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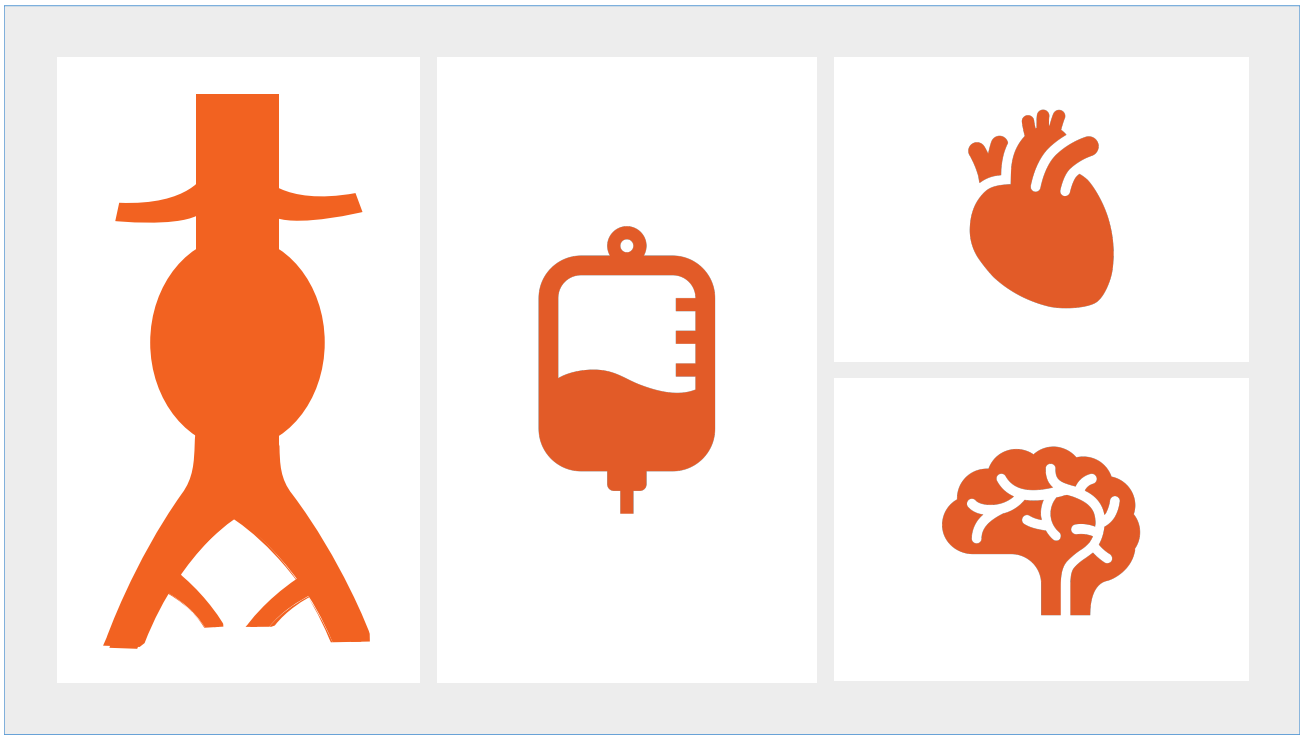

PhD thesis

Anders Møller

Allogeneic blood transfusion in vascular surgery

- a randomized clinical feasibility trial and a nationwide registry study

THIS THESIS HAS BEEN SUBMITTED TO THE GRADUATE SCHOOL OF HEALTH AND MEDICAL SCIENCES, UNIVERSITY OF COPENHAGEN ON FEBRUARY 1ST, 2021

REGION SJÆLLAND
– vi er til for dig

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1 PREFACE

During my first years as a medical doctor and trainee in anaesthesia and intensive care medicine, I often encountered challenging surgical cases ranging from perioperative anaemia, rapid ongoing blood loss to exsanguinating haemorrhage. Management of the latter seemed well protocolized in my departments with the use of balanced 1:1:1 red-cell:plasma:platelet transfusion. However, the *grey-zone* cases of persistent ongoing blood loss that neither justified balanced transfusion nor could be considered "stable patients" clearly challenged my senior colleagues. The essential question "How do we replace surgical blood loss?" almost prompted a new answer for each consultant you asked. Crystalloids, hydroxy-ethyl starches, fresh-frozen plasma (FFP), human albumin, vaso- or inopressors, red blood cell (RBC) transfusion were "taken off the shelves" in varying sequence depending on the consultant in charge. Clearly, the evidence to guide the interventions was sparse or non-existing.

In 2013, Henning Bay Nielsen put me in contact with Jørn Wetterslev. Both later became my PhD-supervisors as Jørn and Henning pitched the idea of a large-scale randomized clinical trial (RCT) comparing two different haemoglobin (Hb) thresholds for RBC transfusion in vascular surgery. In the run-up for preparation of a protocol, it was debated whether such an RCT would be un-feasible. It became relatively clear that a pilot RCT would be an essential step-stone for large-scale RCTs. It was my luck that Slagelse Hospital's anaesthetic department, my working place, in collaboration with the department of vascular surgery and vascular surgeon Saeid Shahidi showed genuine interest to support development of the Transfusion in Vascular surgery (TV) trial. The TV trial founds the basis of my phd.

There are countless people I need to thank for the support during my PhD and as a principal investigator of the TV-trial: my colleagues at Slagelse Hospital, my supervisors Jørn, Ole, Henning, and Dorthe. A special thanks to Jørn and Henrik Hjalgrim, who have provided me a working place at their scientific institutions and scientific sparing. The significance of the one-on-one discussions and result evaluations was invaluable for my motivation and progress. Lastly, this journey and the COVID pandemic has taken an enormous toll on my spare time, working part time clinically. Nonetheless, my love, Ingvild, has been very supportive and a great motivator.

2 ORIGINAL PAPERS

The PhD thesis is based on the following papers:

I. **Trends in abdominal aortic aneurysm surgery incidence, comorbidity, and mortality: a Danish nationwide cohort, 1996-2018.** Moller A, Eldrup N, Wetterslev J, Hellemann D, Nielsen HB, Rostgaard K, Hjalgrim H, Pedersen OB. [ready for submission]

II. **Low vs. high haemoglobin trigger for transfusion in vascular surgery: protocol for a randomised trial.** Moller A, Nielsen HB, Wetterslev J, Pedersen OB, Hellemann D, Shahidi S. Acta Anaesthesiologica Scandinavica. 2017;61(8):952-961.

III. **Low vs high hemoglobin trigger for transfusion in vascular surgery: a randomized clinical feasibility trial.** Moller A, Nielsen HB, Wetterslev J, Pedersen OB, Hellemann D, Winkel P, Marcussen KV, Ramsing BGU, Mortensen A, Jakobsen JC, Shahidi S. Blood 2019;133(25):2639-2650.

IV. **Effect of low vs. high haemoglobin transfusion trigger on cardiac output in patients undergoing elective vascular surgery: post-hoc analysis of a randomised trial.** Moller A, Wetterslev J, Shahidi S, Hellemann D, Secher NH, Pedersen OB, Marcussen KV, Ramsing BGU, Mortensen A, Nielsen HB. Acta Anaesthesiologica Scandinavica. 2020;00:1–11. https://doi.org/10.1111/aas.13733 [epub ahead of print].

3 LIST OF ABBREVIATIONS

AAA,	Abdominal Aortic Aneurysm
CCI,	Charlson's Comorbidity Index
CI,	Confidence Interval
CO,	Cardiac Output
CVD,	Cardiovascular Disease
DO_2,	Oxygen Delivery
FFP,	Fresh Frozen Plasma
GEE,	Generalized Estimating Equations
Hb,	Haemoglobin
IR,	Incidence Rate
IRR,	Incidence Rate Ratio
LEAD,	Lower Extremity Artery occlusive Disease
MD,	Mean difference
MRR,	Mortality Rate Ratio (Hazard ratio for death)
NIRS,	Near-infrared spectroscopy
PPV,	Positive Predictive Value
RBC,	Red Blood Cell
SAP,	Statistical Analysis Plan
ScO_2,	Frontal lobe oxygen saturation
SmO_2,	Biceps muscle oxygen saturation
SV,	Stroke Volume
TRALI,	Transfusion Related Acute Lung Injury

4 SUMMARY

Background

Allogeneic blood transfusions are frequent and indispensable in major vascular surgery, yet the evidence to guide the transfusion practice is limited. To establish the safety and efficacy of red blood cell transfusion and inform transfusion practice, we need large, randomized trials with the power to detect differences in patient-important outcomes. This Ph.D. thesis is a prerequisite for such a trial and includes a nationwide registry study and the protocol and results of a feasibility trial.

Methods

In the nationwide registry study, we describe the trends in incidence, comorbidity and five-year mortality of abdominal aortic aneurysm (AAA) repair in Denmark using prospectively collected data from population-based registers from 1996 through 2018 (Study I). We assessed comorbidities by Charlson's Comorbidity Index (CCI) score and used multivariable Poisson and Cox regression to compute incidence rate ratios and mortality rate ratios (MRR), respectively.

In the Transfusion in Vascular surgery (TV) feasibility trial, we invited patients scheduled for open AAA- or lower-limb bypass surgery. Fifty-eight patients were randomly assigned, following haemoglobin drop below 9.7 g/dL, to red blood cell transfusion at a haemoglobin below 8 g/dL vs. 9.7 g/dL throughout hospitalization (Study II). The primary outcome was postoperative haemoglobin levels day 0-15. We also report units of red blood cells transfused, protocol adherence, regional frontal lobe oxygen saturation (ScO_2) during surgery, as determined by NIRS, and exploratory patient-important outcomes. In a post-hoc analysis of the TV trial, we present the effect of haemoglobin trigger on cardiac output and oxygen delivery during surgery in patients randomized before end of surgery (Study III).

Results

Study I: The overall AAA repair incidence rate decreased by 24 % from 1996 to 2018. The decrease was mostly explained by a 52 % reduction of ruptured AAA repair incidence in men and a 52–63% incidence rate reduction in the population aged below 70 years. The CCI increased independently of age and sex by an annual 0.9 % in AAA repair patients. Both crude and adjusted five-year mortality was markedly reduced during the study period (Adjusted: $MRR_{ruptured}$, 0.46; 95% confidence interval (CI), 0.39 – 0.54, MRR_{intact}, 0.51; 95% CI, 0.44 – 0.59).

Study II: The low transfusion trigger reduced the mean postoperative haemoglobin levels by 0.87 g/dL (9.46 *vs.* 10.33 g/dL; P = 0.022), units red blood cells transfused (median [interquartile range (IQR)], 1 [0-2] *vs.* 3 [2-6]; P = 0.002) and increased the ScO_2-desaturation from baseline during surgery (median [IQR], 421 [42-888] *vs.* 127 [11-331] minutes x %; P = 0.004) compared with the high transfusion

trigger. The low trigger group associated to a higher rate of death or major vascular complications (19/29 *vs.* 8/29; MRR, 3.20; P = 0.006).

Study III: The mean intraoperative cardiac output was numerically 7.3% higher in the low trigger compared with the high trigger group (mean difference, 0.36 L/min; 95%, −0.05 to 0.78; P = 0.092; n = 42). At the nadir ScO_2 level, the cardiac output was 11.9% higher in the low trigger group (mean difference, 0.58 L/ min; 95% CI, 0.10 - 1.07; P = 0.024). The results did not indicate a difference in oxygen delivery between trial groups (MD, 1.39 dL_{O2}/min; 95% CI, −6.16 to 8.93; P = 0.721).

Conclusion

The incidence of AAA repair decreases in the Danish population due to a reduction among males and a shift to an older age group requiring intervention, which may reflect a cohort effect. Even considering increasing comorbidity, analyses showed that the five-year mortality rate following AAA repair decreased during the study period, but the reasons for this are unclear.

The TV feasibility trial successfully separated trial groups in terms of haemoglobin levels and the number of blood products used. We also demonstrated that the low transfusion trigger resulted in a lower ScO_2 during surgery, which was not explained by an impaired- cardiac output or global oxygen delivery. Exploratory outcomes indicated potential harm with the low trigger strategy, and we need further large-scale trials in vascular surgery to establish a safe transfusion strategy.

5 SUMMARY IN DANISH (DANSK RESUME)

Baggrund

Blodtransfusioner er hyppige og uundværlige ved større karkirurgi, men den videnskabelige evidens til at guide transfusionspraksis er begrænset. For at fastslå sikkerheden og effekten af transfusion med røde blodlegemer, og vejlede transfusionspraksis, har vi brug for store forsøg der kan detektere forskelle i patientvigtige effektmål. Denne ph.d.-afhandling er en forudsætning for et sådant forsøg og inkluderer en registerundersøgelse samt protokollen og resultaterne af et pilotforsøg.

Metode

I en landsdækkende registerundersøgelse beskriver vi udviklingen i incidens-, komorbiditet- og fem-års dødelighed af abdominalt aortaaneurisme (AAA)-operation i Danmark ved hjælp af prospektivt indsamlede data fra befolkningsbaserede registre fra 1996 til 2018 (Studie I). Komorbiditet blev vurderet vha. Charlsons comorbiditetsindeks (CCI). Multivariabel Poisson- og Cox-regression blev anvendt til at beregne henholdsvis incidensrater og mortalitetsrate-ratioer (MRR).

I pilotforsøget Transfusion in Vascular surgery (TV) Trial inviterede vi patienter, der var planlagt til åben AAA-operation eller bypassoperation i benene. Otteoghalvtreds patienter blev, efter hæmoglobin fald under 9,7 g/dL (6 mmol/l), tilfældigt tildelt transfusion med røde blodceller ved hæmoglobinfald under 8 g/dL (5 mmol/L) eller 9,7 g/dL (6 mmol/L) gennem hele indlæggelsen (Studie II). Det primære effektmål var post-operativt hæmoglobinniveau fra dag 0 til 15. Sekundære effektmål var antal enheder røde blodceller transfunderet, protokoloverholdelse, iltning af perifert væv under operationen målt vha. NIRS, samt eksplorative patientvigtige effektmål. I en post-hoc analyse af TV-forsøget præsenterer vi effekten af hæmoglobin-transfusionsgrænse på hjertets minutvolumen og oxygentransport under operationen hos patienter inkluderet inden operationens afslutning (Studie III).

Resultater

Studie I: Incidensraten af AAA-operation faldt med 24% fra 1996 til 2018. Faldet kunne hovedsageligt forklares med en 52 % reduktion i incidensraten af rumperet AAA-operation hos mænd og af en 52-63 % incidensratereduktion i AAA-operationer i befolkningen under 70 år. AAA-operationspatienternes CCI steg uafhængigt af både alder og køn med 0,9% årligt gennem studieperioden. Både ujusteret og justeret fem-års dødeligheden blev markant reduceret hen over studieperioden (Justeret: $MRR_{rumperet}$, 0,46; 95% konfidensinterval (CI), 0,39 - 0,54, MRR_{intakt}, 0,51; 95% CI, 0,44 - 0,59).

