group, 8.3% of the control group was transfused with a mean RBC use of 0.22 U/patient. Autologous blood re-infusion using either DRAIN or cell saver resulted neither in a RBC sparing nor in transfusion avoidance in this stratum. This was similar in the primary surgery group. The revision surgery group was, however, too small and too heterogeneous to draw valid conclusions.

Economic evaluation

For this purpose, the total group of 2442 patients were analysed. When the operation was unexpectedly rescheduled to a date within three weeks after randomisation, no Epo was administered. As a result, 66% of the patients randomised to receive Epo actually received Epo, with average Epo costs of €851 per patient (table 4A, 95% CI 785 to 917). The change in costs for RBC use and hospital stay in stratum I was relatively small compared to the costs for Epo. The average total cost increase for the Epo strategy was estimated at €785 per patient (95% CI 262 to 1309). With a decrease in the proportion of transfused patients by 10.8% (from 26.4% to 15.6%), the cost difference translates to €7300 per avoided transfusion (95% CI 1900 to 24000).

Table 3A. ITT analysis of Epo and autologous blood re-infusion (=combined cell saver/ DRAIN) effect on RBC use of total group, and split by primary / revision surgery

Primary and revision surgery patients (total group)					
N=2442	**Mean RBC use (U)**	**Mean adjusted difference[a] (95% CI)**	**Ratio[b] (95% CI)**	**Proportion transfused (%)**	**Adjustedodds ratio[c] (95% CI)**
Stratum I no Epo					
Autologous blood (n=206)	0.76 (1.6)	0.10	1.2	29	1.3
No autologous blood (n=138)	**0.64 (1.6)**	(-0.25 to 0.45)	(0.7 to 2.0)	**23**	(0.8 to 2.1)
Stratum I with Epo					
Autologous blood (n=214)	0.65 (2.5)	0.34	2.6	19	2.2
No autologous blood (n=125)[d]	0.25 (0.9)	(-0.10 to 0.78) p=0.13	(1.2 to 6.5) p=0.02	10	(1.1 to 4.4) p=0.02
Pooled Epo effects					
With Epo (n=339)	0.50 (2.1)	-0.22	0.71	16	0.5
No Epo (n=344)	0.71 (1.6)	(-0.50 to 0.05) p=0.10	(0.42 to 1.13) p=0.15	26	(0.35 to 0.75) p< 0.001
Stratum II (n=1759)					
Autologous blood (n=1061)	0.19 (0.9)	-0.06	0.9	7.7	0.92
No autologous blood (n=698)	0.22 (0.9)	(-0.15 to 0.02) p=0.15	(0.6 to 1.3)	**8.3**	(0.65 to 1.3)
Primary surgery patients					
N=2258	**Mean RBC use (U) (SD)**	**Mean adjusted difference[e] (95% CI)**	**Ratio[b] (95% CI)**	**Proportion transfused (%)**	**Adjusted odds ratio[c] (95% CI)**
Stratum I no Epo (n=311)					
Autologous blood (n=184)	0.78 (1.7)	0.15	1.3	29	1.4
No autologous blood (n=127)	0.61 (1.6)	(-0.22 to 0.52)	(0.8 to 2.3)	23	(0.8 to 2.3)
Stratum I with Epo (n=302)					
Autologous blood (n=190)	0.36 (1.1)	0.09	1.5	17	2.1
No autologous blood (n=112)[d]	0.24 (0.9)	(-0.15 to 0.32)	(0.7 to 4.0)	9	(1.0 to 4.3) p=0.06
Pooled Epo effects					
With Epo (n=302)	0.32 (1.0)	-0.39	0.45	14	0.45
No Epo (n=311)	0.71 (1.6)	(-0.61 to -0.18) p<0.001	(0.28 to 0.69) p<0.01	26[f]	(0.29 to 0.72) p<0.001
Stratum II (n=1645)					
Autologous blood (n=987)	0.16 (0.7)	-0.08	0.73	7.1	0.86
No autologous blood (n=658)	**0.22 (0.9)**	(-0.16 to -0.01) p=0.04	(0.48 to 1.1) p=0.13	**8.2**	(0.6 to 1.2)

Table 3A. *(continued)*

Revision surgery patients					
N=184	**Mean RBC use (U) (SD)**	**Mean adjusted difference[e] (95% CI)**	**Ratio[b] (95% CI)**	**Proportion transfused (%)**	**Adjusted odds ratio[c] (95% CI)**
Stratum I no Epo (n=33)					
Autologous blood (n=22)	0.59 (1.0)	-0.48	0.54	27	
No autologous blood (n=11)	**1.1 (2.0)**	(-1.63 to 0.68)	(0.15 to 2.6)	**27**	(0.2 to 5.1)
Stratum I with Epo (n=37)					
Autologous blood (n=24)	3.0 (6.5)	2.0	9.6	38	3.3
No autologous blood (n=13)[d]	0.3 (0.8)	(-1.77 to 5.72)	(1.9 to 31.4)	15	(0.6 to 18)
Pooled Epo effects					
With Epo (n=37)	2.0 (5.3)	1.76	2.7	30	1.3
No Epo (n=33)	0.76 (1.4)	(-0.14 to 3.67) p=0.07	(0.7 to 8.0)	27	(0.5 to 3.7)
Stratum II (n=114)					
Autologous blood (n=74)	0.64 (2.2)	0.25	2.0	16	1.8
No autologous blood (n=40)	**0.33 (1.2)**	(-0.49 to 1.0)	(0.5 to 14.0)	**10**	(0.5 to 6.2)

Abbreviations: ITT=intention to treat; Epo=erythropoietin; RBC=red blood cell; U=units; CI, confidence interval; SD=standard deviation. Control groups are outlined in bold.

[a] adjusted for revision/non-revision surgery, hospital and knee/hip surgery; confidence intervals for reference purposes only (assuming normality)

[b] ratio was defined as the quotient of mean RBC values of two groups being compared; all estimates and robust standard errors were obtained via bootstrapping in R

[c] all estimates and standard errors were obtained using the Mantel-Haenszel procedure, stratifying by the pre-specified stratification factors hospital and knee/hip surgery

[d] denotes Epo alone group.

[e] adjusted for hospital and for knee/ hip surgery; confidence intervals for reference purposes only (assuming normality)

[f] 12% absolute difference in transfusion avoidance

Table 3B. AT analysis of primary surgery patients (truly received Epo and truly received device)

Primary surgery patients					
N=2258	**Mean RBC use (U) (SD)**	**Mean adjusted difference[a] (95% CI)**	**Ratio[b] (95% CI)**	**Proportion transfused (%)**	**Adjusted odds ratio[c] (95% CI)**
Stratum I no Epo (n=410)					
Autologous blood (n=240)	0.65 (1.6)	0.03	0.99	27	1.2
No autologous blood (n=170)	**0.65 (1.4)**	(-0.26 to 0.33)	(0.63 to 1.6)	**24**	(0.79 to 1.8)
Stratum I with Epo (n=202)					
Autologous blood (n=81)	0.17 (0.5)	-0.14	0.58	9.9	1.1
No autologous blood (n=121)[d]	0.30 (1.2)	(-0.43 to 0.15)	(0.19 to 1.7)	9.1	(0.42 to 2.9)
Pooled Epo effects					
With Epo (n=202)	0.25 (1.0)	-0.40	0.38	9.4	0.30
No Epo (n=410)	0.65 (1.5)	(-0.62 to -0.17) p=0.01	(0.19 to 0.66)	26	(0.18 to 0.51) P<0.001
Stratum II (n=1639)[e]					
Autologous blood (n=887)	0.14 (0.6)	-0.08	0.63	6.2	0.69
No autologous blood (n=752)	**0.22 (0.9)**	(-0.15 to 0.0) p=0.04	(0.42 to 0.95)	**8.8**[f]	(0.47 to 1.0) p=0.05

Abbreviations: ITT=intention to treat; Epo=erythropoietin; CS=cell saver; DR=postoperative drain re-infusion; RBC=red blood cell; U=units; CI=confidence interval; SD=standard deviation. Control groups are outlined in bold.

[a] adjusted for hospital and knee/hip surgery; confidence intervals for reference purposes only (assuming normality).

[b] ratio was defined as the quotient of mean RBC values of two groups being compared; all estimates and robust standard errors were obtained via bootstrapping in R.

[c] all estimates and standard errors were obtained using the Mantel-Haenszel procedure, stratifying by the pre-specified stratification factors hospital and knee/hip surgery.

[d] denotes Epo alone group.

[e] of 7 patients, it was not known whether the device was truly received.

[f] 2.6% absolute difference in transfusion avoidance.

Autologous blood re-infusion was associated with a significant decrease in the use of Epo by 4% (table 4B, 95% CI 2% to 7%) and increased the length of the non-ICU hospital stay by 0.56 days (95% CI 0.23 to 0.90, similar in both strata). The total cost increase for the autologous blood re-infusion strategy was estimated at €378 per patient (95% CI 161 to 595), without RBC reduction.

Study protocol adherence

A total of 284 patients did not receive the intended intervention. Of the 339 patients assigned to Epo, 114 received no Epo (34%), 225 patients assigned to Epo received at least one dose and of those 97% received at least three Epo doses. Sixty-two of 484 (13%) assigned patients did not receive cell saver (with or without Epo) and 110 of 997 (11%) assigned patients did not receive DRAIN (with or without Epo). Most common reasons for

not receiving the intended intervention were earlier rescheduling of surgery in case of Epo, technical problems with the machine (broken or incomplete device) for cell saver and not using the proper drain device or not placing a drain at all.

Table 4. Estimated costs by Epo among patients with low Hb (table 4A) and by autologous blood re-infusion among all patients (table 4B)

Table 4A

	Volumes of health care[a]		Costs (in €)			
	With Epo n=339	**No Epo n=344**	**With Epo n=339**	**No Epo n=344**	**Difference (95% CI)**	
Epo	66%	0.7%[b]	858	8	851	(785; 917)
Cell-saver and/or drain	63%	60%	56	52	4	(-4; 13)
RBC use	16%/0.50	26% / 0.71	418	591	-172	(-401; 57)
ICU care (days)	3.2%/0.04	2.3% / 0.04	100	98	1	(-99; 102)
Non-ICU care (days)	8.87	8.66	4182	4081	101	(-256; 459)
Total costs			5615	4829	785	(262; 1309)

[a] Volume = percentage of patients and/or mean usage
[b]Two patients received Epo while not randomised for Epo

Table 4B

	Volumes of health care[a]		Costs (in €)			
	Autologous blood n=1481	**No autologous blood n=961**	**Autologous blood n=1481**	**No autologous blood n=961**	**Difference (95% CI)**	
Epo	8%	12%	100	152	-53	(-84; -21)
Cell-saver and/or drain	100%	0.3%	89	0	89	(86; 91)
RBC use	12% / 0.34	11% / 0.29	279	238	41	(-38; 121)
ICU care (days)	2.0% / 0.03	1.0% / 0.02	73	37	35	(-9; 80)
Non-ICU care (days)	8.18	7.62	3857	3592	265	(107; 423)
Total costs			4399	4021	378	(161; 595)

[a] Volume=percentage of patients and/or mean usage

Transfusion protocol adherence

In over 95% of the patients, the transfusion protocol was correctly followed according to Hb, age and co-morbidity status (risk evaluation) of the patient before transfusion. Transfusion violations were equally found in all randomisation groups.