Studie II: Den lave hæmoglobingrænse for transfusion reducerede det gennemsnitlige postoperative hæmoglobinniveau med 0,87 g/dL (9,46 vs. 10,33 g / dL; P = 0,022), antal enheder røde blodlegemer transfunderet (median [interkvartilgrænse (IQR)], 1 [0-2] vs. 3 [2-6]; P = 0,002) og øgede omfanget af regional iltdesaturation af frontallappen under operation (median [IQR], 421 [42-888] vs. 127

[11 -331] minutter x%; P = 0,004) sammenlignet med den høje hæmoglobingrænse for transfusion. Lav transfusionsgrænse var forbundet med flere komplikationer eller død (19/29 vs. 8/29; MRR, 3,20; P = 0,006).

Studie III: Det gennemsnitlige hjerteminutvolumen var numerisk 7,3% højere i gruppen med lav transfusionsgrænse sammenlignet med høj transfusionsgrænse (gennemsnitlig forskel, 0,36 L/min; 95%, -0,05 til 0,78; P = 0,092; n = 42) . Ved nadir-iltningsniveauet i frontal-lappen var hjerteminut-volumen 11,9% højere i gruppen med lav transfusionsgrænse (gennemsnitlig forskel, 0,58 L/min; 95% CI, 0,10 - 1,07; P = 0,024). Resultaterne indikerede ikke en forskel i oxygentransport mellem forsøgsgrupperne (gennemsnitlig forskel, 1,39 dL_{O2}/min; 95% CI, -6,16 til 8,93; P = 0,721).

Konklusion

Incidensen af AAA-operation er faldende i den danske befolkning på grund af en reduktion blandt mænd og et skift til en ældre aldersgruppe, der kræver intervention, hvilket kan afspejle en kohorteeffekt. Selv i betragtning af stigende komorbiditet viste analyserne, at den femårige dødelighed efter AAA-operation faldt gennem studieperioden, men årsagerne til dette er uafklaret.

TV-pilotforsøget adskilte succesfuldt forsøgsgrupper med hensyn til hæmoglobinniveau og antal blodprodukter transfunderet. Vi demonstrerede også, at den lave transfusionsgrænse resulterede i en lavere iltning i frontallappen under operationen, hvilket ikke blev forklaret af et nedsat hjerteminutvolumen eller global oxygentransport. TV-forsøget viste potentiel skade med den lave transfusionsgrænse, og vi har brug for store forsøg inden for karkirurgi for at fastlægge en patientsikker transfusionsstrategi.

6 INTRODUCTION

Allogeneic blood transfusions play a vital role in the perioperative management of patients undergoing vascular surgery. Arterial reconstructions often provoke blood loss that exceed the human physiology's compensatory reserve; both in terms of maintaining tissue oxygenation and coagulation competence as blood is shed and the essential O_2 carrying red blood cells (RBC) and coagulation factors are lost. Severe comorbidity, including cardiac disease, is frequent,[1,2] which in turn associates with poor anaemia tolerance.[3,4] Furthermore, major vascular surgery, such as open abdominal aortic aneurysm (AAA) repair, involves comprehensive *surgical trauma* that increases metabolism and O_2 requirements following surgery. So, these patients have several odds against them when facing surgery, which is also reflected in frequent postoperative ischaemic events and deaths following surgery that has classified vascular surgery as *high-risk*.[5,6]

Blood O_2 carrying capacity and coagulation competence may be maintained with allogeneic RBC- and plasma transfusions. However, transfusion carry risk of circulatory overload, infection, severe lung injury and donor-recipient incompatibility reaction,[7] and the balance of risk and benefits of allogeneic blood transfusion in vascular surgery is unknown.[4,8-10] To establish the safety and efficacy of either intervention, a large, randomised trial is needed with power to detect differences in patient important outcomes.

This thesis is a prerequisite for such a trial. It includes *i)* a nationwide registry study of trends in incidence and mortality to inform inclusion criteria and sample size for a future trial and to describe the burden of disease, and *ii)* the results of a pilot trial that was initiated to test the feasibility of implementing a trial protocol during major vascular surgery, adhere to the protocol and achieve group separation of Hb levels and units of RBCs transfused.

7 BACKGROUND

7.1 Study population and nomenclature

Vascular surgery is a surgical specialty where artery or vein diseases are managed by open surgery, endovascular repair, or medicines. Common indications include aneurysmal disease, occlusive artery disease, and variceal veins. The target and study population of this thesis is patients presenting with the following two conditions: *i)* infrarenal abdominal aortic aneurysm (AAA), and *ii)* Lower Extremity Artery occlusive Disease (LEAD) of the aorto-iliac-, femoral-, and popliteal arteries, table 1. Arterial occlusive disease may also be referred to as Peripheral Artery Disease (PAD), stenotic artery disease, or atherosclerotic stenosis.

LEAD is staged according to symptom severity, and more recently, with a corresponding Ankle-brachial index (ABI); table 2.[11]

Table 1	**Registry study**	**Randomized trial**
Study	I	II – III
Paper	I	II – IV
Method	Register study	Randomized trial
AAA		
Intact	√	√
Ruptured	√	%
Endovascular repair	√	%
LEAD		
Central (Aorto-iliac)	√	√
Peripheral (fem-pop)	√	√
PTA	√	%
Age	> 40 years	> 40 years
Location	Nationwide, Denmark	Region Zealand, Denmark

Note: Acute limb ischemia patients were not included in either study.

Table 2. Fontaine Classification

Stage	Symptoms	Ankle-brachial index (ABI)
I	Asymptomatic	ABI > 0.95
II		
IIa	Mild claudication	ABI > 0.80
IIb	Moderate-severe claudication	ABI > 0.40
III	Ischaemic rest pain	ABI < 0.40
IV	Ulceration/Wound or gangrene	Toe pressure < 30 mmHg

7.2 Historical perspective

Key events in the history of transfusion and vascular surgery are summarized in figure A. Blood transfusion has developed since the late 19th century. Most revolutionizing were the Nobel prize-winning discoveries of the ABO- and rhesus-blood groups that lead to a dramatic reduction in donor-recipient transfusion reactions and the development of assays to detect transmissible diseases such as HIV and hepatitis.[12]

Vascular surgery for AAA was mostly unsuccessful in the early 1900s. In 1914, Kummell described a repair of a ruptured thoracic aneurysm by oversewing the defect. The procedure took only 1 hour, but the 52-year-old patient soon “died of exhaustion”. First successful AAA treatment was a ligation of a ruptured syphilitic AAA in 1923 on a 28-year-old female plantation worker. The patient survived 17 months and then died of tuberculosis. Both AAA and LEAD surgery was revolutionized in the 1950s when the insertion of synthetic grafts and homografts became a success. The first PTA was performed in 1964 and the first EVAR in 1987.[13]

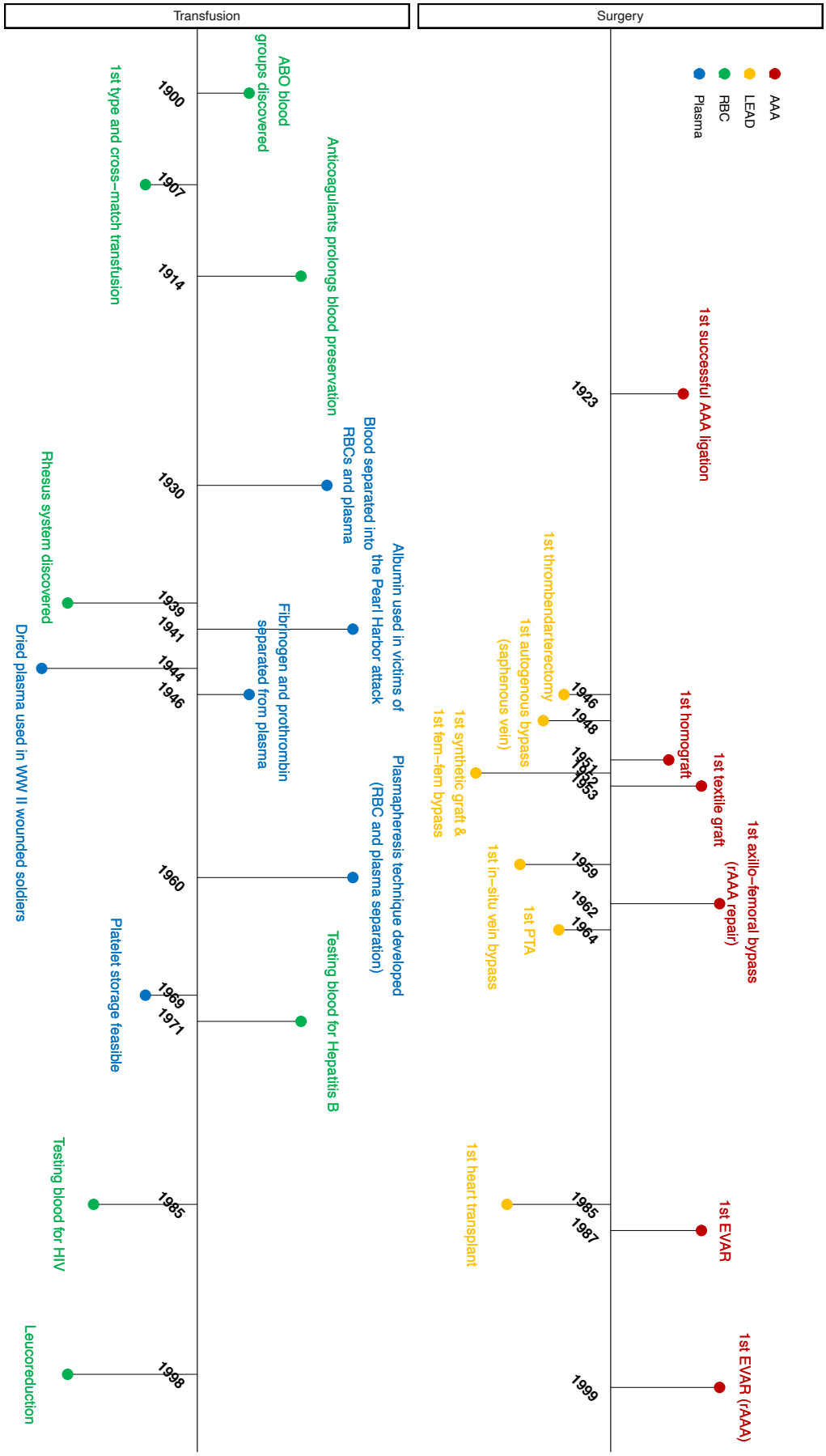
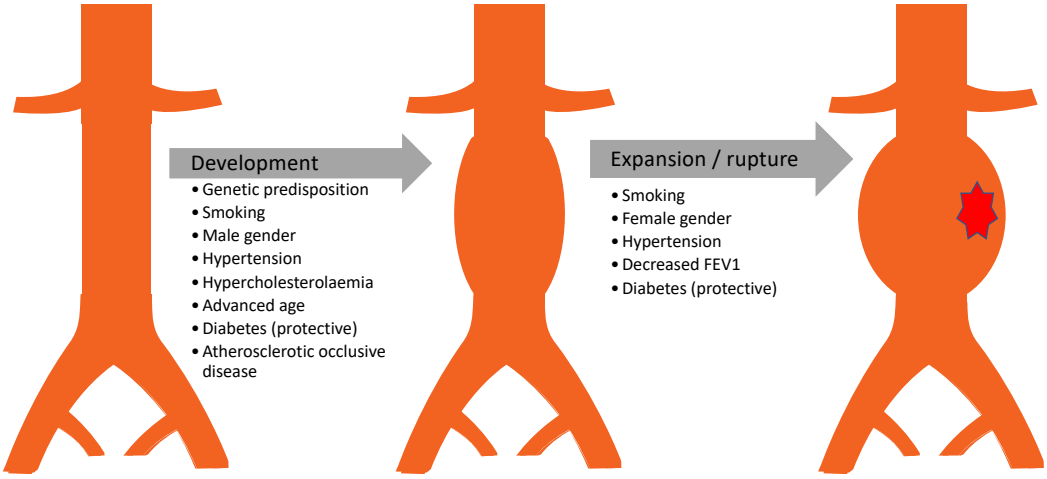


Figure A. Landmarks in blood transfusion and vascular surgery, A. Møller. Data source[12-14].

7.3 Aetiology and risk factors

The development and expansion of AAA have separate sets of risk factors with some overlap Figure B:



Smoking has been identified as the principal modifiable risk factor for AAA development.[15] Once AAA has developed, smoking also accelerates expansion and the risk of rupture.[16] Other risk factors for AAA expansion include hypertension, decreased forced expiratory volume in one second (FEV1), and female sex.[17-19] Historically, Denmark has had a high prevalence of smoking. In the 1950s, 80 % of men were smokers, and in the 1960s, Danish females had the highest recorded smoking prevalence in the world.[20] Diabetes mellitus is a negative risk factor for both AAA development and expansion.[11,21]

The most significant risk factors for LEAD development are diabetes mellitus and smoking.[11]

7.4 Epidemiology

7.4.1 Incidence

From 1950 to 1990, AAA was a disease rising rapidly in the Western world, as demonstrated by increasing AAA-related deaths.[22-26] In Denmark, incident hospital admissions where a AAA *diagnosis* was made, increased from 7.1 per 100,000 to 25.8 per 100 000 person-years, from 1977 to 1990. This corresponded to an average annual incidence increase of 10 %,[22] whereas data from Australia and Minnesota showed an annual increase in the incidence of 4 % and 11 %, resp.[27] The Danish incidence of AAA *repair* also increased from 1977-1990, which was observed in both sexes and across age groups.[22] A WHO data-based study reported a reversing

trend in AAA mortality in most Western countries from 1994 to 2010, but this was not demonstrated in Denmark, Austria, Romania and Hungary.[25,27] The Danish epidemiology in terms of incident AAA repairs and diagnoses has not been described since 1993. However, three regional screening trials by Lindholt and colleagues have estimated the AAA prevalence, table 3.

7.4.2 Prevalence

The prevalence of screening-detected AAAs is decreasing in Sweden[28] and England[29]. In Denmark, the prevalence has been stable or increasing, from 3.9 % (95 % CI = 3.4 - 4.5%) in the Viborg trial of 1994–98[30] to 5.1 % (95% CI = 4.7 - 5.5 %) in the DANCAVAS trial of 2014-17[31]. However, participants in the DANCAVAS trial were screening by computed tomography angiography, which may overestimate the AAA diameter. Other screening trials presented in table 3 used ultrasound. Besides, persons with already known AAAs were invited and are included in the prevalence reporting of the DANCAVAS trial, which might bias the prevalence because men who are aware that they have a AAA may be more motivated to participate. The trial included both arteriography of the thoracic- and abdominal aorta and the coronaries. Thus, the DANCAVAS estimate might be slightly higher than if solely "AAA naive" persons were invited. The Danish data may be taken to reflect, that a rise in the AAA prevalence is a cause of either increased incidence, decreasing expansion rates and/or improved survival (Figure C) Lindholt also screened for LEAD in the VIVA trial, which was prevalent in 10 % of Danish males aged 65-75 years.[32]

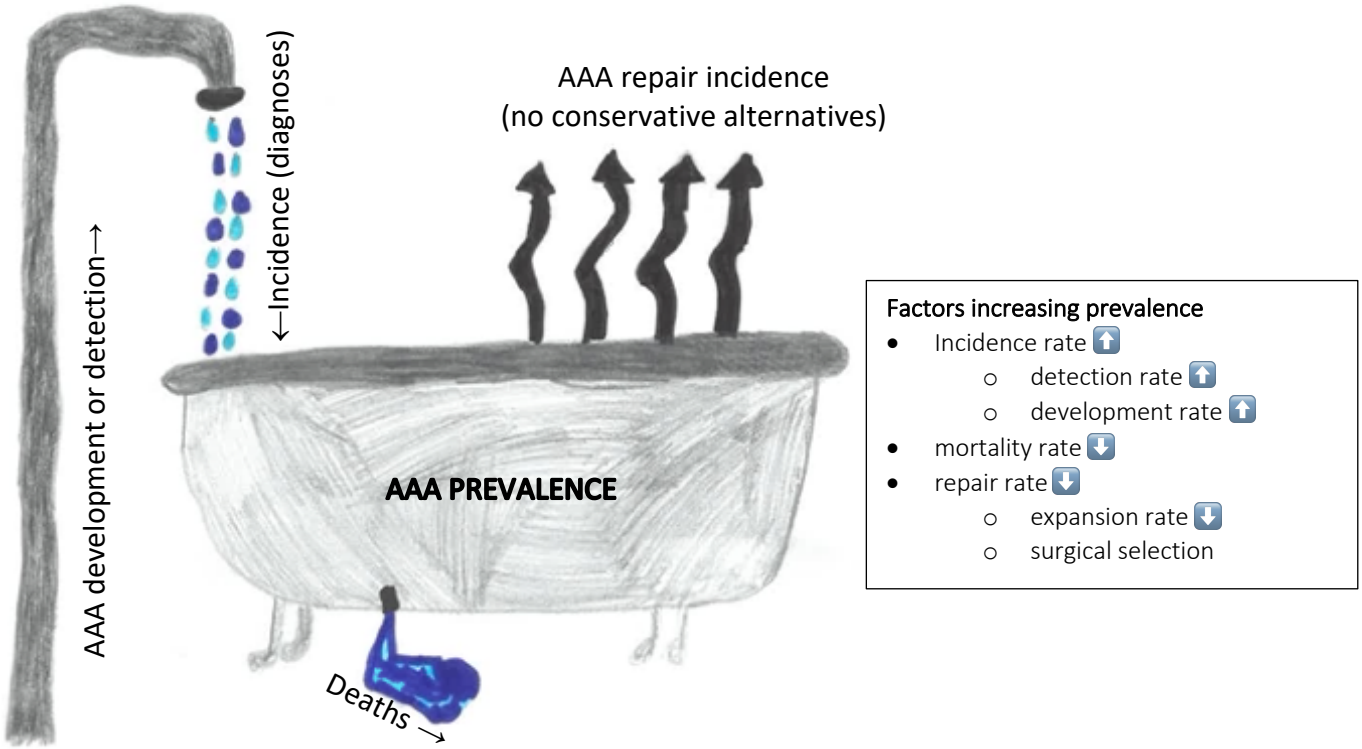
In summary, the epidemiology has reversed in many Western countries from the 20th to the 21st century, but the epidemiology in Denmark is less clear and the incidence of AAA repair has not been reported since 1993.

Table 3. AAA prevalence in men, aortic diameter > 3.0 cm

MALE PREVALENCE	1990-2000	2000-2010	2010-2020	AGE (YEARS)
DK	4.4 %[30]	3.3 %[32]	5.1 %[31]	65-73[30] 65-74[31,32]
SWEDEN		1.7 %[28]		65
ENGLAND, GLOUCESTERSHIRE[29]	5.0 %	2.7 %	1.3 %	65

Note: In the DANCAVAS screening trial[31] AAA screening was by computed tomography and men with previously known AAAs were also included in the prevalence reporting. All other screening studies used ultra-sound. When already repaired- or under surveillance AAAs were included in the Swedish study, the prevalence was 2.2 %.[28]

Figure C. "Epidemiologist's bathtub" - Factors affecting AAA prevalence.
Drawing by Nora Møller Ramberg, my daughter. (With permission)



7.4.3 Mortality

Long term follow-up on AAA repair is essential when comparing surgical techniques and calendar periods because endovascular repair was introduced in 2000-2010 and associates to lower perioperative mortality than open repair. The two techniques associates to similar long-term mortality (5-10 years).[33] Five-year mortality data following AAA repair has not been reported in Denmark.[34]

Another limitation concerning the available evidence is that most, if not all, studies focus on the outcome following first-time surgery, which is, of course, relevant to AAA as recurrent surgery on the AAA indication is rare. However, LEAD surgery is notoriously recurrent because of loss of patency and incident atherosclerotic lesions on new anatomic locations. Thus, the mortality risk associated with LEAD surgery, which is exclusively based on first-time surgery, may not reflect the real mortality in consecutive LEAD patients screened for a trial and may, in turn, underestimate the statical power when planning the trial.

7.4.4 The Danish Vascular Registry

An important data source for research in vascular surgical epidemiology is the Danish Vascular Registry (DVR).[35] The DVR originated in 1989 as a local initiative to monitor outcomes following vascular surgery and promote research. The DVR reached nationwide coverage in 1996. About 200,000 procedures have been registered, including both endovascular and open techniques, central/aortic, carotid and peripheral arterial and vein reconstruction. Key variables include surgical technique, operation type and anatomic localization as per the Scandinavian SKS-coding, and indication for the reconstruction. Patient variables include various comorbidities, care dependency, tobacco/alcohol abuse, cause of death, discharge details, patency of reconstructions, and complications during current index surgery hospitalization. The DVR does not hold information on AAA diameter (an otherwise standard variable in foreign vascular registries) or on time from surgery to complication. The DVR has been integrated into the international VASCUNET initiative for comparison of outcomes across countries. The surgeon enters data digitally, but at some departments, paper forms are used and later entered into the database by secretaries. International validation of the DVR established an external validity of 98 % for AAAs and a high data quality superior to local administrative data.[36,37]

7.5 Anaemia and oxygen delivery

Anaemia is defined as a decrease in the total amount of red blood cells (RBC) or haemoglobin (Hb) and results in a lowered arterial oxygen (O_2) carrying capacity. Anaemia is often observed in vascular surgical patients due to pre-existing chronic anaemia or major surgical haemorrhage.[4,38-41]

The aetiology of postoperative ischaemic events is complex and multifactorial, but an imbalance between oxygen delivery and consumption plays an important role.[42] During surgical haemorrhage, global oxygen delivery (DO_2) can be maintained by increasing cardiac output (CO), usually through haemodilution, or restoring arterial oxygen content (CaO_2). If DO_2 decreases, tissue oxygenation may be maintained by increasing tissue oxygen extraction.

$$DO_2 = CO \times \underbrace{CaO_2}_{1.39 \times Hb \times SaO_2 + 0.003 \times paO_2}$$

Because an increase in the arterial partial pressure of oxygen (paO_2) has little impact on CaO_2 in individuals with normal arterial oxygen saturation (SaO_2), RBC transfusion, either allogeneic or through a blood recovery device, is most effective in restoring CaO_2 during haemorrhage. The integrative human cardiovascular response to RBC transfusion is complex. RBCs increase CaO_2 and may also augment cardiac output (CO) by increasing cardiac preload. However, RBCs also have significant opposing mechanisms on the CO through an increase in afterload and blood viscosity.[43] Furthermore, RBCs play a significant role in the regional flow regulation through the release of vasoactive agents, such as nitric oxide, when haemoglobin deoxygenates.[44,45]

Whole-body oxygen consumption (VO_2) can provide information on whether RBC administration increases global tissue O_2 utilization (VO_2 increase) or reduces oxygen extraction (VO_2 unaltered). However, VO_2 ignores or hides regional differences in oxygen consumption and tissue oxygenation. This is relevant to the vascular surgical population as they often present with multisite atherosclerotic lesions, especially in the coronary, carotid, and lower limb arteries. Near-infrared spectroscopy (NIRS) is a non-invasive technique developed to estimate regional tissue haemoglobin oxygen saturation which can be termed tissue oxygenation. NIRS can be used to monitor regional brain oxygen saturation (ScO_2), which is otherwise a "black box" and challenging to monitor for clinicians who need dynamic assessment of cerebral O_2 supply. NIRS is typically used during coronary artery bypass graft (CABG) and carotid surgery. A low ScO_2 predicts poor outcome in cardiac surgical patients, but more evidence is needed to demonstrate whether ScO_2 targeted algorithms improve patient outcome.[46]

Given the complexity of how different physiological parameters and patient characteristics interact and respond to RBC transfusion, a randomized trial design is required to balance risk factors in order to infer on the impact of different transfusion strategies on circulatory efficacy. However, few perioperative RCT report such data or have a large proportion of patients going through surgery without the need for RBCs. Also, further research is needed to investigate how tissues oxygenate in response to different RBC transfusion strategies.

7.5.1 Allogeneic blood transfusions

A standard blood donation contains RBCs, plasma and platelets. When centrifuged, the blood separates into three layers shown in the picture on the right: RBC (dark red, bottom), plasma (yellow, top) and buffy coat in the middle layer where platelets are situated (foto taken by O. B. Pedersen. With permission).

Blood donations are screened for transmissible infections and are leuco-reduced to minimize the risk of transfusion reactions, especially transfusion related acute lung injury (TRALI). The incidence of TRALI has markedly been reduced after the exclusion of female donors of products with high plasma volume.[47]

8 Aim of studies

This Ph.D. aimed to test the feasibility of an RCT that compares two Hb triggers for RBC transfusion throughout hospitalization for vascular surgery, and to describe the trends in AAA incidence and mortality to inform the inclusion criteria and power calculation for a future trial.

In TV feasibility trial, we observed that the low trigger for RBC transfusion resulted in a lower ScO_2. Thus, in the post-hoc analysis of the TV trial, we aimed to determine whether this finding was associated with or explained by other haemodynamic factors such as the CO.

9 Study outline

9.1 Study I: Trends in Danish AAA epidemiology (Paper 1)

- Paper 1: Trends in abdominal aortic aneurysm surgery incidence, comorbidity, and mortality: a Danish nationwide cohort, 1996-2018

9.2 Study II: The transfusion in vascular surgery (TV) trial, protocol and main results (Paper 2 - 3)

- Paper 2: Low vs. high haemoglobin trigger for transfusion in vascular surgery: protocol for a randomized trial
- Paper 3: Low vs high haemoglobin trigger for transfusion in vascular surgery: a randomized clinical feasibility trial.

9.3 Study III: Post-hoc analysis of the TV trial (Paper 4)

- Paper 4: Effect of low vs. high haemoglobin transfusion trigger on cardiac output in patients undergoing elective vascular surgery: post-hoc analysis of a randomized trial.

10 Study I: Trends in Danish AAA epidemiology

Paper: Trends in abdominal aortic aneurysm surgery incidence, comorbidity, and mortality: a Danish nationwide cohort, 1996-2018

10.1 Methods

10.1.1 Study design and patients

This cohort study was based on prospectively collected data from nationwide Danish registries: the Danish Vascular Registry (DVR), the population-based Danish Civil Registration System (CPR) and Danish National Patient Registry (DNPR). The CPR holds information on vital status, demographics and migration for all residents in Denmark since 1968 an onwards. The DNPR, established in 1977, holds information on all in-patient diagnoses and surgeries registered out-patient visits since 1977. In 1995, information from hospital outpatient and emergency visits were added to the DNPR.

The DVR was used to identify Danish patients undergoing first-time aorto-iliac aneurysm repair or LEAD revascularization from January 1st, 1996 to December 31st, 2019. The paper that reports data on LEAD repair is not included in this thesis, but a few key findings are mentioned.

Comorbidity was assessed by Charlson's Comorbidity Index (CCI) using the DNPR, updated December 31st, 2018. The CPR was up to date as of October 31st, 2019. CCI items and their corresponding International Classification of Diseases (ICD) coding are available in the appendix.

10.1.2 Statistical analysis

The CPR was used to calculate person-years at risk for vascular surgery by age (integer years), sex, and calendar year, of the Danish population. Standardized incidence rates (IRs) were computed by means of direct standardization. Poisson regression modelling was used to obtain IR ratios with 95 % confidence interval (95 % CI) and to test for covariate interaction. Furthermore, by adding information from the DNPR, we calculated person-years at risk by age, sex, year, and CCI score, in order to *i)* compute comorbidity specific risks for vascular surgery; and describe trends in *ii)* comorbidity and *iii)* mortality rates of the Danish residents and the vascular surgery study population.

We computed mortality rate ratio (MRR, via hazard ratios for death) using Cox regression that was adjusted for calendar year, age, sex, CCI, geographic health care region, and stratified for open *vs.* endovascular repair. Crude five-years mortality risk was computed for each calendar period using the Kaplan-Meier estimator.

For the purpose of informing a sample size calculation of future trials, using short-term mortality as an outcome, in this study also presents the 90-day mortality risks.

10.2 Results

10.2.1 AAA Incidence

Using the criteria provided in the flow chart, Figure D, 15,395 patients undergoing first-time AAA repairs were identified. The standardized AAA incidence decreased by 24 % from 25.1 to 19.1 per 100,000 person-years (IRR, 0.76; 95% CI, 0.68 - 0.85; Figure 1 panel a). The decrease was mainly due to a drop in ruptured AAA incidence in the population.

The incidence was highest in persons aged 71 - 80 (table 1). Significant age heterogeneity in the IR trend was observed for both intact and ruptured AAA repair, Figure 1 panel b. The IR decreased by 63 % in persons aged 51-60, by 52 % in age group 61-70, and increased by 81 % in persons aged 81-90 years (p-value for interaction < 0.001, eTable 1). In LEAD surgery the age heterogeneity showed a 50% decrease in age <70 years, 20 % increase in 80-85, 85 % increase in 85-90 (p-value for interaction < 0.001).

The sex specific trend in intact AAA incidence was the same in men and women. In ruptured AAA the male age-standardized IR ranged from 15.1 to 18.7 cases per 100,000 person-years in 1996-1998 and decreased to ranging 5.5 to 7.9 cases per 100,000 person-years in 2017-2019. In females, the age-standardized IR ranged 1.2 - 2.2 in 1996-1999 and 1.1 - 1.5 in 2017-2019 per 100,000 person-years. This corresponded to average annual ruptured AAA IR reduction of 4 % in males and 2 % in females (p interaction < 0.001).

Throughout the study period, intact AAA and LEAD surgery incidence was most frequent in the population registered with very severe CCI, whereas ruptured AAA incidence was highest in the population with modest CCI (table 1).

10.2.2 Patient characteristics

Table 2 presents the baseline characteristics of the patients. Noticeably, 50% of patients had cardio- or cerebrovascular disease at baseline. AAA and LEAD patient shared several risk factors. Diabetes mellitus, however, was considerably more prevalent in LEAD (25 %) compared to AAA (9-11 %).

From calendar period 1996-1999 to 2015-2018, the mean age increased by 3.4 years in intact AAA and 2.4 years in ruptured AAA surgery patients, resp.