As Treated analysis

In table 3B the AT analysis, where the actual use of Epo and the actual use of the autologous blood re-infusion devices are analysed, shows the primary surgery group only. Patients who actually received Epo ("pooled effects with Epo" group) showed a larger reduction in mean RBC use of 62% (ratio 0.38, 95% CI 0.19 to 0.66) and a reduction in proportion transfused patients of 70% (adjusted OR 0.30, 95% CI 0.18 to 0.51). In this low Hb stratum, the actual use of the autologous blood re-infusion devices did not result in a mean RBC reduction or in a reduction in percentage transfused patients. In the patient group with normal pre-operative Hb levels (stratum II), a significant mean RBC reduction of 37% (ratio 0.63, 95% CI 0.42 to 0.95) and a reduction in transfused patients of 31% (adjusted OR 0.69, 95% CI 0.47 to 1.0) from 8.8% to 6.2% (2.6% absolute difference) was found. The AT analysis for the revision surgery patients, and the AT analysis for both separate cell saver and DRAIN are presented in eTables 2 and 3. No significant RBC reduction was found in the revision surgery group as well as no difference in effect of cell saver compared to DRAIN devices.

Serious adverse events (SAEs)

A total of 112 SAEs were reported in 103 patients (eight patients suffered 2 or more SAEs) (Table 5A and 5B). Eighty SAEs were registered within one month postoperatively and 32 SAEs were reported later within the three months of follow up. Categorisation according to intention-to-treat analysis (table 5A) and as treated analysis (table 5B), and occurrence (less or more than one month after surgery) is shown. One patient did not undergo surgery and was not further evaluated because of a stroke after one Epo dose (Hb value of 12.2 g/dL) and one patient was not further evaluated due to assignment of a wrong randomisation number. These patients were included in table 5. Total numbers of reported SAEs by group are outlined. A total of 31 thrombo-embolic (TE) events occurred: nine myocardial infarctions (MI), twelve strokes or TIA's, four deep venous thrombosis of the leg (diagnosed by ultrasound), five pulmonary emboli and one arterial occlusion of a bypass graft in the leg. Five TE events (three MIs and two strokes) occurred in the Epo-group (1.5%), all in patients with Hb levels of 12.2.g/dL or less, two of these events occurred after only one Epo dose. The proportion of TE events (1.5%) in the Epo-group was not significantly different from the non-Epo group (1.2%) (OR 1.2, 95% CI 0.46 to 3.1; p=0.72). In the as treated analysis, 1.8% in the Epo group suffered a TE event (table 5B) increasing the OR (not significantly) compared to the non-Epo group to 1.5 (95% CI 0.50 to 4.2; p=0.49). Non-TE related SAEs were: prosthesis related (n=33) (hip dislocation (n=10), prosthesis infections (n=4) or wound infections (n=7), limited knee flexion needing manipulation (n=5), fracture (n=3) or non-specified (n=4)), cardiovascular events (n=22) (arrhythmia, blood pressure instability etc), allergic events (n=3), non prosthesis related infections or sepsis (n=7), bleeding (n=3), malignancy (n=1) and other (n=12). Autologous blood re-infusion related complications were not specifically sepsis- or infection related. A relatively high proportion of SAEs were

Table 5. Reported numbers Serious Adverse Events (SAEs) by ITT (Table 5A) and by AT group (table 5B): thrombo-embolic (TE) complications[a], non-TE complications[b] and total numbers

Table 5A

ITT group (numbers)	TE complications (%)	Myocardialinfarction (<1 mo/ (1-3 mo)[c]	Stroke/TIA (<1 mo/ (1-3 mo)	DVT (<1 mo/ (1-3 mo)	Pulmonary emboli (<1 mo/ (1-3 mo)	Other (<1 mo/ (1-3 mo)	Non-TE complications	Total numbers of reported SAEs (%)
Epo/CS (n=72)	0 (0%)	0	0	0	0	0	3	3 (4.2%)
Epo/DR (n=142)	1 (2.4%)	1 (1/0)	0	0	0	0	8	9 (6.3%)
Epo (n=125)	4 (3.2 %)	2 (2/0)	2 (2/0)	0	0	0	1	5 (4.0%)
Epo groups (n=339)	**5 (1.5%)**						**12**	**17 (5.0%)[d]**
CS (n=412)	2 (0.5%)	0	2 (2/0)	0	0	0	14	16 (3.9%)
DR (n=855)	15 (0.2%)	4 (2/2)	5 (5/0)	1(0/1)	4 (2/2)	1(1/0)[e]	29	44 (5.1%)
Control group (n=836)	9 (1.1%)	2 (2/0)	3 (2/1)	3(0/3)	1 (0/1)	0	26	35 (4.2%)
Non Epo groups (n=2103)	**26 (1.2%)**						**69**	**95 (4.5%)[d]**
Totals (n=2442)	31 (1.3%)	9 (7/2)	12 (11/1)	4 (0/4)	5(2/3)	1 (1/0)	81	112 (5%)

Table 5B

AT group (numbers)	TE complications (%)	Myocardial infarction (<1 mo/ (1-3 mo)[c]	stroke/TIA (<1 mo/ (1-3 mo)	DVT (<1 mo/ (1-3 mo)	Pulmonary emboli (<1 mo/ (1-3 mo)	Other (<1 mo/ (1-3 mo)	Non-TE complications	Total numbers of reported SAEs (%)
Epo/CS (n=24)	0 (0%)	0	0	0	0	0	2	2 (8.3%)
Epo/DR (n=50)	1 (2.0%)	1 (1/0)	0	0	0	0	8	9 (18%)
Epo (n=153)	3 (1.9 %)	2 (2/0)	1 (1/0)	0	0	0	1	4 (2.6%)
Epo groups (n=227)	**4 (1.8%)**						**11**	**15 (6.6)**[f]
CS (n=275)	0 (0%)	0	0	0	0	0	12	12 (4.4%)
DR (n=638)	15 (2.4%)	4 (2/2)	5 (5/0)	1(0/1)	4 (2/2)	1(1/0)[e]	29	44 (6.9%)
Control group (n=1302)	12 (0.9%)	2 (2/0)	6 (5/1)	3(0/3)	1 (0/1)	0	29	41 (3.1%)
Non Epo groups (n=2215)	**27 (1.2%)**						**70**	**97 (4.4%)**[f]
Totals (n=2442)	31 (1.3%)	9 (7/2)	12 (11/1)	4 (0/4)	5(2/3)	1 (1/0)	81	112 (5%)

Abbreviations: ITT, intention to treat; AT, as treated; mo, months; TIA, transient ischemic attack; DVT, deep venous thrombosis; Epo, erythropoietin; CS, cell saver; DR, postoperative drain re-infusion. Percentages are calculated within ITT or AT group (rows). Six SAE patients from the ITT analysis (Table 4A) did not actually receive the intervention. These patients were included in the control group in the AT analysis (Table 4B). Therefore, the control group comprised of 41 SAE patients in table 4B and 35 SAE patients in table 4A.

[a] TE complications were categorized in: myocardial infarction, stroke/TIA, DVT, pulmonary emboli or other

[b] non-TE complications were prosthesis related events (hip dislocations, prosthesis infections, wound infections, knee contractures, fractures), cardiovascular events (arrhythmia, blood pressure instability etc), allergic events, infection/sepsis not prosthesis related, bleeding etcetera

[c] categorized before and after one month (mo) post-operatively

[d] Chi2 test: p=0.69 (OR 1.1, 95% CI 0.66 to 1.9)

[e] denotes an arterial occlusion

[f] Chi2 test: p=0.13 (OR 1.5, 95% CI 0.88 to 1.9)

reported in the group that actually received Epo and DRAIN (table 5B: as treated group) (18%; n=9), but these were mostly non TE related. Six of them were due to cardiac failure in patients with a known cardiac history. One serious anaphylactic reaction occurred in the DR group after post-operative re-fusion of 50 mL, which was treated with adrenalin and fluid resuscitation, and resolved uneventfully.

DISCUSSION

In elective knee-and hip-arthroplasty patients, three widely used RBC transfusion alternatives were compared while using a baseline restrictive transfusion threshold. Only 11.6% of all patients were transfused. Within the control groups, 23% of patients with a low preoperative Hb (between 10 and 13 g/dL) and 8.3% in patients with a higher Hb level were transfused. In patients with the low preoperative Hb level (stratum I), Epo contributed significantly in avoiding RBC transfusions, but not in decreasing mean RBC reduction. In both strata I and II, the separate and combined use of cell saver and DRAIN did not result in a clinically significant decrease in RBC use. Since the revision surgery group was too small and effects were too heterogeneous, valid conclusions could only be made for the large primary surgery group (93% of the total cohort). Use of Epo in primary surgery patients resulted in a significant 12% absolute reduction and a 55% relative reduction in transfused patients irrespective of the use of cell saver or drain re-infusion. These results confirmed earlier reports that Epo has a significant benefit as a transfusion avoiding strategy (avoidance of exposure to allogeneic RBC transfusions) as well as a significant blood sparing effect (mean units RBC reduction). Our finding that neither cell saver nor DRAIN resulted in a clinically relevant RBC reduction may be explained by the low volume of recovered shed blood in combination with the applied restrictive transfusion threshold. This finding is consistent with a recent survey among 20 hospitals in the United States, in which the effect of blood salvage programs was investigated. The authors also observed that the volume of returned blood in orthopaedic joint surgery was small [27]. The development of better surgical techniques (i.e. less extensive incisions) to minimise blood loss may also have contributed to this effect.

Neither Epo nor blood salvage were cost-effective. From a hospital perspective, the additional costs for the Epo strategy in patients with low Hb levels were estimated at €785 per patient, mainly consisting of the additional Epo costs. Epo avoided a transfusion in about one in every nine patients, translating the cost estimate to €7300 per avoided transfusion. To justify such costs from a health economic perspective, transfusion would have to be associated with a considerable health risk. Specifically, at a cost-effectiveness acceptability threshold of €40.000 per quality adjusted life year, one in every hundred transfused patients would have to incur an average life expectancy loss of approximately

20 years (100 x 7300 / 40.000). According to haemovigilance registers, blood transfusion currently seems considerably safer than that [28]. In our trial, autologous blood re-infusion using cell saver or DRAIN did not reduce allogeneic RBC transfusions and from a health economic perspective the associated cost increase is not justified.

Strengths and limitations of the study

Our study has several strengths and limitations. Strengths were that the study was randomised, the study power was 90% and sufficient numbers of patients were included and evaluated. The design of the study was chosen to be optimally consistent with current clinical practice, allowing to evaluate the combined and separate effect of three types of transfusion alternatives. Despite this complex study design, patients were well balanced across the randomisation groups. Adherence to the restrictive transfusion protocol was over 95%. This high protocol adherence was in contrast to the non-adherence to the randomisation arms that occurred in all participating centres. Non-adherence to Epo randomisation in stratum I was high, namely 34% (n=114) and was mainly due to the surgery date being brought forward when surgery time became suddenly available. This resulted in lack of time to prescribe three weeks of Epo therapy with subsequent protocol violation in the assignment to Epo. This situation may be typical for the Netherlands: at the time of this study the waiting lists for elective orthopaedic surgery were short (less than two months). In the analysis of the effect of autologous re-infusion, we observed that patients randomised to receive autologous re-infusion showed an unexpected, statistically significant, 4% lower use of Epo than patients randomized not to receive autologous re-infusion (Table 4B: autologous versus no autologous: 8% versus 12%). This may have biased our analysis at the expense of autologous re-infusion. However, since the transfusion rate among patients with low Hb was 26%, the overall influence of this imbalance on the transfusion rate cannot have been more than 1% (i.e. 26% of 4%), which is insufficient to alter our negative conclusion on autologous re-infusion.