Figure 3 (see Paper I) presents the time-trend in the comorbidity of AAA patients and the Danish Population, standardized to the age- and sex-distribution of the Danish population aged 41-99 years in 2000. The annual CCI increase was by 0.9 % in patients presenting for intact (95% CI, 0.7-1.1%) and by 0.9 % in ruptured (95% CI, 0.5-1.3%) AAA repair, compared to 2.4 % in the Danish population (CI 95%, 2.4-2.4%).

10.2.3 Mortality

Comparing calendar period 2015 – 2018 to 1996 – 1999, the adjusted 5-year mortality rate reduced by 49 % following intact- and by 54 % following ruptured AAA repair (MRR_{intact}, 0.51; 95% CI, 0.44 – 0.59 *vs.* $MRR_{ruptured}$, 0.46; 95 % CI, 0.39 – 0.54), table 3. Increasing age and CCI explained the difference between crude and adjusted MRR for both ruptured and intact AAA.

The sex-, age- and CCI-adjusted mortality rate was also reduced in the Danish population aged 41-99 in the same period (MRR, 0.56; 95 % CI, 0.56-0.56).

In calendar period 2015-2018, the 90-day mortality risk was 26.4% (95% CI: 22.8 - 29.8%) following ruptured and 3.4% (95% CI: 2.7 - 4.2%) following intact AAA repair.

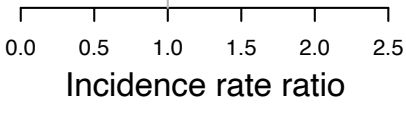
10.3 Conclusion

The AAA repair incidence is decreasing in Denmark due to reduction among males and a shift in presentation to an older population. Comorbidity increased independently of age during the study period. Both crude and adjusted mortality was markedly reduced. Thus, the reason(s) for the mortality reduction is not fully elucidated.

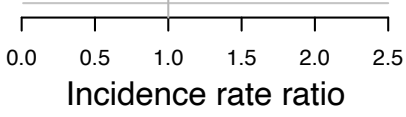
10.3.1 Table 1. Incidence rate of Intact (top) and Ruptured (middle) AAA repair, and LEAD (bottom) table, Study I.

Intact AAA	Cases	Person–Years	Crude IR per 100,000	IRR (95% CI)
Calendar year				
1996 – 1999	1,529	9,800,052	15.6 (14.8–16.4)	Reference
2000 – 2004	1,824	12,697,911	14.4 (13.7–15.0)	0.85 (0.79–0.91)
2005 – 2009	2,499	13,392,843	18.7 (17.9–19.4)	0.99 (0.93–1.06)
2010 – 2014	2,792	14,075,863	19.8 (19.1–20.6)	0.93 (0.88–0.99)
2015 – 2018	2,243	11,712,246	19.2 (18.4–19.9)	0.80 (0.75–0.85)
Age, years				
41 – 50	75	18,192,142	0.4 (0.3–0.5)	0.03 (0.03–0.04)
51 – 60	849	16,844,751	5.0 (4.7–5.4)	0.26 (0.25–0.28)
61 – 70	3,961	13,398,250	29.6 (28.6–30.5)	Reference
71 – 80	5,076	8,678,501	58.5 (56.9–60.1)	1.49 (1.43–1.55)
81 – 90	918	3,947,749	23.3 (21.7–24.8)	0.56 (0.52–0.60)
91 – 99	8	617,522	1.3 (0.4–2.2)	0.04 (0.02–0.08)
Sex				
Male	9,072	29,731,151	30.5 (29.9–31.1)	Reference
Female	1,815	31,947,764	5.7 (5.4–5.9)	0.19 (0.18–0.20)
CCI group				
Normal	589	43,644,149	1.3 (1.2–1.5)	0.04 (0.04–0.05)
Moderate	3,972	8,162,109	48.7 (47.2–50.2)	Reference
Severe	2,687	5,332,614	50.4 (48.5–52.3)	0.97 (0.92–1.02)
Very Severe	3,639	4,540,043	80.2 (77.5–82.8)	1.29 (1.23–1.35)

Ruptured AAA	Cases	Person–Years	Crude IR per 100,000	IRR (95% CI)
Calendar year				
1996 – 1999	880	9,800,052	9.0 (8.4–9.6)	Reference
2000 – 2004	1,114	12,697,911	8.8 (8.3–9.3)	0.96 (0.88–1.05)
2005 – 2009	1,000	13,392,843	7.5 (7.0–7.9)	0.78 (0.71–0.85)
2010 – 2014	907	14,075,863	6.4 (6.0–6.9)	0.63 (0.57–0.69)
2015 – 2018	607	11,712,246	5.2 (4.8–5.6)	0.46 (0.42–0.51)
Age, years				
41 – 50	26	18,192,142	0.1 (0.1–0.2)	0.02 (0.01–0.02)
51 – 60	293	16,844,751	1.7 (1.5–1.9)	0.17 (0.15–0.20)
61 – 70	1,395	13,398,250	10.4 (9.9–11.0)	Reference
71 – 80	2,088	8,678,501	24.1 (23.0–25.1)	2.24 (2.09–2.40)
81 – 90	692	3,947,749	17.5 (16.2–18.8)	1.80 (1.64–1.97)
91 – 99	14	617,522	2.3 (1.1–3.5)	0.31 (0.18–0.52)
Sex				
Male	3,923	29,731,151	13.2 (12.8–13.6)	Reference
Female	585	31,947,764	1.8 (1.7–2.0)	0.12 (0.11–0.13)
CCI group				
Normal	1,727	43,644,149	4.0 (3.8–4.1)	0.46 (0.43–0.49)
Moderate	1,278	8,162,109	15.7 (14.8–16.5)	Reference
Severe	746	5,332,614	14.0 (13.0–15.0)	0.81 (0.74–0.89)
Very Severe	757	4,540,043	16.7 (15.5–17.9)	0.77 (0.71–0.85)



LEAD	Cases	Person–Years	Crude IR per 100,000	IRR (95% CI)
Calendar year				
1996 – 1999	6,517	9,800,052	66.5 (64.9–68.1)	Reference
2000 – 2004	8,369	12,697,911	65.9 (64.5–67.3)	0.88 (0.86–0.91)
2005 – 2009	9,621	13,392,843	71.8 (70.4–73.3)	0.87 (0.84–0.89)
2010 – 2014	9,823	14,075,863	69.8 (68.4–71.2)	0.75 (0.73–0.78)
2015 – 2018	7,109	11,712,246	60.7 (59.3–62.1)	0.60 (0.58–0.62)
Age, years				
41 – 50	1,987	18,192,142	10.9 (10.4–11.4)	0.27 (0.25–0.28)
51 – 60	7,185	16,844,751	42.7 (41.7–43.6)	0.67 (0.65–0.69)
61 – 70	13,501	13,398,250	100.8 (99.1–102.5)	Reference
71 – 80	13,040	8,678,501	150.3 (147.7–152.8)	1.04 (1.02–1.07)
81 – 90	5,274	3,947,749	133.6 (130.0–137.2)	0.76 (0.74–0.79)
91 – 99	452	617,522	73.2 (66.4–79.9)	0.42 (0.38–0.46)
Sex				
Male	22,802	29,731,151	76.7 (75.7–77.7)	Reference
Female	18,637	31,947,764	58.3 (57.5–59.2)	0.78 (0.77–0.80)
CCI group				
Normal	2,464	43,644,149	5.6 (5.4–5.9)	0.04 (0.04–0.04)
Moderate	14,345	8,162,109	175.8 (172.9–178.6)	Reference
Severe	8,450	5,332,614	158.5 (155.1–161.8)	0.86 (0.84–0.89)
Very Severe	16,180	4,540,043	356.4 (350.9–361.9)	1.87 (1.82–1.91)



Note: The incidence ratios ratios are computed from a Poisson regression model including the covariates calendar year, age, sex, and CCI group offset by the logarithm of person-years at risk. **Abbreviations**: CCI, Charlson's Comorbidity Index; IR, Incidence Rate; IRR, Incidence Rate Ratio; CI, Confidence Interval.

10.3.2 Table 2. Baseline characteristics of patients undergoing surgery for lower extremity artery disease in Denmark 1996-2018, Study I.

	Intact AAA (n=10,887)	Ruptured AAA (n=4,508)	First-time LEAD (n=41,439)
Age – mean (sd)	71 (7)	73 (8)	69 (10)
Male sex – no. (%)	9,072 (83)	3,923 (87)	22,802 (55)
CCI group – no. (%)			
Normal	589 (5)	1,727 (38)	2,464 (6)
Moderate	3,972 (36)	1,278 (28)	14,345 (35)
Severe	2,687 (25)	746 (17)	8,450 (20)
Very Severe	3,639 (33)	757 (17)	16,180 (39)
Chronic pulmonary disease – no. (%)	2,445 (22)	1,053 (23)	9,391 (23)
Diabetes – no. (%)	1,225 (11)	425 (9)	10,527 (25)
Renal disease – no. (%)	368 (3)	108 (2)	1,712 (4)
Cardiovascular disease – no. (%)			

Any	5,554 (51)	2,109 (47)	19,263 (46)
Angina history	2,680 (25)	823 (18)	7,936 (19)
Stroke or TCI	1,718 (16)	731 (16)	7,088 (17)
CABG or PCI	1,954 (18)	531 (12)	5,381 (13)
Congestive heart failure	870 (8)	355 (8)	4,598 (11)
Heart valve disease	548 (5)	170 (4)	2,040 (5)
Acute myocardial infarction	2,139 (20)	782 (17)	6,047 (15)
Pacemaker or ICD	446 (4)	119 (3)	1,552 (4)
Other	822 (8)	381 (8)	3,321 (8)
Cancer history – no. (%)	1,841 (17)	499 (11)	5,536 (13)
Care dependency – no. (%)			
Independent	10,131 (93)	3,572 (79)	33,397 (81)
Home care	547 (5)	317 (7)	6,190 (15)
Nursing home & "other"	28 (0)	88 (2)	862 (2)
missing	181 (2)	531 (12)	990 (2)
Tobacco use – no. (%)			
None	1,790 (16)	705 (16)	5,855 (14)
Previous (>6 weeks)	4,225 (39)	907 (20)	14,062 (34)
Current smoker	4,527 (42)	1,514 (34)	20,002 (48)
Missing	345 (3)	1,382 (31)	1,520 (4)
Alcohol abuse – no. (%)			
None	10,022 (92)	3,628 (80)	37,487 (90)
More than > 5 units/day	216 (2)	131 (3)	1,076 (3)
Missing	649 (6)	749 (17)	2,876 (7)
Priority – no. (%)			
Elective	8,943 (82)	58 (1)	35,440 (86)
Subacute	996 (9)	341 (8)	3,757 (9)
Acute	569 (5)	3,928 (87)	610 (1)
missing	379 (3)	181 (4)	1,632 (4)
Technique – no. (%)			
Open	8,209 (76)	4,400 (98)	19,634 (47)
Endovascular	2,663 (24)	106 (2)	20,801 (50)
Hybrid	0 (0)	0 (0)	1,004 (2)

Note: Data source for Charlson's comorbidity (CCI) index was the Danish National Patient Registry. Other disease items were the union set of data in the DNPR and the DVR. Dependency, tobacco and alcohol history was based on the DVR. Other cardiac disease included: cardiomyopathy (DNPR); unspecified cardiac surgery history with no

current symptoms (DVR); AMI< 6 weeks, unstable angina or congestive heart failure (DVR); AMI> 6 weeks or asymptomatic arrythmia (DVR), stabile angina or heart medication (DVR). Full list of ICD codes uses from the DNPR is available in the supplementary digital content as well as a full baseline table of the 19 CCI items. When excluding non-melanoma skin cancer, the prevalence of patients with a history of cancer was 14 % in intact AAA, 8 % in ruptured AAA and 11 % in LEAD surgery.

4,686 (11 %) of open LEAD repair were aorto-iliac reconstructions, 20,801 (50 %) were PTA, and 472 (1 %) were axillo-bifemoral bypass.

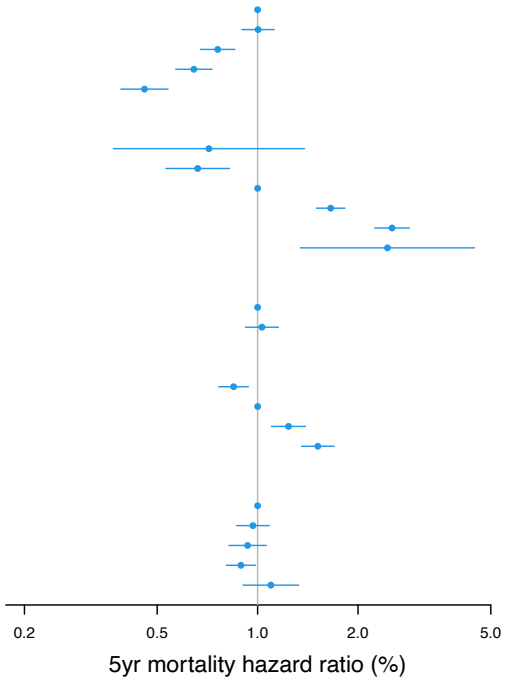
10.3.3 Table 3. Five-year mortality following AAA surgery in Denmark 1996-2018, Study I.

Calendar period of surgery	No. of deaths	No. of patients	Mortality risk % (95 % CI)	Mortality rate ratio (95% CI)	
				Crude	Adjusted[a]
Intact AAA					
1996 - 1999	539	1,529	35.3 (32.8 - 37.6)	Reference	Reference
2000 - 2004	584	1,824	32.0 (29.8 - 34.1)	0.88 (0.79 - 0.99)	0.84 (0.74 - 0.94)
2005 - 2009	741	2,499	29.7 (27.8 - 31.4)	0.80 (0.72 - 0.90)	0.67 (0.60 - 0.75)
2010 - 2014	678	2,792	24.3 (22.7 - 25.9)	0.63 (0.56 - 0.71)	0.51 (0.46 - 0.58)
2015 - 2018	337	2,243	24.7 (22.4 - 27.0)	0.65 (0.56 - 0.74)	0.51 (0.44 - 0.59)
Ruptured AAA					
1996 - 1999	555	880	63.1 (59.7 - 66.1)	Reference	Reference
2000 - 2004	716	1,114	64.3 (61.3 - 67.0)	1.04 (0.93 - 1.16)	1.00 (0.90 - 1.12)
2005 - 2009	550	1,000	55.0 (51.8 - 58.0)	0.81 (0.72 - 0.91)	0.76 (0.67 - 0.86)
2010 - 2014	474	907	52.3 (48.9 - 55.4)	0.71 (0.63 - 0.81)	0.64 (0.57 - 0.73)
2015 - 2018	207	607	39.7 (35.4 - 43.8)	0.50 (0.43 - 0.59)	0.46 (0.39 - 0.54)

Note: [a]Adjusted for age, sex, Charlson's comorbidity index, surgical center in a Cox regression stratified for open versus endovascular repair.

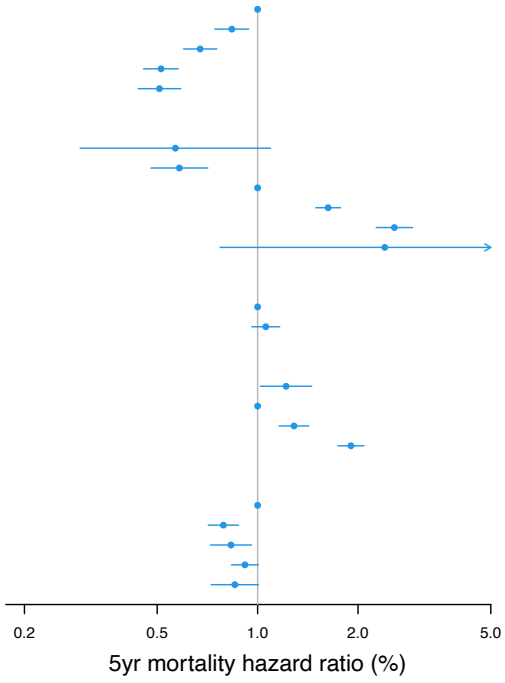
10.3.4 eTable 3a. Ruptured AAA, Study I.

Ruptured AAA	No. Dead	No. Patients	Mortality risk (%)	Crude HR (95% CI)	Adj. HR (95% CI)
Year					
1996 – 1999	555	880	63.1 (59.7 – 66.1)	Reference	Reference
2000 – 2004	716	1,114	64.3 (61.3 – 67.0)	1.04 (0.93 – 1.16)	1.00 (0.90 – 1.12)
2005 – 2009	550	1,000	55.0 (51.8 – 58.0)	0.81 (0.72 – 0.91)	0.76 (0.67 – 0.86)
2010 – 2014	474	907	52.3 (48.9 – 55.4)	0.71 (0.63 – 0.81)	0.64 (0.57 – 0.73)
2015 – 2018	207	607	39.4 (34.0 – 44.4)	0.50 (0.43 – 0.59)	0.46 (0.39 – 0.54)
Age, years					
41 – 50	9	26	35.4 (13.8 – 51.6)	0.74 (0.38 – 1.42)	0.71 (0.37 – 1.38)
51 – 60	93	293	32.3 (26.7 – 37.5)	0.68 (0.55 – 0.85)	0.66 (0.53 – 0.82)
61 – 70	594	1,395	43.6 (40.9 – 46.2)	Reference	Reference
71 – 80	1,267	2,088	62.4 (60.2 – 64.4)	1.69 (1.53 – 1.86)	1.66 (1.50 – 1.83)
81 – 90	528	692	78.2 (74.7 – 81.2)	2.59 (2.30 – 2.91)	2.53 (2.24 – 2.85)
91 – 99	11	14	81.0 (39.7 – 94.0)	2.45 (1.35 – 4.45)	2.45 (1.34 – 4.47)
Sex					
Male	2,148	3,923	56.1 (54.4 – 57.6)	Reference	Reference
Female	354	585	62.1 (57.8 – 65.9)	1.17 (1.05 – 1.31)	1.03 (0.92 – 1.15)
CCI group					
Normal	791	1,727	46.9 (44.5 – 49.3)	0.80 (0.72 – 0.88)	0.85 (0.76 – 0.94)
Moderate	695	1,278	55.5 (52.6 – 58.2)	Reference	Reference
Severe	467	746	64.3 (60.6 – 67.7)	1.25 (1.11 – 1.41)	1.24 (1.10 – 1.39)
Very Severe	549	757	74.4 (70.9 – 77.4)	1.54 (1.38 – 1.72)	1.52 (1.35 – 1.70)
Region					
Capital	894	1,527	59.5 (56.9 – 61.9)	Reference	Reference
Central	463	857	55.1 (51.6 – 58.4)	0.91 (0.82 – 1.02)	0.97 (0.87 – 1.08)
Northern	315	559	57.8 (53.4 – 61.8)	0.95 (0.84 – 1.08)	0.93 (0.82 – 1.06)
Southern	710	1,380	53.4 (50.6 – 56.1)	0.83 (0.76 – 0.92)	0.89 (0.81 – 0.98)
Zealand	120	185	65.0 (57.4 – 71.2)	1.15 (0.95 – 1.40)	1.10 (0.90 – 1.33)



10.3.5 eTable 3b. Intact AAA, Study I

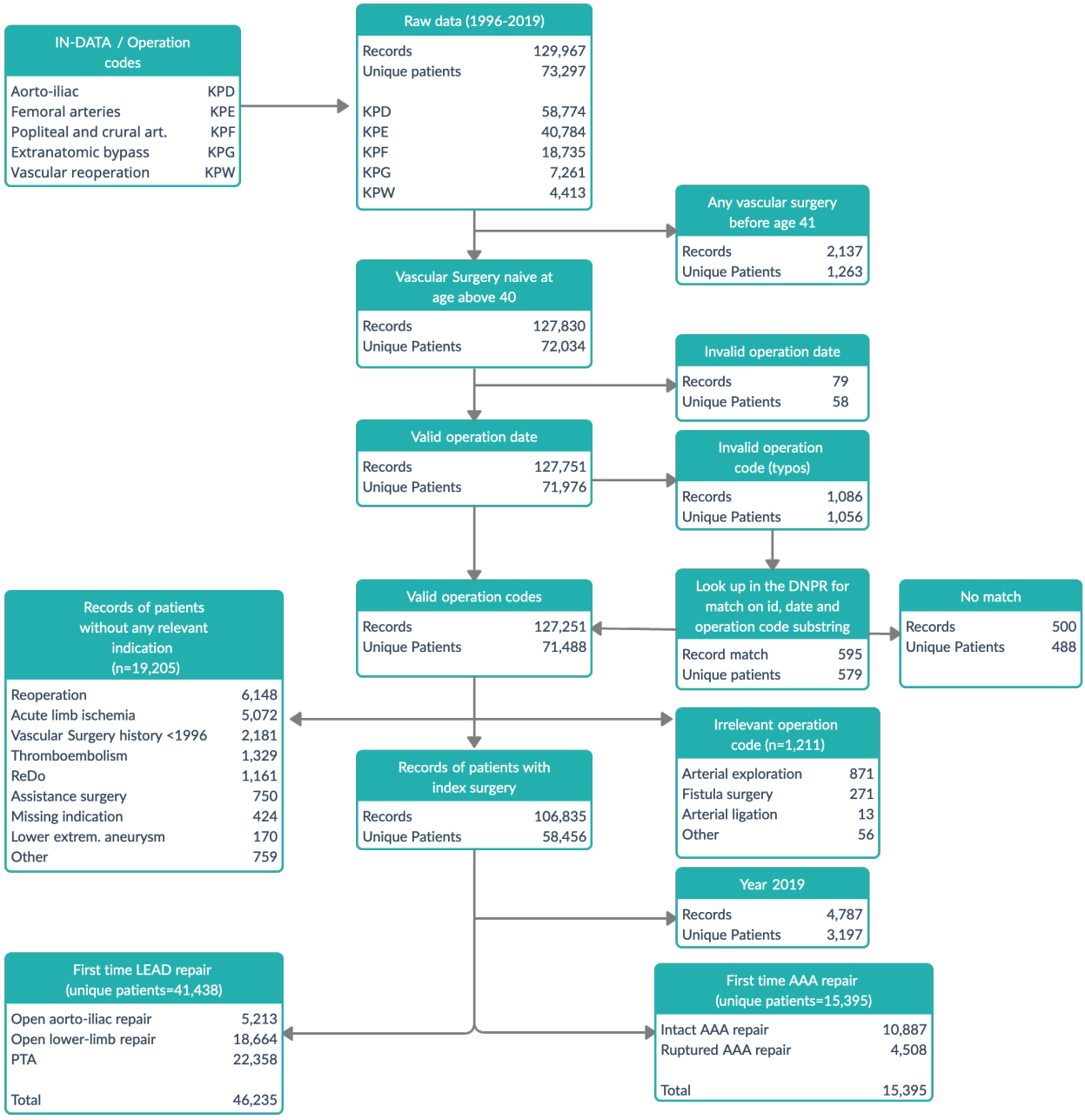
Intact AAA	No. Dead	No. Patients	Mortality risk (%)	Crude HR (95% CI)	Adj. HR (95% CI)
Year					
1996 – 1999	539	1,529	35.3 (32.8 – 37.6)	Reference	Reference
2000 – 2004	584	1,824	32.0 (29.8 – 34.1)	0.88 (0.79 – 0.99)	0.84 (0.74 – 0.94)
2005 – 2009	741	2,499	29.7 (27.8 – 31.4)	0.80 (0.72 – 0.90)	0.67 (0.60 – 0.75)
2010 – 2014	678	2,792	24.3 (22.7 – 25.9)	0.63 (0.56 – 0.71)	0.51 (0.46 – 0.58)
2015 – 2018	337	2,243	23.3 (20.5 – 26.0)	0.65 (0.56 – 0.74)	0.51 (0.44 – 0.59)
Age, years					
41 – 50	<5	–––	12.8 (4.6 – 20.2)	0.55 (0.28 – 1.06)	0.57 (0.29 – 1.09)
51 – 60	114	849	14.3 (11.8 – 16.7)	0.61 (0.50 – 0.74)	0.58 (0.48 – 0.71)
61 – 70	825	3,961	22.3 (20.9 – 23.6)	Reference	Reference
71 – 80	1,557	5,076	34.1 (32.6 – 35.4)	1.64 (1.51 – 1.78)	1.63 (1.49 – 1.77)
81 – 90	371	918	45.9 (42.2 – 49.3)	2.47 (2.18 – 2.79)	2.57 (2.27 – 2.91)
91 – 99	<5	–––	53.3 (0.0 – 81.9)	2.24 (0.72 – 6.95)	2.41 (0.77 – 7.50)
Sex					
Male	2,366	9,072	28.6 (27.6 – 29.5)	Reference	Reference
Female	513	1,815	30.4 (28.1 – 32.6)	1.11 (1.01 – 1.22)	1.06 (0.96 – 1.17)
CCI group					
Normal	146	589	25.7 (22.0 – 29.2)	1.21 (1.01 – 1.44)	1.22 (1.02 – 1.45)
Moderate	808	3,972	22.1 (20.8 – 23.5)	Reference	Reference
Severe	677	2,687	27.6 (25.8 – 29.4)	1.30 (1.17 – 1.43)	1.29 (1.16 – 1.42)
Very Severe	1,248	3,639	37.9 (36.2 – 39.6)	1.92 (1.75 – 2.09)	1.90 (1.74 – 2.08)
Region					
Capital	1,013	3,364	32.4 (30.7 – 34.1)	Reference	Reference
Central	564	2,454	24.7 (22.9 – 26.5)	0.74 (0.67 – 0.82)	0.79 (0.71 – 0.88)
Northern	256	1,044	27.0 (24.1 – 29.8)	0.82 (0.72 – 0.94)	0.83 (0.72 – 0.96)
Southern	869	3,297	29.5 (27.8 – 31.2)	0.90 (0.82 – 0.98)	0.92 (0.84 – 1.01)
Zealand	177	728	26.1 (22.7 – 29.3)	0.80 (0.68 – 0.93)	0.85 (0.73 – 1.01)



10.3.6 eTable 3c, 90-day mortality, 2015-2018 vs. 1996-1999

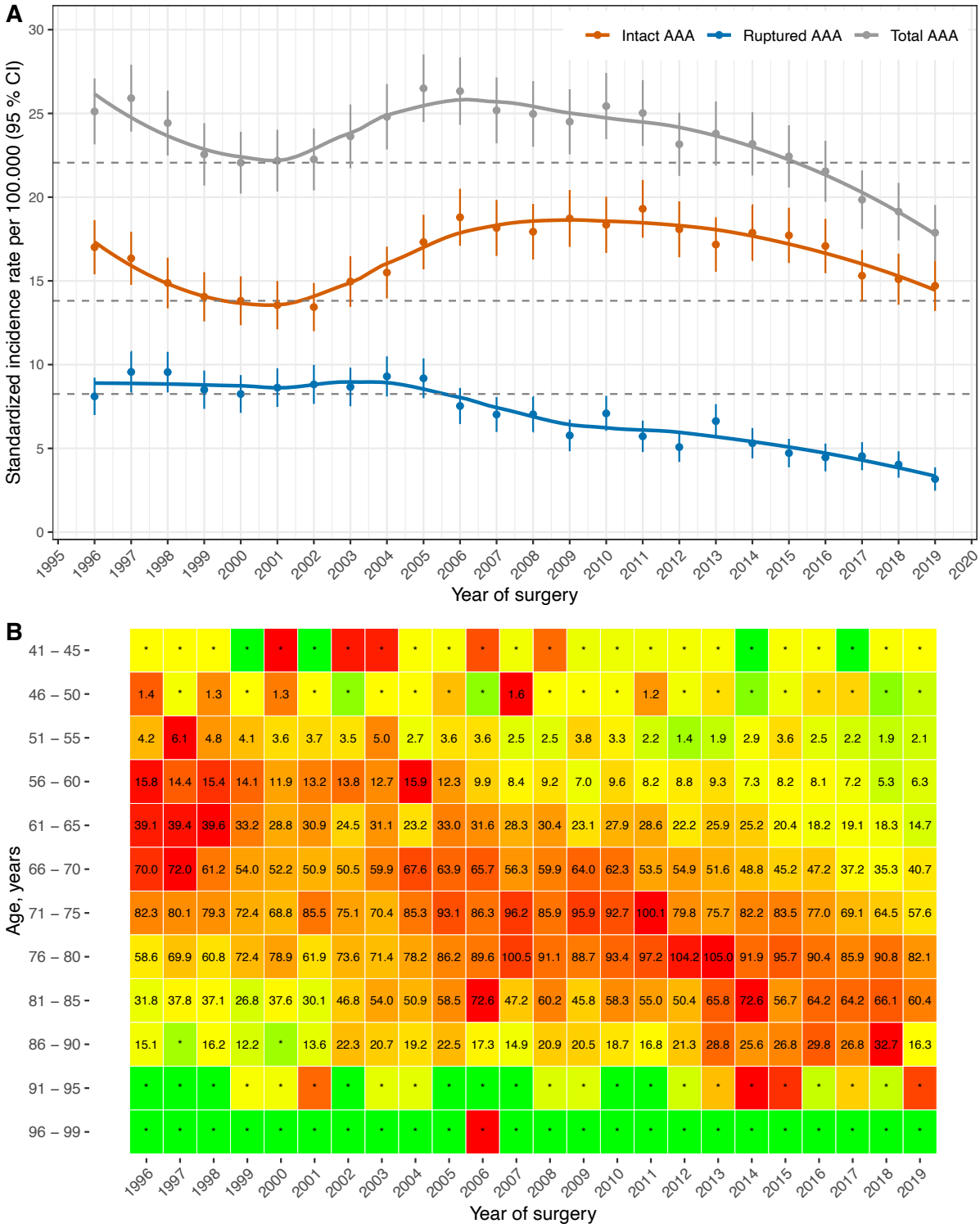
Surgery	No_dead	No_patients	Mortality risk (%)	Crude HR (95% CI)	Adj. HR (95% CI)
Ruptured AAA	160	607	26.4 (22.8 - 29.8)	0.49 (0.41 - 0.59)	0.46 (0.38 - 0.55)
Intact AAA	77	2,243	3.4 (2.7 - 4.2)	0.39 (0.29 - 0.52)	0.43 (0.32 - 0.57)
Open Aorto-iliac	19	462	4.1 (2.3 - 5.9)	0.64 (0.39 - 1.05)	0.57 (0.34 - 0.93)
Open Peripheral	134	3,151	4.3 (3.5 - 5.0)	0.66 (0.53 - 0.82)	0.57 (0.46 - 0.72)
PTA	229	4,679	4.9 (4.3 - 5.5)	1.55 (1.18 - 2.04)	0.93 (0.70 - 1.23)

10.3.7 eFigure D. Flow chart, (efigure 0 in supplemental material of Study I).