Non-adherence to the cell saver and to the DRAIN was present in 13% (n=62) and 11% (n=111) of patients, respectively. Despite use of these devices, some patients did not receive any autologous blood due to insufficient drainage and/or collection of shed blood. Of the patients who did receive the intended intervention (as treated analysis), use of Epo in primary surgery patients showed that RBC reduction was larger, but still did not reach the 75% reduction level as hypothesized. In this analysis, use of blood salvage devices did result in a significant decrease in RBC use in primary surgery patients, who had a normal preoperative Hb level. However, since the absolute reduction was only 0.08 RBC units, it is questionable whether this is clinically relevant.

Another limitation of the study may be that only the study investigators were blinded and not the clinical team, who was informed of the assigned randomisation arm in order to avoid protocol violations. The non-blinding of Epo may have resulted in transfusion

bias, however this was not likely, since clinicians adhered to the transfusion protocol and violations were equal in all randomisation groups.. Furthermore, since the study was not powered for safety evaluation, we are unable to draw valid conclusions on the incidence of complications. All patients in our study received thrombosis-prophylaxis, which may have an effect on the low proportion of thrombo-embolic complications in the Epo group. This finding is in contrast to a safety study in orthopaedic spine surgery patients not receiving thrombosis-prophylaxis that reported a higher incidence of post-operative thrombotic events (deep vein thrombosis in particular) in patients after Epo treatment compared to a control group [29]. Finally, all transfusion trials are flawed due to the fact that randomisation occurs prior to surgery, while the majority of included patients do not reach the trigger for transfusion. This however does not invalidate in any respect the intention-to-treat approach [30].

Implications for clinicians and other researchers

This study may serve as a valid estimate for the primary hip- and knee surgery population in the Netherlands (16.6 million inhabitants), where approximately 50.000 total hip and knee replacements are performed annually, which is expected to rise to over 100.000 in 2030 [31]. Considering the fact that use of autologous blood re-infusion devices are used in up to 80% of Dutch hospitals (year 2007) [11], and our findings that they have no blood sparing benefit, omission of these devices from blood management protocols may result in a considerable decrease in health care costs.

Our results confirm that patients with a low preoperative Hb were more likely to receive a RBC transfusion (23% of 138 control group patients in stratum I compared to 8.3% of 698 non-anaemic control group patients in stratum II) and the patients with overt pre-operative anaemia according to the WHO criteria even required a RBC transfusion in 32.4% [32-36]. For these anaemic patients, Epo is recommended in recently published guidelines, after excluding treatable causes of anaemia [33]. In our study, we did not investigate the cost-effectiveness of Epo in the anaemic subpopulation (210 patients in this study), nor corrected for anaemia, and propose to wait for more data to decide on the use of Epo in this subpopulation. Future research to aim for optimal blood management should rather focus on cheaper alternatives to Epo, such as iron supplements for the anaemic patient who is most at risk of being transfused.

CONCLUSIONS

In elective knee-and hip-arthroplasty patients with preoperative Hb levels between 10 and 13 g/dL, even with a restrictive transfusion policy, Epo contributed as a significant transfusion alternative, but at unacceptably high costs. No clinically relevant decrease in RBC

use was found using autologous blood salvage by cell saver or DRAIN, which consequently only increased costs. These findings may have a substantial impact for current blood management protocols in which Epo usage and autologous blood re-infusion devices are frequently embedded.

What is already known on this topic:

- In elective hip- and knee- replacement surgery, the use of Erythropoietin (Epo) and autologous blood re-infusion as red blood cell alternatives are widely accepted and embedded in daily practice.
- However, the effect sizes differ in literature and are smaller when a transfusion protocol is present.
- Since transfusion protocols have become more restrictive, it is questionable whether these alternatives are still in place in blood management protocols.

What this study adds:

Even with a restrictive transfusion policy, Epo significantly decreased red blood cell use in elective knee-and hip-arthroplasty patients with a preoperative Hb value of 13 g/dL or less, but at unacceptably high costs. The use of cell saver or postoperative drain re-infusion device did not result in a red blood cell reduction and consequently only increased costs.

Based on costs without apparent clinical benefit, the findings of this study do not support the use of Epo or autologous blood re-infusion by cell saver or post-operative drain re-infusion device as transfusion alternatives and support the use of a restrictive transfusion policy in this study population.

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Contributors

AB was principal investigator. CS, AK and RN were lead clinical investigators. CS was study coordinator. RB was lead investigator for statistics, study design and management. CS, RN, JH and AB obtained ethical approval of the study and obtained funding. AB and RN supervised the study. All authors were members of the project management team. RN, EK, RO, RP, CS

and AK participated in recruitment of centres or patients, or both. TJ was responsible for the design of the data base and the data (quality) management as well as basic reporting of data to the investigators. WH was responsible for the economic evaluation. All authors participated in data interpretation and in reporting of results. All authors have seen and approved the final version.

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ONLINE-ONLY MATERIAL

Includes:

- Sample size calculation (text)
- eFigure 1 Statistical design of the study
- Legend to eFigure 1
- eTable 1 ITT analysis of separate cell saver and DRAIN effect by surgery group (primary or revision)
- eTable 2 AT analysis of revision surgery patients
- eTable 3 AT analysis of separate cell saver and DRAIN effect by surgery group (primary or revision)

SAMPLE SIZE CALCULATION

The actual design is made keeping in mind that theoretically there could be an interaction between the Epo, the cell saver and the DRAIN effect on the outcome. If such an interaction is clinically irrelevant (and statistically absent) power can be gained without introducing any bias, by making the univariate comparisons as indicated in the design chart (eFigure 1), through pooling the unbiased effects within two or more stratification categories, leading to a smaller sample size. However, we have designed the trial to have sufficient power even in the case of (severe) interaction, i.e. the situation in which for example the effect of "DRAIN with or without cell saver" versus "Neither DRAIN nor cell saver" would in itself depend on the Epo-stratum. If that were the case, we would have to report this effect in the three strata separately. It should be noted that only in our statistical design (eFigure 1) the cell saver device is denoted as cell saver+DRAIN+ for statistical convenience, but for convenience of the reader the cell saver group is denoted in the print article as cell saver+DRAIN-. Likewise, the Epo versus no-Epo effect is an "intention-to-treat" estimate so one could argue that it is only necessary to compare both arms without regard for the other randomisation consequences (DRAIN and cell saver). However, the randomisation of the three components takes place at the same time, i.e. it is actually a randomisation into 6 different treatment modalities (depending on the stratification variables). Hence it would be prudent to anticipate a possible interaction between the Epo effect and the cell saver/DRAIN effect. In a worst case scenario the "pure" Epo effect could then only be estimated by comparing the Epo versus non-Epo in the no-DRAIN, no-cell saver situation, thus reducing the sample size for this comparison.

To accommodate all these scenario's and realizing that this clinical design should answer the various comparisons in one study and also if assumptions of no-interaction will turn out not to be met, we decided to safeguard the power of the trial such that at the end a decision among all scenario's can be made with 90% power. The following assumptions are made:

1. 1/3 is eligible for Epo, randomisation for EPO is 1:1
2. Mean transfusion rate is 1.0 RBC Unit, with SD=1.4 (medium risk scenario SD=1.6, worst case scenario SD=1.8)
3. Power of the trial =90%
4. Hypothesis 1: Epo versus no Epo, Hypothesis 2: DRAIN with or without cell saver versus none (any autologous blood re-infusion device versus no autologous re-infusion). Hypothesis 3: cell saver versus no cell saver in case of autologous re-infusion (intra-and postoperative re-infusion by cell saver versus postoperative re-infusion by DRAIN).

In case of hypothesis 1: for a 75% reduction in blood use (from 1,0 to 0,25 U RBC) 125 patients are needed per group. Therefore, 2 times 125 patients are needed. In a worst case scenario (SD= 1.8) 3 times 2 times 125=750 patients are needed.

Please note that we do not compare percentages but average amounts of blood used.

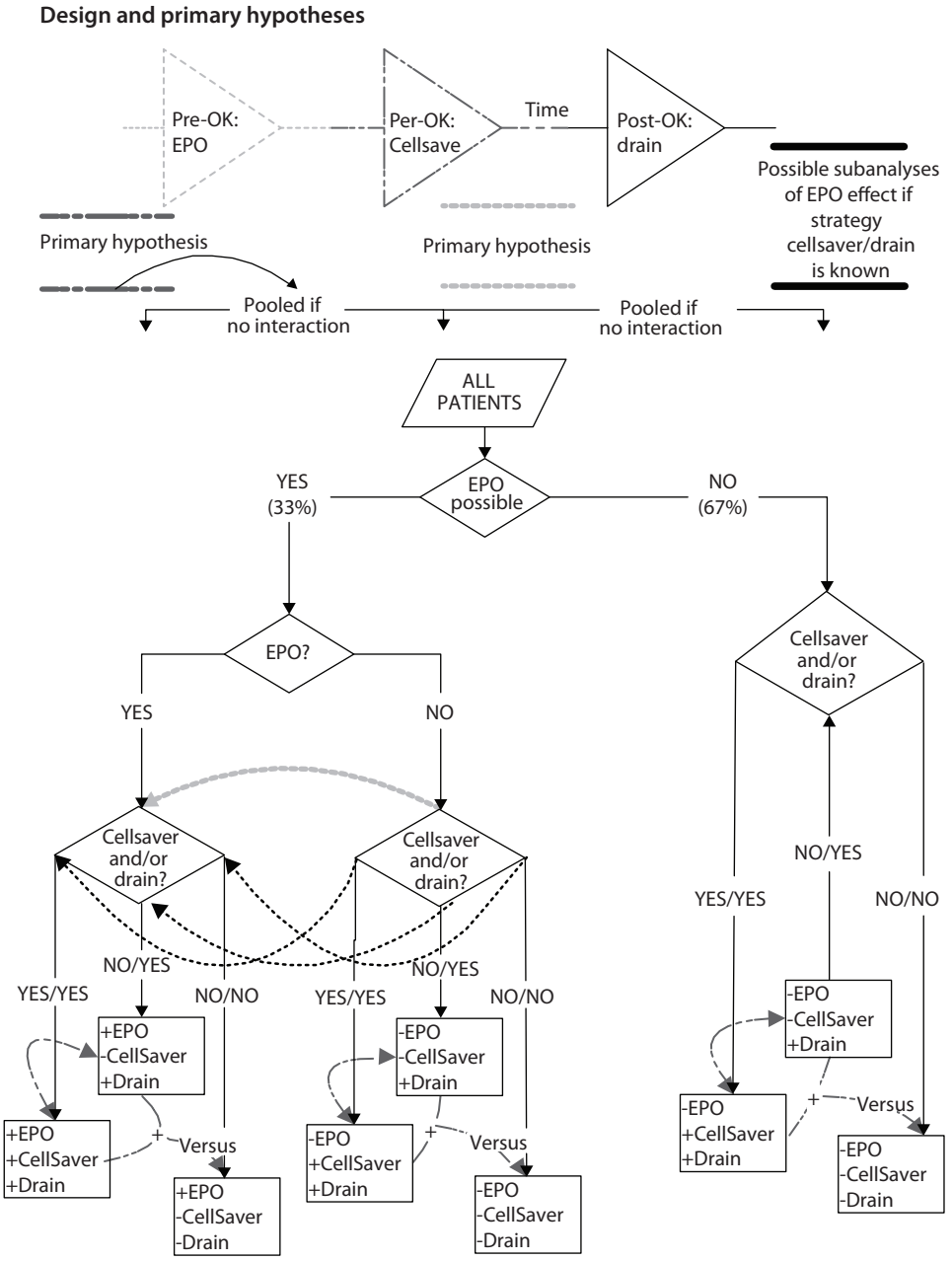
In case of hypothesis 2 and hypothesis 3: this involves 2/3 of all patients included (sum of "DRAIN with cell saver" and "DRAIN without cell saver"). For a reduction of 30% in blood use (from 1,0 to 0,7 U RBC) we need 1000 patients in a worst-case scenario, so for this group it will be 3/2 times1000=1500 patients. We then protect the trial against severe clinical interactions between the various components of the distinguished scenario's:

However, in case of interaction between Epo and DRAIN/cell saver the analysis must be performed within the no Epo group (this consists of 5/6 of the total number of patients), so 6/5 times 1500=1800 patient inclusions are needed. In the worst case, analysis can only be performed in ''no Epo not eligible for Epo group"=2/3 of total inclusions, that is: 3/2 times 1500=2250 patients.