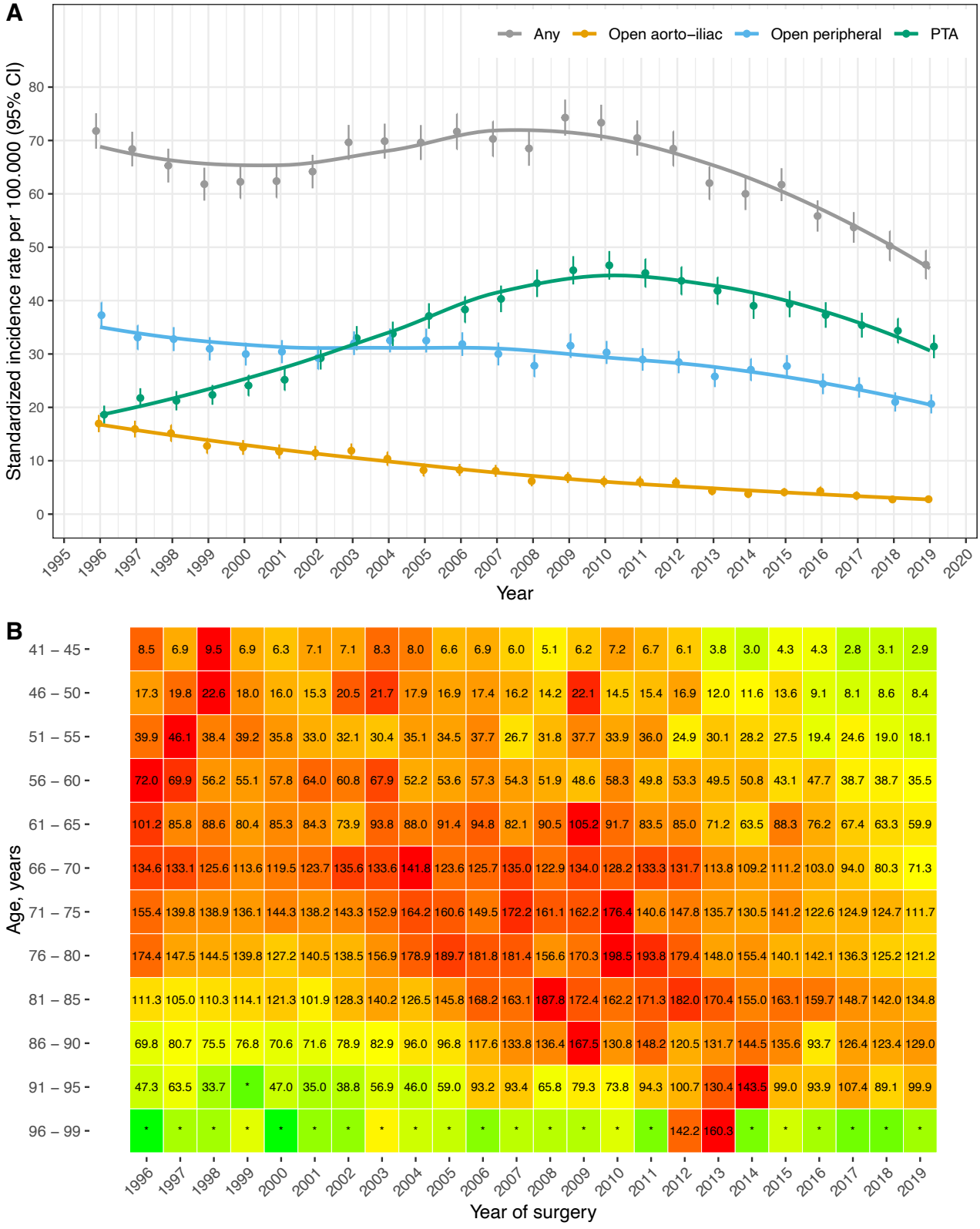


Note: the paper on Danish trends in first time LEAD repair is not a part of this thesis.

10.3.8 Figure 1. Standardized incidence rate (A) and age specific incidence (B) for AAA surgery in Denmark 1996-2019, Study I.



10.3.9 Figure 1b. Standardized incidence rate (A) and age specific incidence (B) for first-time LEAD surgery in Denmark 1996-2019



11 Study II: The transfusion in vascular surgery (TV) trial, protocol and main results

11.1 Methods

11.1.1 Trial Design

The Transfusion in Vascular surgery (TV) trial was an investigator-initiated, single-centre, open-label, randomized clinical trial (RCT) with web-based randomization. The trial protocol was approved by the Regional Scientific Ethical Committee of Region Zealand, Denmark (project-ID: SJ-426), the Danish Data Protection Agency, and was registered at www.clinicaltrials.gov (identifier: NCT02465125) before enrolment of the first patient. The protocol including the detailed Statistical Analysis Plan (SAP) adhere to the *The Standard Protocol Items: Recommendations for Interventional Trials* (SPIRIT)-guideline and was published in a peer-review journal before the trial database was established[48].

11.1.2 Patients

Potential participants were invited to participate ahead of surgery if they fulfilled 1 and 2 of the following. Randomization was performed on Hb decline below 9.7 g/dL (6 mmol/L), criteria 3.

Inclusion criteria

1. Age > 40 years AND
2. Scheduled for open AAA repair or lower limb bypass (infra-inguinal bypass or femoro-femoral cross-over surgery) AND
3. Hb < 9.7 g/dL

Exclusion criteria

1. RBC transfusion refusal OR
2. Previous severe adverse reaction to RBC transfusion OR
3. Unable to understand the benefit and risks of testing OR
4. Previously randomized into the TV trial

The participant was excluded if the Hb remained at or above 9.7 g/dL for 30 days following surgery.

11.1.3 Randomization and blinding

The randomization system was centralized and set up by The Copenhagen Trial Unit, Rigshospitalet, Denmark. The allocation sequence was computer-generated in a 1:1 ratio and stratified for open AAA *vs.* lower limb bypass repair with fixed block-sizes of six.

The patients, trial statisticians, and outcome assessor were blinding to the allocated treatment.

11.1.4 Intervention

Randomization and implementation of trial group assignment occurred immediately following Hb drop below 9.7 g/dL to one of the following two Hb thresholds for RBC transfusion:

1. experimental intervention: Hb < 8 g/dL ~ "low trigger"
2. control intervention: Hb < 9.7 g/dL ~ "high trigger"

corresponding to the 2014 Danish Health Authority guidelines for patients with cardiovascular disease (5 mmol/L) *vs.* 2014 Danish anaesthesia practice for patients undergoing vascular surgery (6 mmol/L), respectively. Protocol specifics for anaesthesia and fluid therapy is described in Study III.

11.1.5 Outcome measures

Primary outcome

1) Postoperative Hb day 0-15 following surgery

Secondary outcomes

1) Units of RBCs transfused
2) Randomization rate
3) Proportion of patients with protocol suspension
4) Adherence to transfusion trigger allocation
5) Intraoperative tissue oxygenation as determined by Near-infrared spectroscopy (NIRS)
   a) Lowest tissue oxygenation saturation of the frontal-lobe (ScO_2) and muscle (SmO_2) before an RBC transfusion (%)
   b) Desaturation load, defined as the cumulative area under baseline (minutes x %)
6) Serious adverse events at day 30

Exploratory outcome measures at day 90

1) Death

2) Major vascular complications (renal replacement therapy, stroke, acute myocardial infarction, serious adverse transfusion reaction, vascular reoperation or amputation)
3) Days alive and out of hospital within 90 days

11.1.6 Statistical methods

Statistical Analyses

All analyses *i)* adhere to the published SAP, *ii)* were based on the intention to treat population, *ii)* were adjusted for age, the stratum (type of operation) and, when relevant, the baseline value of the outcome.[49] The primary outcome was longitudinal and analysed using Generalized Estimating Equations (GEE) and the mixed model. Continuous outcomes were analysed with linear regression. Count data were analysed using Van Elteren's test (adjusted for the stratum). We used logistic regression analysis for binary outcomes and Cox regression for major-vascular-complication-free-survival analysis with right censoring 90 days following randomisation of the last patient, March 8th, 2017.

11.2 Results

Fifty-eight patients were randomized between July 2015 and December 2016. The patient flow chart is presented in Figure 1. Patient characteristics were comparable at baseline as shown in table 1. The mean Hb from dat 0 to 15 was lower in the low trigger group compared with the high trigger (mean difference, -0.91 g/dL; 95% CI, -1.21 to -0.61; $P_{GEE} = 0.022$; $P_{mixed\text{-}model} < 0.001$; Table 2). The Hb separation was pronounced on arrival in the recovery room, day 0, (mean difference, -1.35 g/dL; P < 0.001) and at 30 days following surgery (mean difference, -1.07 g/dL; P = 0.004). Also, units of RBCs transfused were reduced with the low trigger strategy (median [IQR], 1 [0-2] *vs.* 3 [2-6]; P = 0.002). Any non-adherence occurred in 28 % in patients allocated to the low trigger vs. 34 % of patients in the high trigger. While non-adherent RBC transfusion was more common in the low trigger, failure to transfuse was most common in the high trigger.

The cerebral desaturation load was more pronounced with the low trigger strategy (median [IQR], 421 [42-888] *vs.* 127 [11-331] minute x %; P = 0.004). We observed no trial group difference in nadir ScO_2, SmO_2, or muscle desaturation load during surgery.

Figure 4 shows the major vascular complication-free survival and relative risk for death or major vascular complications at 90 days. Death or vascular complication occurred in 19/29 (66%) of patients in the low trigger *vs.* 8/29 (28%) of patients in the high trigger group at

90 days (HR, 3.20, P = 0.005). Days alive out of hospital within 90 days following surgery corroborated this finding (median [IQR], 76 [67-82] days *vs.* 82 [76-84] days; P = .049).

11.3 Conclusion

This feasibility trial demonstrated that a protocol restricting RBC transfusion to an Hb below 8 g/dL, as compared with 9.7 g/dL, significantly reduced postoperative Hb levels, units of RBCs transfused, and increased the cerebral desaturation load during surgery. The exploratory outcomes were indicative of potential patient harm with the low trigger strategy and warrants further trials in the vascular surgery population before a restrictive RBC transfusion can be relied upon as best practice.

11.3.1 Figure 1. Trial flow chart, Study II.

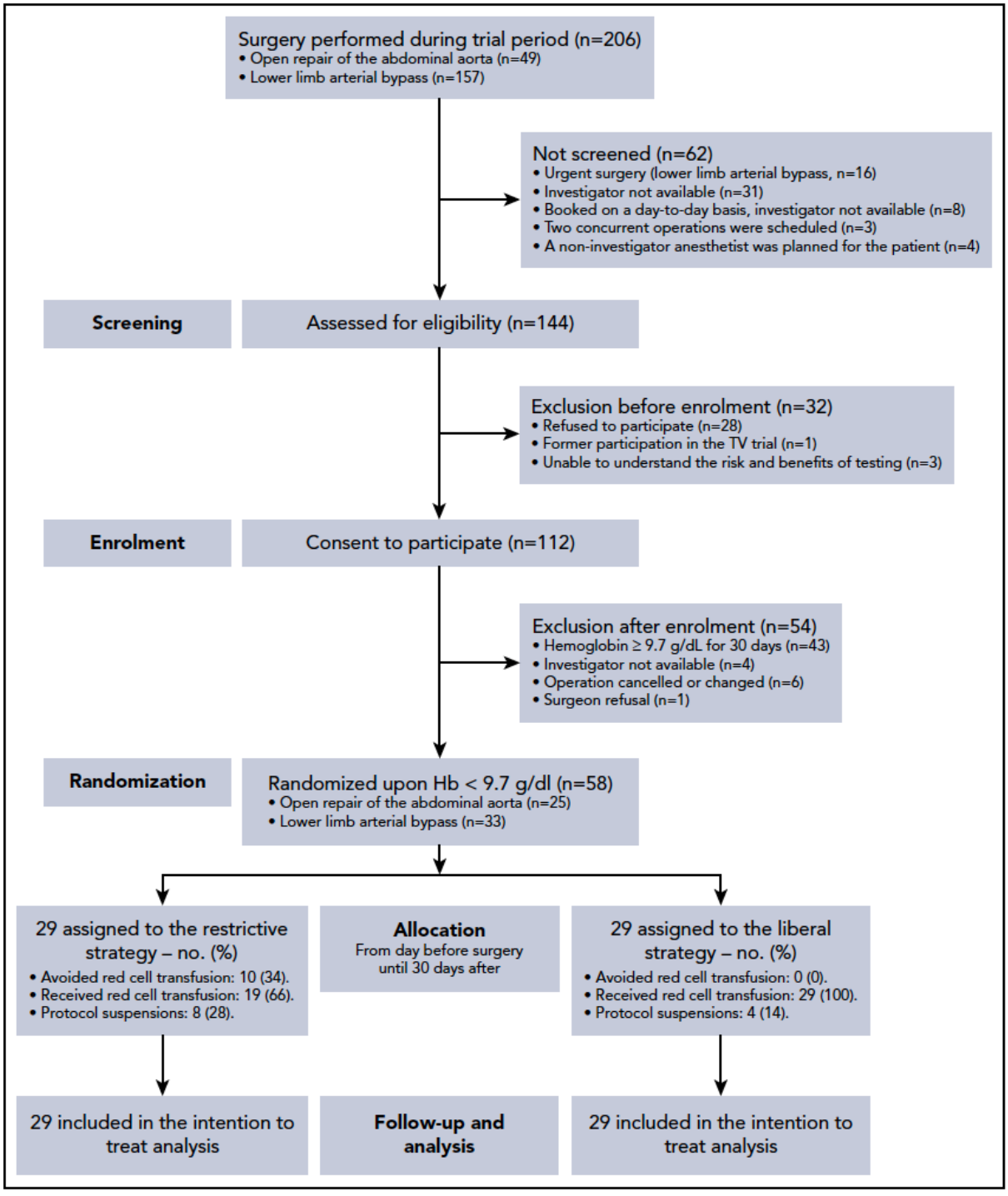


Figure 1. Screening, enrolment, randomization, and follow-up. Randomization occurred in 46 (79%) patients before end of surgery, in 53 patients (91%) within or on the second postoperative day, and in the remaining 5 patients on the third, fourth, sixth, ninth, and fifteenth day.