Since we have more than one test involved in reaching a final recommendation for a scenario, multiple testing should be taken into account: to protect against multiple testing a Bonferroni correction is used and in case of a SD of 1.4 and 30% mean RBC reduction we need 1800 patient inclusions. When analysis has to be restricted to the non-Epo group we need 6/5 times 1800=2200 inclusions.

In conclusion when 2250 patients are included we expect sufficient power for all hypotheses 1 to 3, even in a worst case scenario. When interaction is not found, then pooling is allowed and much less than 2250 patients are needed.

An interim analysis will be performed at 1000 patients by an independent Data Safety and Monitoring Committee. Study-stop criteria are: 1. p smaller than 0.025 for the primary endpoint; 2. p smaller than 0.025 for less than 30% reduction by Epo and less than 15% by transfusions by shed blood (cell saver/DRAIN).



eFigure 1. Statistical design of the study

Since the cell saver device (OrthoPAT®) collected and re-infused both intra-and postoperative wound blood, the DRAIN notation in combination of cell saver (cell saver+DRAIN+) denotes the use of the cell saver device only. This notation is used only in this figure and has been used for statistical purposes only to calculate sample sizes and to construct the hypotheses. The light gray arrow denotes hypothesis 1 (Epo versus no Epo). The dark gray arrows denote hypothesis 2 (autologous blood versus no autologous blood) and hypothesis 3 (cell saver device versus DRAIN device within the autologous blood re-infusion groups). In case of no interaction, groups were pooled.

eTable 1. ITT analysis of separate cell saver (CS) and DRAIN (DR) effect by surgery group (primary or revision)

Primary surgery patients					
N=2258	**Mean RBC use (U) (SD)**	**Mean adjusted difference[a] (95% CI)**	**Ratio[b] (95% CI)**	**Proportion transfused (%)**	**Adjusted odds ratio[c] DR versus CS (95% CI)**
Stratum I no Epo (n=311)					
Autologous blood (n=184)	0.78 (1.7)				
CS (n=56)	0.93 (1.8)	0.13	1.3	30	1.1 (0.6 to 2.2)
DR (n=128)	0.71 (1.6)	(-0.46 to 0.73)	(0.64 to 2.4)	28	
Stratum I with Epo (n=302)					
Autologous blood (n=190)	0.36 (1.1)				
CS (n=64)	0.36 (0.7)	-0.12	0.98	20	1.4 (0.7 to 3.1)
DR (n=126)	0.37 (1.2)	(-0.49 to 0.25)	(0.44 to 2.2)	15	
Stratum II (Normal Hb) (n=1645)					
Autologous blood (n=987)	0.16 (0.7)				
CS (n=322)	0.13 (0.5)	-0.12	0.74	7.1	1.0 (0.6 to1.7)
DR (n=665)	0.17 (0.7)	(-0.22 to -0.02) p=0.02	(0.41 to 1.2)	7.1	
Revision surgery patients					
N=184	**Mean RBC use (U) (SD)**	**Mean adjusted difference[a] (95% CI)**	**Ratio[b] (95% CI)**	**Proportion transfused (%)**	**Adjusted odds ratio[d] (95% CI)**
Stratum I no Epo					
Autologous blood (n=22)	0.59 (1.0)				
CS (n=8)	0.63 (1.2)	-0.20	1.1	25	0.83 (0.12 to 6.0)
DR (n=14)	0.57 (0.9)	(-1.63 to 1.22)	(0.0 to 4.8)	29	
Stratum I with Epo					
Autologous blood (n=24)	3.0 (6.5)				
CS (n=8)	1.3 (2.8)	-3.14	0.33	25	0.43 (0.07 to 2.8)
DR (n=16)	3.8[4] (7.6)	(-9.4 to 3.1)	(0.0 to 2.2)	44	
Stratum II (Normal Hb)					
Autologous blood (n=74)	0.64 (2.2)				
CS (n=26)	0.46 (1.3)	-0.81	0.63	15	0.91 (0.25 to 3.4)
DR (n=48)[e]	0.73 (2.5)	(-2.0 to 0.40) p=0.18	(0.05 to 3.0)	17	

Abbreviations: ITT=intention to treat; CS=cell saver; DR=postoperative drain re-infusion; Epo=erythropoietin; RBC=red blood cell; U=units; CI=confidence interval; SD=standard deviation.

[a] adjusted for hospital and for knee/ hip surgery; confidence intervals for reference purposes only (assuming normality)

[b] ratio was defined as the quotient of mean RBC values of two groups being compared; all estimates and robust standard errors were obtained via bootstrapping in R

[c] all estimates and standard errors were obtained using the Mantel-Haenszel procedure, stratifying by the pre-specified stratification factors hospital and knee/hip surgery

[d] included 2 hip surgery patients with respectively 17 and 27 RBC transfusions; when analysed as treated, these patients did not receive the drain device and ended in the epo only group. (see eTable 3)

[e] mean of hip surgery group (n=26) was 1.27 (3.3) and mean of knee surgery group (n=22) was 0.09 (0.4)

eTable 2. AT analysis of revision surgery patients (combined effect of cell saver and DRAIN denoted as autologous blood)

Revision surgery patients					
N=184	**Mean RBC use (U) (SD)**	**Mean adjusted difference[a] (95% CI)**	**Ratio[b] (95% CI)**	**Proportion transfused (%)**	**Adjusted odds ratio[c] (95% CI)**
Stratum I no Epo (n=45)					
Autologous blood (n=26)	1.62 (3.6)	0.91	1.92	39	1.8
No autologous blood (n=19)	**0.84 (1.6)**	(-0.99 to 2.8)	(0.53 to 8.8)	**26**	(0.48 to 6.4)
Stratum I with Epo (n=25)					
Autologous blood (n=11)	1.0 (2.5)	-0.92	0.45	18	0.82
No autologous blood (n=14)[d]	2.2 (7.2)	(-5.8 to 3.9)	(0.0 to 9.0)	21	(0.11 to 6.0)
Pooled Epo effects					
With Epo (n=25)	1.7 (5.6)	0.88	1.3	20	0.60
No Epo (n=45)	1.3 (2.9)	(-1.12 to 2.88)	(0.11 to 4.6)	33	(0.20 to 1.8)
Stratum II (n=113)[e]					
Autologous blood (n=62)	0.56 (2.2)	0.04	1.2	13	0.80
No autologous blood (n=51)	**0.49 (1.4)**	(-0.68 to 0.75)	(0.25 to 4.5)	**16**	(0.28 to 2.3)

Abbreviations: AT=as treated; RBC=red blood cell; U=units; CI=confidence interval; SD=standard deviation; Epo=erythropoietin. Control groups are outlined in bold

[a] adjusted for hospital and for knee/ hip surgery; confidence intervals for reference purposes only (assuming normality)

[b] ratio was defined as the quotient of mean RBC values of two groups being compared; all estimates and robust standard errors were obtained via bootstrapping in R

[c] all estimates and standard errors were obtained using the Mantel-Haenszel procedure, stratifying by the pre-specified stratification factors hospital and knee/hip surgery

[d] denotes Epo alone group.

[e] in one patient it was unknown whether the device was truly received

eTable 3. AT analysis of separate cell saver (CS) and DRAIN (DR) effect by surgery group (primary or revision)

Primary surgery patients					
N=2258	**Mean RBC use (U) (SD)**	**Mean adjusted difference[a] (95% CI)**	**Ratio[b] (95% CI)**	**Proportion transfused (%)**	**Adjusted odds ratio[c] DR versus CS (95% CI)**
Stratum I no Epo (n=410)					
Autologous blood (n=240)					
CS (n=70)	0.79 (1.5)	0.09	1.34	31	1.4
DR (n=170)	0.59 (1.4)	(-0.35 to 0.54)	(0.72 to 2.3)	25	(0.73 to 2.5)
Stratum I with Epo (n=202)					
Autologous blood (n=81)					
CS (n=27)	0.19 (0.6)	0.01	1.11	11	1.2
DR (n=54)	0.17 (0.5)	(-0.30 to 0.31)	(0.0 to 5.1)	9.3	(0.27 to 5.6)
Stratum II (n=1645)					
Autologous blood (n=888)[d]					
CS (n=282)	0.13 (0.5)	-0.15	0.83	6.4	0.94
DR (n=606)	0.15 (0.7)	(-0.26 to -0.04) p=0.01	(0.42 to 1.5)	6.3	(0.3 to 1.7)
Revision surgery patients					
N=184	**Mean RBC use (U) (SD)**	**Mean adjusted difference[a] (95% CI)**	**Ratio[b] (95% CI)**	**Proportion transfused (%)**	**Adjusted odds ratio[c] (95% CI)**
Stratum I no Epo (n=45)					
Autologous blood (n=26)					
CS (n=12)	2.5 (5.1)	0.94	2.92	33	0.67
DR (n=14)	0.86 (1.0)	(-2.68 to 4.56)	(0.26 to 9.6)	43	(0.14 to 3.3)
Stratum I with Epo (n=25)					
Autologous blood (n=11)					
CS (n=5)	1.6 (3.6)	0.01	3.2	20	1.3
DR (n=6)	0.50 (1.2)	(-5.5 to 5.5)	(0.0 to 9.6)	17	(0.06 to 26.9)
Stratum II (n=113)					
Autologous blood (n=62)					
CS (n=26)	0.58 (1.4)	-0.24	1.0	19	2.4
DR (n=36)	0.56 (2.7)	(-1.66 to 1.19)	(0.16 to 13.3)	8.3	(0.54 to 11.1)

Abbreviations: AT=as treated; CS=cell saver; DR=postoperative drain re-infusion; RBC=red blood cell; U=units; CI=confidence interval; SD=standard deviation; Epo=erythropoietin.

[a] adjusted for hospital and for knee/ hip surgery; confidence intervals for reference purposes only (assuming normality)

[b] ratio was defined as the quotient of mean RBC values of two groups being compared; all estimates and robust standard errors were obtained via bootstrapping in R

[c] all estimates and standard errors were obtained using the Mantel-Haenszel procedure, stratifying by the pre-specified stratification factors hospital and knee/hip surgery

[d] one patient received CS (intra-operatively) AND drain (postoperatively)

Chapter 8

Further improvement in Patient Blood Management

We showed that despite a restrictive transfusion trigger still a quarter of the patients receive transfusions for elective total hip- and knee surgery. Autologous re-infusion by use of a cell saver or a post-operative drain re-infusion device was not effective to further decrease RBC use. Pre-operative use of Epo, however, is an effective transfusion alternative, but against unacceptably high costs. Therefore, these transfusion alternatives should not be used for the average elective orthopaedic patient. Further improvement in Patient Blood Management may be gained by looking at patient-specific factors. In this chapter, risk indicators for receiving a red blood cell (RBC) transfusion and for adverse clinical outcome (morbidity and mortality) in elective hip-and knee surgery are discussed. Since preoperative anaemia has been identified as an independent risk indicator for blood transfusion and has been associated with adverse outcome, one of the strategies for optimal blood management is to treat preoperative anaemia in order to aim for normal Hb values before surgery. This is discussed in the light of future studies (i.e. more attention to the preoperative anaemic patient, including the evaluation of the use of intravenous iron). Finally, the issues for further improvement in Patient Blood Management by prognostic modelling are discussed.

RISK FACTORS FOR TRANSFUSION IN ELECTIVE HIP-AND KNEE REPLACEMENT SURGERY

The need for a red blood cell (RBC) transfusion in patients scheduled for hip or knee surgery may depend on several factors: surgical factors (e.g surgical time, type of surgery and surgical technique), patient factors (e.g. co-morbidity) and blood management protocols. A restrictive RBC transfusion trigger is a powerful tool to reduce RBC transfusions. This has been investigated in two randomised trials, reported in chapters 2,3 and 7.These studies showed that the majority of these patients are relatively healthy and do not need RBC transfusions, and subsequently should not be treated with blood sparing modalities. Therefore, it is necessary to look for patient characteristics that can be identified as risk indicators for RBC transfusions. Of all reported risk indicators, a low preoperative Hb value was found to be a strong independent predictor for RBC transfusions [1-7].

In Table 1, other risk indicators are reported as well, but these were not identified consistently. Our own data showed, that patients with Hb levels of 8.1 mmol/L (=13 g/dL) or lower, are three times more often transfused than patients with Hb levels above 8.1 mmol/L (24% versus 8%) (the TOMaat study data). In patients with a preoperative Hb of 8.1 mmol/L or lower, Epo reduced the transfusion rate to 12% (OR 0.50; 95% CI 0.3 to 0.7), but at unacceptably high costs per avoided transfusion. Studies investigating other, less costly alternatives for Epo, are therefore necessary. Furthermore, by identifying more predictors for the use of a RBC transfusion, selective application of blood saving measures to a certain well-defined group of patients would be possible for optimal cost-efficiency.

Table 1. Preoperative risk indicators for red blood cell (RBC) transfusions in hip-and knee replacement surgery

Author (year)	Type of surgery (numbers)	Study design	Risk factor (if available OR and CI is included)
Keating (1998) [2]	Unilateral TKR (n=279) Bilateral TKR (n=280)	Retrospective Logistic multivariable regression analysis	Preop Hb 10-13 g./dL vs >13 g/dL
Faris (1999) [6]	THR/TKR (n=276)	Retrospective Logistic regression curve	Preoperative Hb 10-13 g/dL
Rosencher (2003) OSTHEO study [32]	THR n=2640 TKR n=1305	Prospective Logistic regression plot	Inverse relation Hb and RBC transfusion: transfusion risk if Hb=8 g/dL 75% for women and 69% for men; if Hb=13 g/dL: 32% for women and 22% for men
Bong (2004) [3]	TKR (n=1402)	Retrospective Multivariable regression analysis all p<0.05	Preop Hb: 10-13 g/dL OR 1.83 <10 g/dL OR 4.17 Age: 65-74 OR 1.54 75-84 OR 2.88 >85 OR 4.50 use of LMWH: OR 2.08
Guerin (2007) [4]	THR and TKR (n=162)	Prospective Multivariable regression analysis	Preop Hb level < 13 g/dL
Walsh (2012) [7]	Revision THR (n=210)	Prospective Multivariable regression analysis	Preoperative Hb (change per g/dL increase in Hb): OR. 0.44 [0.33-0.58] Weight (change per kg, increase): OR 0.98 [0.96-1.00] blood loss (change per mL increase in blood loss): OR1.002 [1.002-1.003] re-infusion of perioperative salvaged blood (yes/no): OR 0.31 [0.11-0.82]

Abbreviations: n=numbers; TKR=Total Knee Replacement; OR=Odds Ratio; CI=Confidence Interval; THR=Total Hip Replacement; LMWH= Low Molecular Weight Heparin

PREOPERATIVE ANAEMIA AS A RISK INDICATOR FOR ADVERSE CLINICAL OUTCOME IN ELECTIVE HIP-AND KNEE SURGERY

Besides having an increased transfusion risk, preoperative anaemia has also been identified as an independent risk indicator for mortality and morbidity after surgery. Beattie and co-workers reported a strong association between anaemia and peri-operative mortality

in a large non-cardiac surgery cohort of more than 7000 patients. However, the subgroup of orthopaedic surgery patients was not specified (Table 2) [8]. Musallam and co-workers investigated a large cohort of 227.425 patients including 69.227 anaemic patients (10.758 were orthopaedic surgery patients), and found an increased risk for morbidity and mortality 30 days after surgery in the anaemic patients compared to the non-anaemic patients [9]. This study found an increased risk for composite morbidity (e.g. myocardial infarction (MI), stroke, pneumonia, renal insufficiency, wound infection, sepsis, thrombo-embolism) of 53% (OR1.53 [95% CI 1.23 to 1.90]). Complications were increased in 42% of the sample of 10.000 anaemic orthopaedic patients .This association, however, could not be confirmed by Mantilla and co-workers who performed a case-control study of hip-and knee replacement surgery patients (50% elective, 50% emergency), and matched for type of surgery, age and sex [10]. The investigators found that preoperative Hb value was not a risk for mortality or MI, but identified other existing co-morbidities such as cardiovascular, cerebro-vascular or pulmonary disease as the most important risk indicators. This risk model applied to both emergency as well as to elective surgery subgroups. The authors discussed that their population included patients with a relatively high mean age (78 years) with a high prevalence of co-morbidities (65% cardiovascular diseases) compared to other studies. In an earlier and descriptive study on a large study population of 10.244 primary total hip and knee arthroplasty patients over a 10 year period, the same authors (Mantilla (2002) reported on the frequency of myocardial infarction, pulmonary embolism, deep venous thrombosis and postoperative death and found a frequency of 2.2% of these complications within 30 days after surgery, mainly in the older age group (>70 years). However they did not evaluate the association between these complications and anaemia [11].

Since high age and co-morbidities are also identified as risk indicators for adverse outcome in hip fracture surgery patients, it seems that some overlap exists in risk indicators for patients scheduled for elective orthopaedic surgery and patients who had more acute orthopaedic surgery after a hip fracture (Table 3) [10,11]. These latter patients are also referred to as "the frail elderly" in contrast to the vital healthy elderly. The frail elderly group has been associated with impaired physical function, gait speed and impaired cognition and have a higher risk of death and disability. An alternative to identify frailty is to estimate the biological age of the patient, which has been performed in 1000 randomly recruited ambulatory 75-year old women in Sweden. In that study, the biological age was predictive for both future fractures (OR 7.52: oldest tertile compared to youngest tertile), and overall mortality (OR 3.65) [12].

Anaemia may also be associated with "frailty", and the presence of anaemia, whatever its cause, may well be a proxy for "the frail elderly". So by identifying and treating the anaemic preoperative patient, the surgical outcome may be influenced in a positive way.

Table 2. Risk indicators for adverse outcome in (elective) hip-and knee replacement surgery

Author (year)	Type of surgery (numbers)	Study design / type of analysis	Risk factors/ predictors
Mantilla (2002) [11]	Elective THR/TKR (n=10244)	Retrospective, descriptive data	Age (>70 y) (higher frequencies) Outcome: MI, pulmonary embolism, death
Beattie (2009) [8]	Non-cardiac surgery (n=7759) (orthopaedic surgery not specified)	Retrospective, multivariable analysis	Preoperative anaemia OR 2.36 [1.57-3.41] Outcome: mortality
Musallam (2011) [9]	Non-cardiac surgery (n=227425) (orthopaedic surgery not specified)	Retrospective, multivariable analysis	Preoperative anaemia OR 1.42 [1.31-1.54] Outcome: 30-day morbidity and mortality
Sabate (2011) [33]	Non-cardiac surgery (n=3387) (34% was orthopaedic surgery)	Prospective, multivariable analysis	Existing co-morbidities, blood transfusion: Coronary artery disease: OR 2.2 [1.3-3.5]; Congestive heart failure: OR 2.3 [1.4-3.9]; Chronic kidney disease: OR 1.9 [1.2-3.2] Cerebrovascular disease: OR 2.9 [1.7-4.7] RBC transfusion: OR 2.7 [1.9-4.1] Outcome: major cardiac and cerebrovascular events
Mantilla (2011) [10]	Elective and emergency THR/TKR (n=391+391)	Case-control, multivariable analysis	Cardiovascular disease: OR 3.27 [2.27-4.72]; cerebrovascular disease: OR 1.99 [1.24-3.19]; pulmonary disease: OR 1.62 [1.00-2.61] Outcome: MI, death

Abbreviations: n=numbers; Y=Years; TKR=Total Knee Replacement; THR=Total Hip Replacement; OR [CI]=Odds Ratio [Confidence Interval]; MI=Myocardial Infarction

Table 3. Risk indicators or predictors for adverse outcome after hip fracture surgery

Author (year)	Type of surgery (numbers)	Study design/ data analysis	Risk factor
Lu-Yao (1994) [34]	Hip fractures -femoral neck n=13167 -per-trochanteric n=13767 Total n= 26424 Age ≥65 years	Cross-sectional multivariable analysis Outcome: 90-d (here shown) and 3y-mortality	Age (1-y increase): OR 1.07 [1.06-1.07] Male: OR 2.21 [2.04-2.40] Nursing home OR 1.39 [1.28-1.52] Pertrochanteric fracture site OR 1.18 [1.06-1.30] Charlson* co-morbidity score >0 OR 1.89 [1.75-2.04]
Nettleman (1996) [35]	Hip fracture (not specified) n=390 all ages	Retrospective Multiple logistic regression analysis Outcome: 30-d mortality	Predictors: - CHF OR 32.2 [5.4-92] - angina 25.7 [3.6-184] - COPD 11.1 [2.0-62]
Gruson (2002) [36]	Hip fracture (femoral neck and intertrochanteric) n=395 age ≥65 years	Prospective, multivariable logistic regression analysis Outcome: 3-,6-,12-m mortality	Predictive factor: preoperative anaemia: OR 1.4 [0.5-4.0] n.s. (3-month mortality) OR 2.9 [1.2-7.3] (6-m mortality) OR 2.6 [1.2-5.5] (12-m mortality)
Richmond (2003) [37]	Hip fractures (not specified) n=836 age ≥65 years	Prospective Standardised Mortality Ratio (SMR) Outcome: 2 year-mortality	ASA 3-4 in age 65-84: SMR 3.2 Not increased in: ASA 1-2 and/or age >84
Roberts (2003) [38]	Femur neck fractures n=32590 age ≥65 years	Retrospective Case Fatality Rates (CFR) Outcome: 30-d, 90-d and 365-d mortality (CFR)	CFR by age, and by sex: from OR 7.2 in men 65-69y to OR 33.7 in men >90y (30-d) compared to women (from OR 2.7 to OR 22.7 (30-d); Social class IV and V (adjusted for age and sex): OR. 2.47 [1.79-3.42] (30-d) ORs are further increased after 90-d and 365-d mortality
Halm (2004) [39]	Hip fracture (femoral neck, inter- and sub-trochanteric) n=550 all ages	Prospective Multivariable regression analysis Outcome: death 60-d after discharge	Preoperative Hb level: OR 0.69 [0.49-0.95]