11.3.2 Table 1. Patients characteristics at baseline, Study II.

Table 1. Patient characteristics

	Trigger < 8 g/dL (n = 29)	Trigger < 9.7 g/dL (n = 29)
Age, mean ± SD, y	71.3 ± 9.4	73.7 ± 7.3
Male sex, n (%)	19 (65.5)	18 (62.1)
BMI, mean ± SD, kg/m²	25.3 ± 5.8	24.8 ± 3.8
Operation, n (%)		
Lower limb bypass	17 (58.6)	16 (55.2)
Abdominal aortic aneurysm*	12 (41.4)	13 (44.8)
Time of randomization, n (%)		
The day before surgery	2 (6.9)	3 (10.3)
During surgery	20 (69.0)	21 (72.4)
After end of surgery	7 (24.1)	5 (17.2)
Any cardiovascular disease, n (%)	26 (89.7)	23 (79.3)
Lower extremity artery disease	20 (69.0)	17 (58.6)
Claudication	4 (13.8)	1 (3.4)
Pain at rest	4 (13.8)	5 (17.2)
Wound/gangrene	12 (41.4)	11 (37.9)
Angina	3 (10.3)	5 (17.2)
Stroke or TIA	8 (27.6)	5 (17.2)
Previous CABG or PCI	3 (10.3)	5 (17.2)
Congestive heart failure	1 (3.4)	2 (6.9)
Heart valve disease	3 (10.3)	4 (13.8)
Acute myocardial infarction	0 (0.0)	6 (20.7)
Pacemaker	0 (0.0)	0 (0.0)
Any cardiovascular risk factor, n (%)	29 (100.0)	28 (96.6)
Arterial hypertension	19 (65.5)	22 (75.9)
Smoker	12 (41.4)	11 (37.9)
COPD	6 (20.7)	8 (27.6)
Hypercholesterolemia	22 (75.9)	26 (89.7)
Chronic renal failure	3 (10.3)	6 (20.7)
Diabetes mellitus	8 (27.6)	6 (20.7)
ASA score, mean ± SD	3.0 ± 0.27	2.9 ± 0.41
ASA class, n (%)		
ASA1	0 (0.0)	0 (0.0)
ASA2	1 (3.4)	4 (13.8)
ASA3	27 (93.1)	24 (82.8)
ASA4	1 (3.4)	1 (3.4)
Preoperative Hb, mean ± SD, g/dL	12.3 ± 1.8	12.4 ± 2.2
Preoperative creatinine, median (IQR), µmol/L	73 (62 to 98)	87 (66 to 108)
NIRS at baseline†, mean ± SD		
Regional cerebral oxygenation, %	60.8 ± 9.6	59.5 ± 9.1
Regional biceps muscle oxygenation, %	69.8 ± 8.9	68.2 ± 9.8

There was no between-group difference in race, as all patients were white. The only statistically significant baseline difference between groups was previous AMI ($P = .023$).

ASA, American Society of Anesthesiologists; BMI, body mass index; CABG, coronary artery bypass grafting; COPD, chronic obstructive pulmonary disease; PCI, percutaneous coronary intervention; TIA, transient ischemic attack.

*One patient in the AAA-stratum of the high-trigger (< 9.7 g/dL) group underwent aorto-iliac bifurcated prosthesis surgery on the indication aorto-iliac occlusive disease. Otherwise, all patients in the AAA-stratum had aneurysmal disease.

†Baseline was defined as the point at which the patient was normovolemic according to the goal-directed fluid strategy after anesthesia was induced and before starting surgery. The NIRS readings before O_2-supplementation and induction of anesthesia were similar to this baseline (supplemental Table 3.0).

11.3.3 Table 2. Hb levels, red cell units transfused and adherence, Study II.

Table 2. Hb, red cell transfusions and adherence

	Trigger<8 g/dL (n = 29)	Trigger<9.7 g/dL (n = 29)	Treatment effect (95% CI)	P
Hb (day 0 – 15)				
Grand mean, g/dl and GEE coefficient*	9.46	10.33	GEE coefficient: −0.45 (−0.84 to −0.07)	.022
Modeled mean, g/dL†	9.70	10.61	−0.91 (−1.21 to −0.61)	<.001
Hb in recovery room (day 0)‡				
Mean, g/dl (n = 46)†	9.17	10.51	−1.35 (−1.74 to −0.95)	<.001
Hb at follow-up (day 30)				
Mean, g/dL (n = 45)†	10.75	11.83	−1.07 (−1.79 to −0.36)	.004
Hb below lowest trigger, n (%)				
≥1 Hb measurement below 8 g/dL	18 (62)	4 (14)	48% (27% to 70%)	<.001
Red cell transfusions				
Median no. of units (interquartile range)¶				
Overall	1 (0 to 2)	3 (2 to 6)		.002
During surgery§	0 (0 to 1)	1 (1 to 2)		<.001
After surgery	1 (1 to 2)	2 (1 to 3)		.009
≥1 unit of red cells, n (%)‖				
Overall	19 (66)	29 (100)	−34% (−52% to −17%)	.002
Preoperative	0 (0)	3 (10)	−10% (−21% to 1%)	.236
Intraoperative	10 (34)	24 (83)	−48% (−70% to −26%)	<.001
Postoperative	16 (55)	26 (90)	−35% (−56% to −13%)	.008
Total count of red cell units transfused	57	136		
Protocol suspension red cell transfusions**				
≥1 unit of red cells, n (%)‖	8 (28)	4 (14)	14% (−7% to 34%)	.331
Total count¶	23	5		.311
Nonadherence, n (%)‖				
≥1 nonadherent event, overall	8 (28)	10 (34)	−7% (−31% to 17%)	.777
≥1 nonadherent red cell transfusion ††	6 (21)	4 (14)	7% (−13% to 26%)	.728
≥1 nonadherent failure to transfuse‡‡	3 (10)	10 (35)	−24% (−45% to −4%)	.059

*GEE adjusted for age, baseline Hb, and type of operation. The mean Hb values are unadjusted, as this model does not provide mean ± confidence intervals. GEE was applied on a data frame with the Hb unit in mmol/L.

†Mixed model adjusted for age, baseline Hb, and type of operation. Least squared means are showed along with the mean difference. In the case of time point measurement (eg, day 0 or 30), generalized linear model was adjusted for the same covariates.

‡Hb in patients randomized before end of surgery.

¶van Elteren's test adjusted for type of operation.

§No patient received more than 5 units of red blood cell intraoperatively.

‖χ-square with estimated risk difference.

**Reasons for protocol suspension: uncontrollable hemorrhage during surgery 5 (17%) the low-trigger group vs 4 (14%) in the high-trigger group. Protocol suspension resulting from stroke or extremity ischemia exclusively occurred in 3 (10%) patients in the low-trigger group.

††Red cell transfusion at a Hb level above the allocated trigger or not preceded by a Hb measurement. No nonadherence occurred intraoperatively. Adherence proportion (number of red cell units transfused adherently divided by all red cell transfusions) is provided in the supplemental Table 2.6.

‡‡Omission of red cell transfusion despite an Hb level below the trigger. No red cell transfusions were omitted because of volume overload.

11.3.4 Figure 4. Exploratory outcomes, Study II.

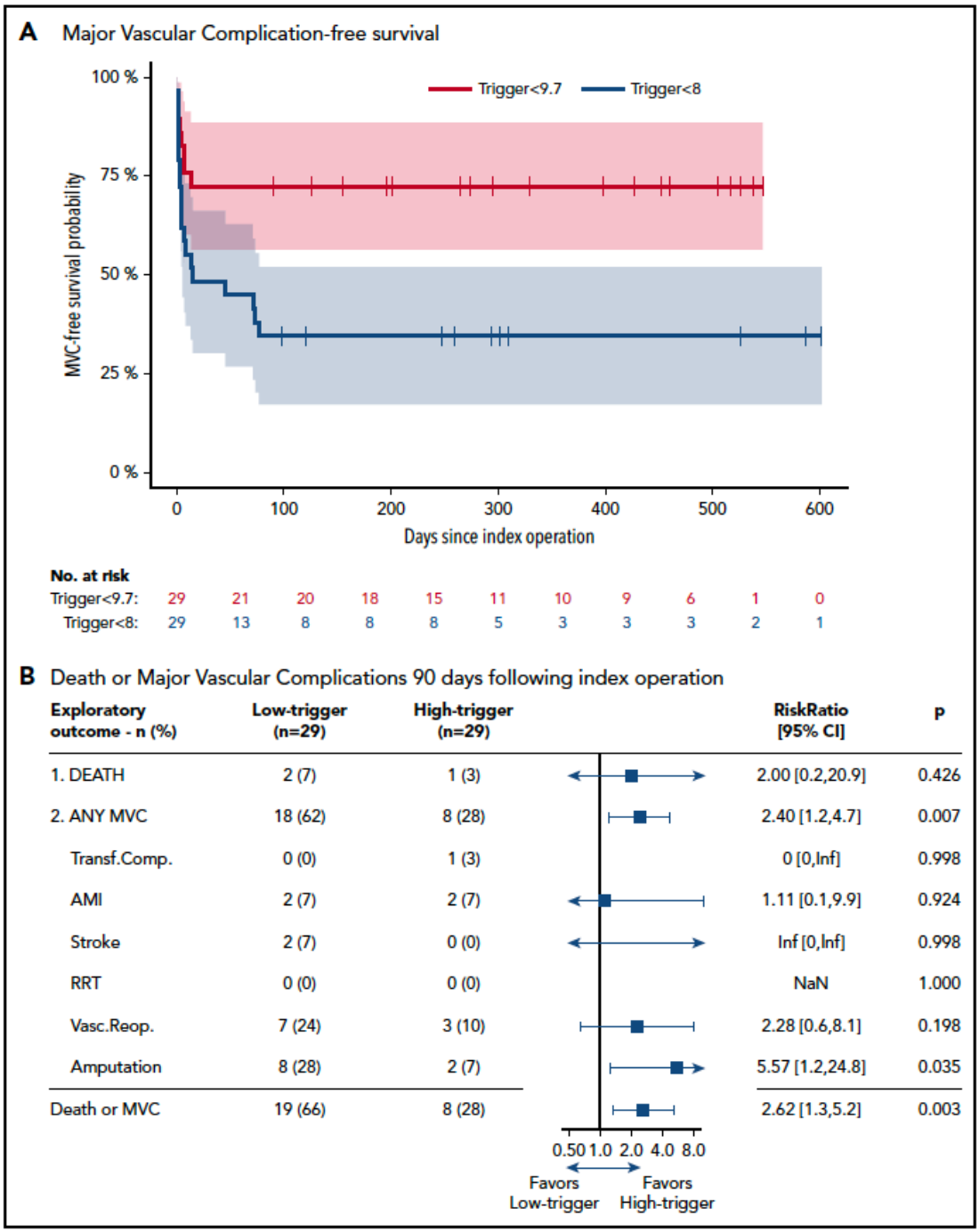


Figure 4. Major vascular complication-free survival and relative risk for death or major vascular complications at 90 days. (*A*) Major vascular complication-free survival probability with 95% CI in the intention-to-treat population. Data were censored 90 days after the last randomized patient. (*B*) shows the relative risks (blue boxes) with 95% CIs (horizontal lines) for the exploratory outcome measures death or major vascular complications at day 90 in the low-trigger group as compared with the high-trigger group. Low-trigger, Hb lower than 8 g/dL; high-trigger, Hb lower than 9.7 g/dL. MVC, major vascular complication. Transf.Compl., severe adverse transfusion reaction (anaphylactic reaction, transfusion-associated circulatory overload, transfusion-related acute lung injury within 6 hours after RBC transfusion or severe acute hemolytic transfusion reaction within 24 hours after RBC transfusion); see trial protocol[21] for further detail. AMI, acute myocardial infarction. RRT, renal replacement therapy. Vasc.Reop., vascular reoperation (specifications provided in supplemental Table 8). Amputation, lower limb amputation from femur to toes. When considering major amputations (femoral or crural) at day 90: 3 (10%) major amputations had occurred in the low-trigger group vs zero (0%) in the high-trigger group; and at right censoring: 7 (24%) vs zero (0%), respectively. *Unadjusted because of lack of model fit with zero events in 1 stratum. All other logistic regressions were adjusted for operation type and age. Odds ratios were converted to RR using the δ-method, where probabilities were derived from the coefficients of the logistic regression using the mean age (73 years) and operation type (0.43); 95% CI was calculated from the standard error.

12 Study III: Post-hoc analysis of the TV trial

12.1 Methods

This is a post-hoc analysis of the TV trial confined to the 46 patient that were randomized before end of surgery. Except for the sample size, the methods in Study II and Study III are similar. In the following, we specify the protocol for anaesthesia and fluid therapy, and data collection during surgery.

12.1.1 Randomization, blinding and data collection

A baseline arterial blood gas was analysed after placement of the arterial line. When the Hb decreased blow 9.7 g/dL, randomization was performed in the operation theatre using the web-based randomisation system. New blood gasses were obtained every half hour during ongoing bleeding and following RBC transfusions.

Circulatory variables were retrieved using CO monitoring platform EV1000 (CO, stroke volume (SV), cardiac index, heart rate, and mean arterial pressure) and NIRS-parameters (ScO_2 and SmO_2) were recorded every 20^{th} second and stored on USB-hubs. The NIRS monitor was set in blinded "study modus" preventing any readings to be visible for the anaesthetic team.

12.1.2 Anaesthesia and fluid therapy

General anaesthesia was with sevoflurane and fentanyl. Rocuronium provided muscle relaxation. AAA patients received perioperative epidural analgesia, in addition. We used invasive arterial uncalibrated waveform analysis (EV1000, FloTrac® ver.4.0) to measure cardiac SV, which was used to guide fluid therapy. When a 250 ml fluid bolus increased SV by less than 10 %, the patient was considered normovolaemic. The baseline was defined as the time point where the patient was normovolaemic for the first time, before starting surgery. When normovolaemic, noradrenaline was infused as needed to keep the mean arterial pressure above 65 mmHg. The inspiratory oxygen fraction was set at 0.5, and the $paCO_2$ targeted at 4.5 - 6.0 kPa.

Ringers acetate solution was the first choice i.v. fluid. If the blood loss surpassed 1000 mL or the total crystalloid administration was above 3000 mL, human albumin (50 g/L) was used, as guided by the SV, until the Hb decreased below the allocated transfusion threshold.

Patients allocated to the high trigger received one unit of RBCs immediately following randomization and additional units as needed to maintain an Hb $\geq$ 9.7 g/dL. Patients allocated to

the low trigger awaited RBC transfusion until the Hb dropped below 8 g/dL to maintain that level or higher.

In case of uncontrollable haemorrhage or severe hypovolaemia, the anaesthesiologist could contravene the protocol and transfuse RBCs at his or her discretion. Hb guided RBC transfusion was resumed when haemodynamic stability was restored.

12.1.3 Outcome measures

The primary outcome was the mean CO during surgery, from baseline to skin closure. For a full haemodynamic profile, we also report CO at the nadir ScO_2 reading (the lowest ScO_2 before an RBC transfusion, a predefine outcome measure in the SAP); the mean level of Hb, DO_2, ScO_2 and SmO_2, paO_2, $paCO_2$, and noradrenaline dosage.