Table 3. *Continued*

Author (year)	Type of surgery (numbers)	Study design/ data analysis	Risk factor
Roche (2005) [40]	Hip fractures (not specified) n=2448 age ≥ 60 years	Prospective, multivariable analysis Outcome: 30-d mortality	Three or more co-morbidities: OR 2.5 [1,6-3.9]; respiratory disease: OR 1.8 [1.3-2.5] Malignancy: OR 1.5 [1.01-2.3]
Maxwell (2008) [41]	Femur neck fractures (n=5162) all ages	Prospective, multivariable analysis Outcome: 30-d mortality	Age >65 y: OR 4.34 [1.34-14.0] Male gender: OR 1.63 [1.15-2.39] Two or more co-morbidities: OR 1.63 [1.15-2.32] MMS score 6 or less: OR 1.55 [1.01-2.39] Malignancy: OR 1.76 [1.13-2.74]
Burgos (2008) [42]	Hip fracture (not specified) n=232 age ≥65 years	Prospective ROC curve: AUC ≥0.7 As acceptable predictive value Outcome: A. Serious complications B. 90-d mortality	Predictive preoperative risk scores (AUC): Risk- VAS: 0.707 (for A) Charlson: 0.833 (for A) POSSUM 0.726 (for A) None were predictive for B
Vochteloo (2011) [43]	Hip fracture (femoral neck, inter- and sub-trochanteric) n=1262 age ≥65 years	Retrospective and prospective, multivariable analysis Outcome: mortality (in-hospital, 3 m and 12 m)	Preoperative anaemia: not predictive for mortality: OR 1.30 [0.96-1.76] RBC transfusion: predictive for in-hospital mortality, 3-m and 12-m

*Charlson index: scores pre-operative co-morbidity as a predictor for adverse postoperative outcome
Abbreviations: n=numbers; OR=Odds Ratio [95% confidence interval]; n.s.=not significant; m=months; d=day; y=year; CHF=Congestive Heart Failure; COPD= Chronic Obstructive Pulmonary Disease; MMS= Minimal Mental State; ROC=Receiver Operation Curve; AUC=Area Under Curve; RISK-VAS=Visual Analogue Scale for Risk; POSSUM=Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity

PREOPERATIVE ANAEMIA IN THE OLDER PATIENT

Several surveys report a relationship of prevalence of anaemia and increasing age [13-15] with an overall prevalence of 11% in males and of 10% in females older than 65 years according to the WHO criteria [16]. One-third can be explained by nutritional causes (of which half was due to iron deficiency), one third by Anaemia of Chronic Disorder (ACD)

and Unexplained Anaemia (UA) in the remaining one third of patients. These latter two categories of patients are characterized by low erythropoietin levels and low levels of pro-inflammatory cytokines [17,18].

If anaemia is due to nutritional causes, correction might be easily performed by supplementing the deficient components. However, if anaemia is due to ACD or UA, it may be necessary to find the underlying disease that causes the anaemia (e.g. kidney failure, malignancy). If a patient is then still eligible for elective surgery, preoperative measures to increase the Hb level can be considered.

OPTIMISING PATIENT BLOOD MANAGEMENT AND POSTOPERATIVE OUTCOME BY OPTIMISING THE PREOPERATIVE HB LEVEL

Since the preoperative Hb level was consistently reported as an independent significant risk factor for a RBC transfusion, it was included in the workup for optimising Patient Blood Management [19,20]. A Patient Blood Management Protocol was developed by a NATA working party aiming for preoperative non-anaemic levels in the elective orthopaedic surgery population. Due to the elective character, the optimal preoperative treatment can be explored, which however can be a problem if the waiting period for surgery is less than 4 weeks. Use of Epo (with oral iron) to increase Hb to normal levels, is very costly. An alternative may be the use of IntraVenous (IV) iron, since IV iron therapy may increase Hb values not only in iron deficient patients, but also in patients with ACD, bypassing the blocking effect of hepcidin that makes iron unavailable for incorporation in red blood cells [21]. Whether patients with UA benefit from either IV iron or Epo therapy or are refractory to both treatments, is unknown and must be further evaluated.

The efficacy and safety of IV iron compared to oral iron, or to placebo had been studied in several randomised trials, and was reported in a systematic review [22]. The authors concluded, that ferric carboxymaltose (Ferinject) significantly increased Hb levels compared to placebo, oral iron and intravenous iron sucrose (Venofer). Furthermore, the use was comparably safe.

In orthopaedic surgery, no randomized trials are published that primarily evaluated the use of IV iron as an transfusion alternative and compared its effect to other blood management modalities. Of four published randomized studies using IV iron, none investigated RBC use as a primary outcome: three of them combined preoperative use with or without Epo and scored the frequency to preoperatively donated autologous blood as primary outcome [23-25], a fourth study evaluated postoperative use of IV iron and its effect on Hb recovery [26]. Another problem of these studies was the low numbers of patients.

Since these randomized studies only investigated IV iron at a limited level and hardly as a blood sparing modality, more insight in the response to preoperative intravenous iron

in the elderly population for elective hip-and knee surgery is important. For this purpose, a new study protocol was designed: the Preoperative Orthopaedic Patients- Iron (POP-I) study.

The POP-I study

In this study, patients with a preoperative Hb value between 6.2 (10 g/dL) and 8.1 mmol/L (13 g/dL) will be equally randomised for IV iron therapy (ferric carboxymaltose), for Epo (+oral iron) and for a control group. Control patients will be supplemented with oral iron in case of an iron deficiency anaemia. Primary outcome is the proportion of transfused patients and cost-effectiveness will be evaluated. IV iron and Epo will be prescribed at least 4 weeks before surgery and Hb levels will be monitored. With this study, we may provide evidence for using IV iron as a cost-effective alternative for Epo.

Interestingly, a same type of study is ongoing in hip fracture patients, comparing IV iron to IV iron+ Epo and to placebo (a multi-centre, randomised study: the PAHFRAC-01project; NCT01154491).

OPTIMISING PATIENT BLOOD MANAGEMENT BY DEVELOPING A PROGNOSTIC MODEL

Optimal tailor-made treatment (in Dutch: Op Maat) can be attained by developing a model in which several outcome results (i.e. to be transfused, or morbidity or mortality) can be predicted with a certain likelihood. Development of such a multivariable prognostic model can be performed best by using data from a prospective cohort study, although data from randomised intervention studies can also be used [27]. If such a model is developed and validated it can also be used to evaluate whether the predictors identified in elective orthopaedic surgery patients are valid for other patients groups, such as the "frail elderly" undergoing hip fracture surgery.

Prediction models for RBC transfusions have been proposed, but none of the models has been widely accepted and used. A systematic review on patient characteristics and its association with perioperative RBC transfusions was published by Khanna and coworkers, who analysed 46 studies of which 13 were among elective knee-and hip replacement surgery patients [28]. They found that a low preoperative Hb level was most frequently associated with RBC transfusions, being identified as a strong predictor in all studies. The other factors commonly associated with risk for transfusion in literature, were advancing age (3 studies), female gender (6 studies), and small body size (4 studies). Only 2 non-orthopaedic studies validated their predictive model for RBC transfusion on other prospective data and confirmed robustness of their model. However, the retrospective nature (lack of data), the small sample sizes and heterogeneity of the studies made it impossible to use the data

in a combined dataset to define a clinically useful prediction model for allogeneic RBC transfusion.

Having access to reliable predictors for adverse outcome, the clinician may outweigh the benefit of an elective lower extremity joint replacement against the risk of an adverse outcome for an individual patient. The PROPER study (PROject PEroperative Risk) compared preoperative risk factors to postoperative adverse outcome during hospital stay in 1471 elective general surgery and orthopaedic surgery patients, and found four predictive factors for adverse outcome in the overall group (orthopaedic patients were not separately specified): a risk-Visual Analogue Score (VAS), ASA score, age and surgical stress (a four point scale scoring minor to extensive procedures, to estimate the magnitude of surgical stress imposed upon the patient). However, a prediction model was not developed, because all predictors had moderate sensitivity and specificity and a too low predictive value for individual patient counselling [29].

By using data from the 2500 randomised patients from our collected TOMaat-study dataset, that included more than 250 transfused patients, we aim to develop a prognostic model for prediction of RBC transfusions, which can further be validated in collected datasets from other orthopaedic surgery patients. For the prediction of adverse postoperative outcome, other databases will be needed to have sufficient numbers of adverse events for input in a model. The most important and significant predictors can then be identified, assigning relative weights to each predictor, and estimating the model's predictive performance with adjusting the model for overfitting. Finally, validating the model will be done in new datasets.

PATIENT BLOOD MANAGEMENT: FUTURE DIRECTIONS

Despite the fact that blood components are safe in the Western world, there is an ongoing aim for "bloodless surgery" [30,31], which refers to optimalisation of peri-operative Patient Blood Management. We demonstrated that in elective knee-and hip surgery, due to the use of a restrictive transfusion trigger and continuously improving surgical techniques, some transfusion alternatives are no longer effective in reducing RBC use (autologous re-infusion devices such as cell saver or postoperative drain re-infusion devices), or are not cost-effectively reducing RBCs, such as Epo, and are thus not considered as appropriate transfusion alternatives. By identifying predictors for transfusion, the use of (other) blood sparing modalities may be further evaluated and applied to the high risk patients only.

Nowadays, the scope for optimal Patient Blood Management has changed to the preoperative setting, in which the anaemic patient, which is a proxy for patients with some kind of co-morbidity, needs more thorough evaluation to prevent adverse postoperative outcome. This is underscored by the identification of preoperative anaemia as a strong

predictor for transfusion. Patients with a preoperative anaemia must be treated to increase Hb levels to normal, thus reducing peri-operative RBC use and possibly also reducing the postoperative complication rate. Use of Intravenous iron can be explored in patients with iron deficiency anaemia and ACD as a cost-effective alternative for Epo.

In conclusion, a clinically relevant prediction model with respect to allogeneic RBC transfusions, will support a Tailor Made (in Dutch: Transfusie Op Maat) Patient Blood Management strategy for a specific group of patients, in which the use of transfusion alternatives may be applied. By identifying predictors for worse outcome (i.e. mortality and high morbidity), a decision model for the clinician may assist in the decision whether the benefits of a joint prosthesis outweighs the risks for adverse postoperative outcome. Future studies must include prognostic modeling leading to optimal Patient Blood Management.

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Chapter 9

Designing a strategy to implement cost-effective blood transfusion management in elective hip and knee arthroplasties: a study protocol

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ABSTRACT

Background: Total hip and knee arthroplasties are two of the most commonly performed procedures in orthopedic surgery. Different blood-saving measures (BSMs) are used to reduce the often-needed allogenic blood transfusions in these procedures. A recent large randomized controlled trial showed it is not cost effective to use the BSMs of erythropoietin and perioperative autologous blood salvage in elective primary hip and knee arthroplasties. Despite dissemination of these study results, medical professionals keep using these BSMs. To actually change practice, an implementation strategy is needed that is based on a good understanding of target groups and settings and the psychological constructs that predict behavior of medical professionals. However, detailed insight into these issuses is lacking. Therefore, this study aims to explore which groups of professionals should be targeted at which settings, as well as relevant barriers and facilitators that should be taken into acount in the strategy to implement evidence-based, cost-effective blood transfusion management and to de-implement BSMs.

Methods: The study consists of three phases. First, a questionnaire survey among all Dutch orthopedic hospital departments and independent treatment centers (n=99) will be conducted to analyze current blood management practice. Second, semistructured interviews will be held among 10 orthopedic surgeons and 10 anesthesiologists to identify barriers and facilitators that are relevant for the uptake of cost-effective blood transfusion management. Interview questions will be based on the Theoretical Domains Interview framework. The interviews will be followed by a questionnaire survey among 800 medical professionals in orthopedics and anesthesiology (400 professionals per discipline) in which the identified barriers and facilitators will be ranked by frequency and importance. Finally, an implementation strategy will be developed based on the results from the previous phases, using principles of intervention mapping and an expert panel.

Discussion: The developed strategy for cost-effective blood transfusion management by de-implementing BSMs is likely to reduce costs for elective hip and knee arthroplasties. In addition, this study will lead to generalized knowledge regarding relevant factors for the de-implementation of non-cost-effective interventions and insight in the differences between implementation and de-implementation strategies.

Total hip and knee arthroplasties are two of the most commonly performed procedures in orthopedic surgery [1,2]. It is expected that the number of these procedures within the Netherlands will increase to more than 100,000 by the year 2030 [3]. During primary hip or knee arthroplasty, the calculated visible and invisible blood loss is 1,500 ml on average, followed by a drop of hemoglobin of approximately 3 g/dl [4]. This leads to high rates of allogenic blood transfusions up to 69% depending on the transfusion threshold [5]. Even though blood transfusions may be necessary, they include the risk for infections and noninfectious transfusion reactions [6].

Many studies on blood-saving measures (BSMs) have therefore been performed, including erythropoietin (EPO) and peri-operative autologous blood salvage (intra-operative use of Cell Saver (CS) and a postoperative drainage and reinfusion device (DR)). Reviews showed that these studies had several limitations, such as a retrospective design, small patient numbers and poor methodological quality [5,7,8]. A multicenter randomized controlled trial (RCT) with adequate power (n=2,442) was therefore performed recently to test the cost effectiveness of using BSMs, including EPO, CS, and DR, in elective primary hip and knee arthroplasties [9]. It was shown that blood salvage (CS and DR) resulted in neither decreased mean red blood cell (RBC) use nor in a decrease in the proportion of transfused patients and was more expensive due to the costs of the devices used and a prolonged hospital stay. EPO showed a significant decrease in the proportion of transfused patients, but costs were considered too high. It was thus concluded that these BSMs were not cost effective in primary hip and knee arthroplasties [10].

Despite this evidence about BSMs not being cost effective, medical professionals keep using these BSMs in daily practice. To decrease costs of care delivery to patients undergoing a hip or knee arthroplasty, cost-effective blood transfusion management needs to be implemented. However, little is known about how to effectively de-implement common practices. To actually change practice, a de-implementation strategy is needed that is based on a good understanding of target groups and settings and the barriers and facilitators that influence the behavior of medical professionals [10,11]. However, detailed insight into these factors is lacking. Psychological theories are used in understanding and predicting intentions and clinical behavior [12] and may help to outline an effective strategy to de-implement these non-cost-effective BSMs.

OBJECTIVE

The Leiden Implementation Study of BlOod management in hip and knee Arthroplasties (LISBOA) aims to explore the target groups, settings, and relevant barriers and facilitators that should be taken into account to develop a strategy directed at all involved medical professionals (target group) and their organizations to implement evidence-based, cost effective transfusion management and to de-implement BSMs.

To reach the aim of this study, the following research questions were formulated:

A. How often and in what settings are BSMs applied in hip and knee arthroplasties?
B. Which barriers and facilitators influence the implementation of cost-effective blood transfusion management and de-implementation of non-cost-effective BSMs among the target group, including orthopedic surgeons and anesthesiologists?
C. What is a tailored implementation strategy for the uptake of cost-effective blood transfusion management given the results of the first two research questions?

METHODS

The study will be subdivided in three study phases to be executed in one year:

A. analysis of current blood transfusion management practice in elective primary hip and knee arthroplasties (months 1 to 3),
B. analysis of barriers and facilitators relevant for the implementation of cost-effective blood transfusion management and de-implementation of non-cost-effective BSMs (months 4 to 8),
C. development of an implementation strategy based on the results of phases A and B (months 9 to 12).

The study design, study population, analysis, and outcome measures are described per study phase.

PHASE A: ANALYSIS OF CURRENT BLOOD TRANSFUSION MANAGEMENT

Study design

To analyze current blood transfusion management practice in hip and knee arthroplasties, a survey among all orthopedic departments of Dutch university, teaching, and general hospitals and independent treatment centers will be performed. A survey in the period 1995-1997 showed that EPO was used rarely in the Netherlands at that time, in only 2% of all hospitals, and that CS was used in 24% of hospitals [13]. A more recent survey in 2007 showed that approximately half of all Dutch orthopedic departments applied EPO and/or autologous blood salvage [14]. However, these surveys neither showed how frequent these BSMs were applied within hospitals nor in what type of setting (university, teaching, or general hospital or independent treatment center). This information is needed to target the implementation strategy to the appropriate professionals and departments.

The current survey will thus include questions about the type and size of the department, the transfusion protocol used, and the frequency of application of BSMs in patients within the last 12 months. Furthermore, questions will be included about the policy of preoperative anticoagulant use. These last questions are added to assess whether these protocols are related to BSM use and should be taken into account in the implementation strategy. The content of the survey will be developed together with an orthopedic surgeon, anesthesiologist, and hematologist specialized in blood transfusions. Reminders to nonresponders will be sent after two weeks and again by telephone after four weeks.

Study population

All heads of orthopedic departments of Dutch university, teaching, and general hospitals and independent treatment centers (n=99) will be approached to participate in the survey. In case of nonresponse, a different orthopedic surgeon within the same department will be approached.

Analysis

Descriptive statistics will be used to describe current blood management practice. Independent t tests or Mann Whitney U tests for continuous variables and Chi-Square tests or Fisher's exact tests for proportions are used to analyze differences in frequency of use between the different settings, department sizes, or other conditions.

Outcome measures

The main outcome measures are the percentage of orthopedic departments applying BSMs per size and type of setting of the orthopedic department and the frequency of BSM use within a department. These results are used in phase C to address the implementation strategy to the appropriate (groups of) orthopedic departments. A secondary outcome measure is the number of days anticoagulants are stopped preoperatively. This is used to analyze whether this is associated with BSM use and should be taken into account in the implementation strategy.

PHASE B: ANALYSIS OF BARRIERS AND FACILITATORS FOR IMPLEMENTATION OF COST-EFFECTIVE BLOOD TRANSFUSION MANAGEMENT

Study design

Two steps will be taken to identify barriers and facilitators associated with the implementation of cost-effective blood transfusion management. First, semistructured interviews will be performed to explore all relevant barriers and facilitators for the uptake of cost-effective blood transfusion management. The interview questions will be based on the Theoretical Domains Interview (TDI) framework [15], complemented by the framework of Cabana, who subdivided largely similar constructs in three "sequences of behavior change" to give a good overview of the used constructs [16]. The TDI framework includes 12 theoretical construct domains derived from 33 health psychology theories (covering 128 theoretical constructs) that help explain clinicians' behavior [15,17].

Second, a survey will be held among a random sample of 400 Dutch orthopedic surgeons and 400 anesthesiologists to rank the barriers and facilitators identified in the interviews both on frequency and importance. The survey will include questions in which

these barriers and facilitators of the identified theoretical domains can be related to specific clinical behavior.

Study population

Orthopedic surgeons and anesthesiologists are key stakeholders in deciding to use allogenic blood transfusions only or BSMs in patients that undergo hip and knee arthroplasty. Based on the analysis of current practice (phase A of this study), we will select a sample of departments that frequently apply BSMs to identify barriers, as well as departments with rare use of BSMs to identify facilitators. In this selection, the setting is taken into account (university, teaching, or general hospital or independent treatment center). In addition, departments with alternative answers (e.g., the use of a different transfusion protocol) will be selected for interviews. In total, ten orthopedic surgeons and ten anesthesiologists will be interviewed to identify barriers and facilitators relevant for the uptake of a cost-effective blood transfusion policy and their motivations to apply BSMs. Data saturation for the interviews is defined as three consecutive interviews without new themes emerging. If there is no data saturation after 10 interviews per specialism, additional interviews will be conducted [18]. The total number of interviews will thus be determined by the number it takes to reach data saturation.

The interviews with orthopedic surgeons and anesthesiologists may reveal that other groups of stakeholders have an important role in deciding to use BSMs. In that case, additional interviews will be held with those stakeholders to elicit their views about relevant barriers and facilitators associated with the uptake of cost-effective transfusionmanagement. For the survey, a random sample (n=400) of all Dutch orthopedic surgeons listed in the registry of the Dutch Orthopedic Association (NOV) (n=595) and a random sample (n=400) of anesthesiologists listed in the registry of the Netherlands Society of Anesthesiologists (NVA) (n≈1200) will be approached for participation in the survey.

Analysis

The interviews will be audiotaped and transcribed in full for analysis. The interview transcripts will be analyzed by two researchers using the TDI framework as a base [15]. Important theoretical domains and the barriers and facilitators within these domains will be coded. This qualitative analysis will be executed using the software package ATLAS.ti (ATLAS.ti Scientific Software Development GmBH, Berlin, Germany).

The subsequent survey data will allow us to rank the importance of barriers and facilitators and their relationships with behavioral intention. These relationships will be assessed using regression analysis.

Outcome measures

The most important barriers and facilitators relevant for the uptake of cost-effective blood transfusion management by medical professionals will be the outcome measures from this phase.

PHASE C: DEVELOPMENT OF AN EFFECTIVE IMPLEMENTATION STRATEGY FOR COST-EFFECTIVE BLOOD MANAGEMENT

Study design

The results from the previous phases will be used to develop a tailored implementation strategy for cost-effective blood transfusion management for elective primary hip and knee arthroplasties. The results from phase A will show to which type of departments the strategy should be aimed. Phase B results will show the most important barriers and facilitators that should be taken into account in the development of the strategy.

From the literature, it is known that, in general, multifaceted strategies are more effective than single strategies [19,20]. Assuming this, and our expectation that several barriers on different theoretical domains will be found, it is very likely that the implementation strategy to be developed will include several components directed at different levels (i.e., professional and organizational context). Furthermore, it is expected that the strategy components will include educational outreach or interactive educational strategy since these are known to be effective [20,21].

In the development process, we will use a method based on the intervention mapping approach of Bartholomew et al [22]. This method begins with the creation of matrices in which the performance objectives are set against the top 10 ranking of factors that hinder or facilitate the implementation of a cost-effective transfusion policy. Subsequently, a brainstorming session will be held about the strategy components needed to achieve the performance objective, in the presence or absence of the hindering or facilitating factor mentioned in the matrix. The cells of the matrices will then gradually be filled with implementation strategy components [23]. Next, the formulated strategy components will be translated into practical strategies at each level (e.g., professional and organizational).

After the implementation strategy is developed, an expert meeting will be held with a panel of key opinion leaders in orthopedic surgery and anesthesiology, delegates of blood transfusion committees, and implementation experts (n=10 to n=20) to discuss the strategy's feasibility and to refine the developed implementation strategy. Their opinion about the strategy and their intention to use the strategy will be taken into account.

Analysis

The expert meeting will be audiotaped and transcribed. The panel members will receive a summary of the formulated implementation strategy and will be asked whether this summary is consistent with the conclusions reached in the meeting.

Outcome measures

The outcome from this phase will be a tailored implementation strategy likely to be effective for implementing cost effective blood transfusion management and de-implementing BSMs in elective primary hip and knee athroplasties.

Ethical approval

The study protocol has been presented to the Medical Ethical Committee of the Leiden University Medical Center. They declared ethical approval was not required under Dutch national law. (CME 11/104)

DISCUSSION

The goal of this study is to develop an implementation strategy for cost-effective blood transfusion management in elective hip and knee arthroplasties in which BSMs are de-implemented. This study is the next step following a RCT on EPO and blood salvage as transfusion alternatives in orthopedic surgery using a restrictive transfusion policy that showed that use of these BSMs is not cost effective [9]. Given the number of hip and knee arthroplasties performed annually in the Netherlands and worldwide, and the accompanied blood loss and transfusion risks, implementing a cost-effective blood transfusion management may reduce costs.

Several studies have been performed to develop and test implementation strategies, including identification of barriers that prevent implementation [10,16,19]. They all conclude that a prior inventory of barriers to develop a tailored implementation strategy is useful and can confirm whether barriers differ in different settings. Prior inventory thereby reduces the number of costly trials evaluating different implementation strategies [11,24,25]. The present study, however, focuses on de-implementation of BSMs known to be cost ineffective. Little is known about barriers and facilitators for de-implementation and whether these are similar to barriers and facilitators for implementation. The knowledge obtained by the present study may thus be further generalized to other practices that need to be de-implemented and contributes to general knowledge regarding differences between de-implementation and implementation strategies.

Strengths and limitations

Possible limitations of the study are biased results due to response bias in the phase A survey [26]. Nonresponse may cause an under- or overestimation of BSM use. The selection for the interviews in phase B is based on the results of phase A, so if non-responders have different intentions or experience different barriers and facilitators for the uptake of cost-effective blood transfusion management, this may influence the resulting barriers and facilitators.
We will try to overcome this by sending reminders by email and telephone, but this will not completely prevent response bias. In addition, response bias may also occur in the phase B survey if nonresponders to this survey rank the selected barriers and facilitators in a different order; this may influence the likelihood of barriers and facilitators being included in the implementation strategy. Again, reminders will be sent to keep bias to a minimum, and we will compare respondents and nonrespondents on demographic variables (e.g., type of hospital) to estimate how likely it is that bias may be introduced.

A strength of this study is that it is one of the first studies to identify barriers and facilitators relevant for de-implementation. The study results will thus lead to generalized knowledge regarding factors that are important for the de-implementation of non-cost-effective interventions and how these differ from relevant factors for implementation.

Future work

The developed implementation strategy should be tested for effectiveness, feasibility, and costs within orthopedic practice in the Netherlands in a future study. As the current implementation strategy will be aimed at de-implementation of the use of EPO, CS, and DR, further research is needed to evaluate the cost effectiveness of other BSMs in hip and knee arthroplasties. Cost effective blood transfusion management implemented in this way is likely to improve efficiency of care.

Competing interests

The authors have no competing interests to declare.

Authors' contributions

LB, AK, RN, CS, and TV designed the study; VV will carry out the study; LB and PM will coordinate the study. VV, LB, and PM drafted the manuscript. The manuscript has been read and approved by all authors.

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Chapter 10

Summary / Samenvatting

Summary

To minimise exposure to allogeneic blood in elective orthopaedic surgery, both restrictive transfusion policies as well as the use of transfusion alternatives are widely recommended. The first step towards optimal Patient Blood Management should be the implementation of a restrictive transfusion policy. With this measure, we managed to save 36% in mean RBC use and 13% in proportion transfused patients (chapters 2 and 3), without differences in quality of life (chapters 3 and 4). Because still 26% was transfused in our study (chapter 2), a second step was appropriate to evaluate, the additional effect on RBC use of transfusion alternatives. Although many studies on transfusion alternatives have been published, inclusion numbers were usually low and often without the use of a strict transfusion policy. In a pilot study, we compared different types of post-operative drain re-infusion devices for its efficacy and feasibility (chapter 5) and found these to be comparable. This pilot-study was performed in order to decide which drain device could be used in a larger study on transfusion alternatives, which included erythropoietin (Epo) as a preoperative modality, cell saver as an intra-operative modality for re-infusion of washed salvaged blood and a drain re-infusion device as a postoperative modality for re-infusion of unwashed salvaged blood. A restrictive transfusion policy was used for all patients according to the Dutch guidelines (chapter 7). In this study, Epo was the only effective transfusion alternative, but it was not cost-effective. Therefore, for optimal efficacy, the use of Epo should be discouraged in elective hip-and knee surgery, as well as use of devices for re-infusion of salvaged blood, like cell savers and post-operative drain re-infusion devices.

Although autologous salvaged blood contains many cytokines from the wound bed (chapter 5), comparable changes in pro-inflammatory cytokine gene expression of IL-8, TNFsf10 and TNFsf13 were seen in the systemic circulation of re-infused and control patients (chapter 6). This finding does not support the hypothesis that salvaged blood in general may reduce postoperative infection risk due to its immuno-modulatory properties. However, re-infusion of washed salvaged blood showed a lower pro-inflammatory cytokine profile in the patient than unwashed salvaged blood.

In order to aim for an optimal and Tailor Made Blood Management Program (in Dutch: Transfusie Op Maat) for elective orthopaedic surgery patients, we need to investigate other (cost-) effective modalities, as well as to distinguish the patient at risk for blood transfusions and for adverse outcome from the normal risk patient (chapter 8). On the other hand, we need to de-implement current blood management policies that use non cost-effective modalities, such as Epo and autologous blood re-infusion devices, starting by first exploring the barriers and facilitators for de-implementation (chapter 9).

Samenvatting

Om de kans op het krijgen van een allogene bloedtransfusie in de electieve orthopaedische chirurgie te minimaliseren, worden zowel een restrictief transfusie beleid als het gebruik van transfusie alternatieven alom aanbevolen. De eerste stap naar optimaal Patient Blood Management zou het implementeren van een strict transfusiebeleid moeten zijn. Met deze maatregel, is het ons gelukt om een 36% reductie in gemiddeld Rode Bloed Cel (RBC) gebruik te bereiken en een daling van 13% in het aantal getransfundeerde patienten (hoofdstukken 2 en 3), zonder aantoonbare verschillen in kwaliteit van leven (hoofdstukken 3 en 4). Omdat in onze studie nog steeds 26% van de patiënten getransfundeerd werd (hoofdstuk 2), was het een logische tweede stap om het additionele effect van transfusie alternatieven op RBC gebruik te evalueren. Hoewel veel is gepubliceerd over het gebruik van transfusie alternatieven, waren de inclusie aantallen in de studies vaak laag en werd vaak geen gebruik gemaakt van een restrictief transfusiebeleid. In een pilot studie vergeleken we verschillende typen post-operatieve drain re-infusie systemen op hun efficiëntie en gebruikersgemak en vonden we deze gelijkwaardig (hoofdstuk 5). Deze pilot studie was verricht om een besluit te kunnen nemen welk drain re-infusiesysteem geschikt zou zijn om te gebruiken in een grotere studie over transfusie alternatieven, waarin tegelijkertijd erythropoietine (Epo) als preoperatieve modaliteit, de cell saver voor re-infusie van gewassen wondbloed als intra-operatieve modaliteit, alsook een drain re-infusie systeem voor re-infusie van ongewassen wondbloed als postoperatieve modaliteit, zou worden onderzocht. Bij alle patiënten werd een restrictief transfusie beleid volgens de huidige Nederlandse Transfusie Richtlijn gebruikt (hoofdstuk 7). In deze studie bleek Epo de enige effectieve bloedbesparende maatregel te zijn, maar was helaas niet kosten-effectief. Om een zo'n optimaal mogelijke kosten-effectiviteit te bereiken, dient het gebruik van Epo in de electieve heup-en knie vervangende chirurgie, ontmoedigd te worden, net zoals het gebruik van wondbloedre-infusie systemen, zoals cell savers en post-operatieve drain re-infusie systemen.

Hoewel autoloog wondbloed veel cytokines vanuit het wondbed bevat (hoofdstuk 5), werden vergelijkbare veranderingen in pro-inflammatoire cytokine gen expressie van IL-8, TNFsf10 en TNFsf13 gevonden in de systemische circulatie van zowel gere-infundeerde patiënten als controle patiënten (hoofdstuk 6). Deze bevinding ondersteunt de hypothese niet dat wondbloed in het algemeen het postoperatieve infectierisico kan verlagen vanwege de immuno-modulatoire eigenschappen. Echter, re-infusie van gewassen wondbloed vertoonde een lager pro-inflammatoire cytokine profiel in de patiënt dan ongewassen wondbloed.

Om een optimaal Bloed Management Protocol Op Maat te bereiken voor de electieve orthopaedische chirurgie patiënt, is het nodig om andere (kosten-) effectieve modaliteiten te onderzoeken, en om de risico patiënt met een verhoogde kans op het krijgen van een RBC transfusie en op het krijgen van complicaties, te kunnen onderscheiden van de patiënt met een gemiddeld risico (hoofdstuk 8). Anderzijds zouden we bestaande bloedmanagement protocollen waarin gebruik gemaakt wordt van niet-kosten effectieve modaliteiten, zoals Epo en autoloog wondbloed re-infusie systemen, onder de loep moeten nemen met als doel deze modaliteiten te de-implementeren. Hiervoor dienen in eerste instantie zowel de drempels als de faciliterende factoren voor de-implementatie inzichtelijk te worden gemaakt (hoofdstuk 9).

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Lieve Ralph, een huishouden met twee medische specialisten heeft zo zijn voor- en zijn nadelen. Ondanks onze drukke agenda's was er gelukkig nooit een gebrek aan quality-time met en zonder de kids. Ik kijk uit naar de mooie momenten die nog komen gaan!

Curriculum Vitae

De auteur van dit proefschrift werd op 17 juli 1966 geboren te Jakarta, Indonesië. In 1968 emigreerde haar familie naar Nederland. Het middelbaar onderwijs werd gevolgd aan de CSG Comenius te Capelle aan den IJssel, alwaar in 1984 het VWO diploma werd behaald. Vanaf 1984 studeerde ze geneeskunde aan de Erasmus Universiteit te Rotterdam, waar het artsexamen werd behaald in 1990. Na een jaar AGNIO-schap startte ze haar opleiding to internist in het Zuiderziekenhuis te Rotterdam (nu Maasstad-ziekenhuis) (opleider dr A. Berghout), welke werd afgerond in het Dijkzigt ziekenhuis, eveneens in Rotterdam (nu EMC-Centrum) (opleider prof. dr S.W.J. Lamberts). Na haar registratie in de Inwendige Geneeskunde in1998 volgde een registratie in de Hematologie (2001) (opleider prof dr B. Löwenberg) en een registratie in de Bloedtransfusiegeneeskunde (2003) (opleider prof dr A. Brand). Gedurende de opleiding tot transfusiespecialist werden de eerste stappen gezet voor dit proefschrift, waarin gestart werd met het opzetten van klinische studies inzake bloedtransfusiegebruik op de afdeling Orthopaedie met prof dr R.G.H.H. Nelissen. Sinds 2002 is zij werkzaam bij Sanquin Bloedvoorziening op de afdeling Klinische Consultatieve Dienst als Bloedtransfusiespecialist. In 2008 werd het Master of Science diploma in de Klinische Epidemiologie behaald (VUMC, Amsterdam).
Cynthia is in 1994 getrouwd met Ralph, anaesthesioloog-intensivist en Manager Kwaliteit in het Albert Schweitzer ziekenhuis te Dordrecht. Ze hebben drie kinderen: Ruben (1999), Lieke (2000) en Vincent (2003).

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