12.1.4 Statistical methods

The statistical analyses adhere to the published SAP.[48] Mean intraoperative levels of the outcome measure were calculated for each patient using area-under-the-curve. Trial groups were compared with linear regression and adjusted for operation type, age, and the baseline value of the outcome.

12.2 Results

We obtained written consent from 112 of 144 screened patients. 58 of the 112 patients were randomized and the 46 randomized before skin closure were included in the post-hoc analysis. Baseline characteristics were balanced among the included patients (see Table 1, Paper IV). The fluid balance, surgery duration and noradrenaline dosage were similar in the two trial groups, Table 2.

The mean intraoperative Hb was reduced with the low trigger strategy (mean difference, -0.76 g/dL; P<0.001; Table 3). The mean CO was numerically higher in the low trigger group (mean difference, 0.36 L/min; -0.05 to 0.78; P = 0.092) and at the nadir ScO_2 reading, the CO was 0.58 L/min higher in the low trigger compared with the high trigger (P = 0.024). There was no difference in mean DO_2 between the two trial groups (P = 0.721). The low trigger strategy resulted in a 3 %-point lower mean ScO_2 during surgery compared with the high trigger (P = 0.029), whereas the SmO_2 was maintained at similar levels in the two trial groups (P = 0.836). Figure 3 presents the relative change in Hb, CO, DO_2, ScO_2 and SmO_2 from baseline. There was no

difference in cumulated norepinephrine dose, arterial O_2-saturation, or arterial partial O_2- or CO_2 pressure.

12.3 Conclusion

In elective patients undergoing vascular surgical, RBC transfusion at an Hb < 8 g/dL compared with an Hb < 9.7 g/dL significantly reduced the intraoperative Hb levels and number of RBCs unit transfused. Despite that the CO was higher in the low trigger group, and that the DO_2 was maintained at similar levels in the two trial groups, the ScO_2 was reduced with the low trigger strategy. Thus, the results did not indicate that the CO response to haemodilution was insufficient to mitigate a drop in arterial oxygen content when RBC transfusion was restricted to Hb levels below 8 g/dL compared with 9.7 g/dL.

12.3.1 Table 2. Fluid balance, noradrenaline dose and duration of anaesthesia, surgery and cross clamp, Study III.

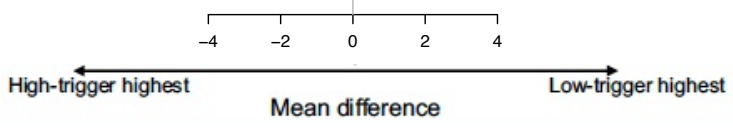
	Trigger < 8 g/dL (n = 22)	Trigger < 9.7 g/dL (n = 24)	P
Fluid input (mL)			
Crystalloids	2300 [1800-3500]	2900 [2075-3704]	0.235
Human albumin 50 g/L	500 [0-875]	250 [0-1000]	0.186
Red blood cells	250 [0-1000]	450 [300-675]	<0.001
Total	3000 [2100-4378]	4025 [2700-5238]	0.083
Fluid loss (mL)			
Blood loss	898 [403-1695]	1650 [590-2160]	0.429
Urine	468 [288-869]	648 [394-964]	0.190
Total	1528 [936-2436]	2260 [1321-3108]	0.368
Fluid balance (mL)			
Total	1190 [1081-2098]	1855 [1499-2300]	0.312
Ratio (mL/mL)			
Total fluid input/blood loss	3.5 [2.6-5.4]	2.9 [2.3-4.6]	0.584
Duration (minutes)			
Anaesthesia	238 [189-282]	252 [207-277]	0.808
Surgery	174 [153-232]	181 [152-213]	0.843
AAA Cross Clamp (n = 8 *vs* n = 11)	89 [64-140]	75 [64-90]	0.225
Noradrenaline (mg)			
Cumulated dose (n = 21 *vs* n = 22)	0.39 [0.05-0.91]	0.44 [0.00-0.74]	0.840

Note: Results presented as median [inter-quartile range]. Test statistic is Van Elteren's test adjusted for operation type. In the low-trigger group 12/22 (55%) avoided RBC transfusion whereas all patients in the high-trigger group received RBCs. One patient in the low-trigger group received fresh frozen plasma transfusion due to massive bleeding caused by iliac vein laceration during insertion of aorto-bifurcated prosthesis. No patients required platelet transfusion. No cases of non-adherence to transfusion strategies were registered during surgery, but protocol suspension was used in five patients in the low-trigger and in four patients in the high-trigger group due to rapid haemorrhage.[1] An additional patient in the low-trigger group received RBCs under protocol suspension due to extremity ischaemia. See Supplementary Material for further data. Three patients had missing data on cumulated noradrenaline dose because the change in infusion rate was not recorded in the electronic anaesthesia files.

Reference 1 in the table legend corresponds to reference no.[8] of this Ph.D. thesis (Paper II).

12.3.2 Table 3. Haemodynamic profile, Study III.

Haemodynamic variables (n 22 vs 24)	Trigger<8 mean (sd)	Trigger<9.7 mean (sd)	MD	95 % CI	P
Haemoglobin (g/dL)					
Nadir	8.4 (0.78)	9.12 (0.34)	−0.75	[−1.10 to −0.39]	<0.001
Mean	9.54 (0.85)	10.36 (0.67)	−0.76	[−1.07 to −0.45]	<0.001
Recovery room	9.23 (0.76)	10.49 (0.63)	−1.35	[−1.73 to −0.96]	<0.001
Cardiac output (L/min), (n 20 vs 22)					
CO at nadir ScO2	5.6 (1.1)	4.8 (0.92)	0.58	[0.10 to 1.07]	0.024
Mean	5.49 (1.15)	4.85 (0.88)	0.36	[−0.05 to 0.78]	0.092
Skin Closure	5.84 (1.28)	5.16 (1.31)	0.42	[−0.17 to 1.01]	0.172
DO2 (dL_O2/min), (n 20 vs 22)					
Nadir	63.4 (16.2)	58 (11.1)	2.64	[−4.48 to 9.76]	0.472
Mean	71.3 (14.9)	67.3 (13.5)	1.39	[−6.16 to 8.93]	0.721
Last Measurement	70.8 (18.2)	68.2 (17.3)	−0.86	[−10.20 to 8.49]	0.858
ScO2 (%), (n 22 vs 23)					
Nadir	57.45 (8.74)	57.66 (8.5)	−2.11	[−5.48 to 1.27]	0.228
Mean	59.77 (7.76)	61.25 (7.01)	−2.86	[−5.34 to −0.38]	0.029
Skin Closure	59.77 (7.76)	61.22 (7.03)	−2.84	[−5.31 to −0.36]	0.030
SmO2 (%), (n 22 vs 23)					
Nadir	71.21 (8.4)	67.32 (9.65)	1.50	[−1.66 to 4.67]	0.358
Mean	71.34 (8.3)	69.34 (9.05)	−0.26	[−2.73 to 2.21]	0.836
Skin Closure	71.33 (8.3)	69.34 (9.04)	−0.27	[−2.74 to 2.20]	0.833
MAP (mmHg), (n 20 vs 22)					
MAP at nadir ScO2	69.34 (12.34)	65.95 (7.85)	4.23	[−2.38 to 10.83]	0.218
Mean	69.24 (6.08)	69.04 (4.51)	0.92	[−2.25 to 4.10]	0.572
Skin Closure	70.97 (10.31)	66.75 (11.63)	4.71	[−2.52 to 11.94]	0.209
Respiration (kPa) (n 20 vs 21)					
Mean paO2	19.7 (5.2)	19.5 (6.2)	0.22	[−3.50 to 3.93]	0.910
Mean paCO2	5.4 (0.4)	5.3 (0.5)	0.12	[−0.16 to 0.40]	0.411



Abbreviations: Cardiac output, CO. DO_2, oxygen delivery. MAP, mean arterial pressure. ScO_2, frontal lobe tissue oxygenation. SmO_2, biceps muscle oxygenation.

The trial group means are presented as grand means (standard deviation, sd). MD, modelled mean difference. CI, confidence interval. The vertical line indicates zero MD. CO data were missing from two patients in each group (the first three patients were randomized before we had the option to download the CO-data from the EV1000-platform and one patient had missing CO-data because the EV1000-power supply short-circuited as iv-fluid entered by accident and the data was lost). DO_2 was calculated based on Hb bound O_2 alone. Results from DO_2 based on Hb-bound and plasma dissolved O2 is in the results section. DO_2 at skin closure could not be calculated because an arterial blood gas was not consequently obtained at skin closure. Conversely, recovery room DO_2 could not be calculated because CO was not recorded in the recovery room. Thus, last DO_2 obtained during surgery is presented. Recovery room Hb, and nadir ScO_2 and SmO_2 have been reported previously1 but are also presented in this table for comparison.

12.3.3 Figure 3. Relative change in haemodynamic parameters from baseline, Study III.

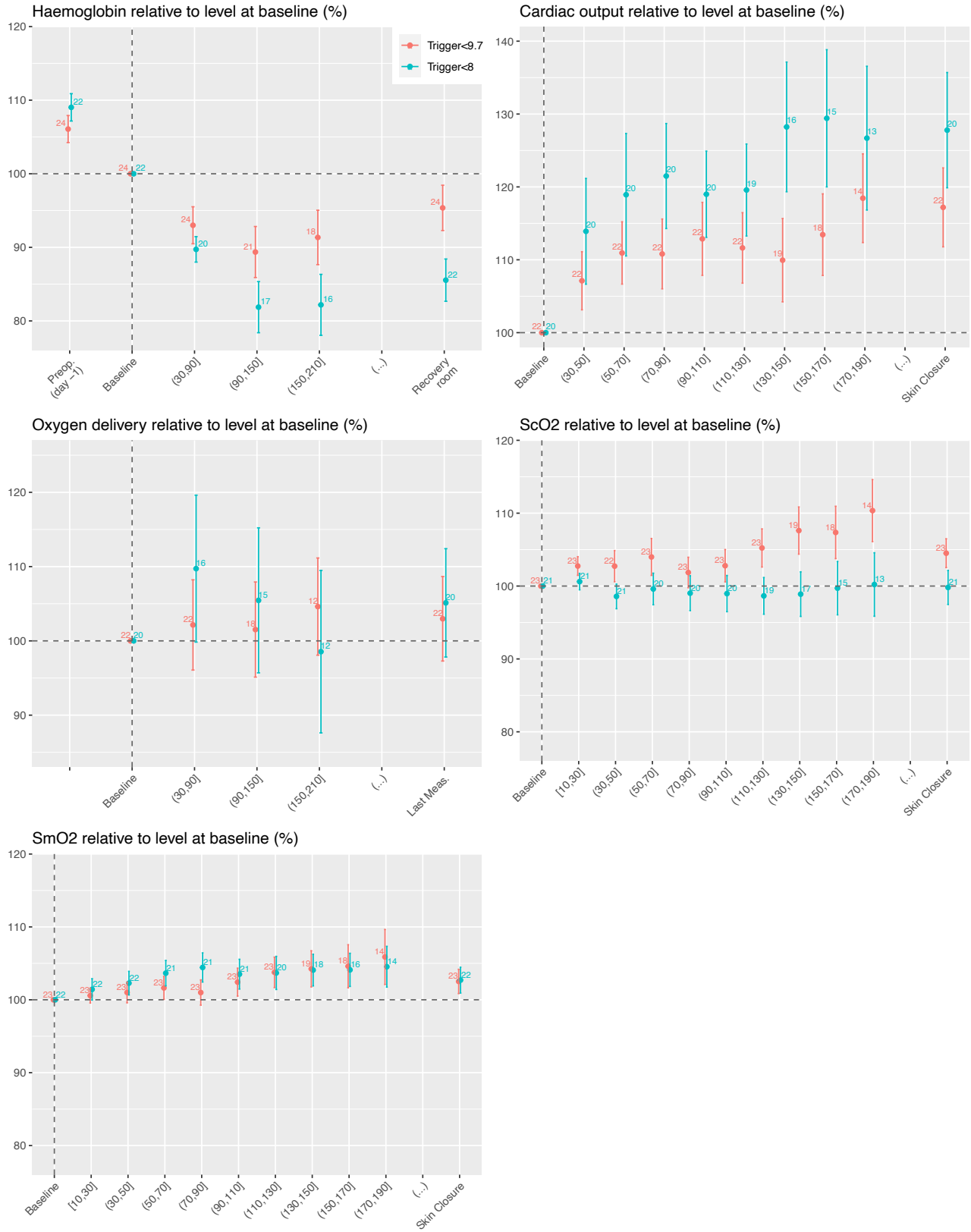


FIGURE 3 Haemoglobin, cardiac output, oxygen delivery and tissue oxygenation during surgery. *X*-axis: time elapsed since baseline (minutes). *Y*-axis: mean ± SE (%). Numerals indicate the number of patients observed. The *x*-axis is truncated at 180 minutes, the mean duration of surgery. Baseline was defined as the time point when the patient's stroke volume was unresponsive to further fluid administration. Oxygen delivery calculation was based on Hb bound O_2

13 Discussion

13.1 Principle findings

In the registry study I, we observed that the incidence in AAA surgery is decreasing in Denmark, which was mostly explained by a significant drop in incident ruptured AAA repairs in men, and in persons aged < 70 years. We suggest that the sex- and age heterogeneities in incidence reflect a cohort effect of Danish smoking patterns in the 20th century. Both crude and adjusted mortality was markedly reduced over calendar time and the explanation for this remains unclear.

In the randomized feasibility TV trial (Study II), we found that a protocol aiming to restrict RBC transfusion until the Hb had decreased below 8 g/dL compared with 9.7 g/dL, successfully separated trial groups in terms of postoperative Hb levels, units of RBCs transfused and ScO_2. Explorative outcomes suggested potential harm with restrictive RBC transfusion.

In the post-hoc analysis of the TV trial (Study III), we established that the lower ScO_2 observed in the low trigger group occurred despite a higher CO than the high trigger. The DO_2 was comparable in the two groups. The data point to that vascular surgical patients, despite cardiovascular disease, have a sufficient physiologic reserve in terms of global circulatory parameters. We suggest that the explanation for the ScO_2 difference may be found in the regional/peripheral tissue's response to haemodilution.

13.2 Strengths and limitations

13.2.1 Study I

Design

This was a retrospective observational study based on prospectively collected data. A major strength is the population- and registry-based longitudinal design in a country with universal healthcare access. A unique person-identification number enables both individual-level linkage across registries and calculation of comorbidity specific incidence rates in the population. Data from both the DVR, CPR and DNPR are prospectively collected, well validated,[35,36,50] and allow for complete follow-up on hospitalisations, deaths, and migration. The DVR was deliberately established for quality improvement and research purposes, as opposed to studies that use

administrative databases.[24,51] The latter may have limitations in reporting accuracy, lack of case-mix adjustment and comorbidity information.

Patient selection

We chose to describe the epidemiology of AAA *surgery* using the DVR, not AAA *diagnosed*, because we needed data on the surgery specific mortality. The DVR provides multiple key variables that may enhance specification of the surgery performed as follows.

When reporting incidence rates in a longitudinal study, various bias or imprecision source may affect the numerator and/or denominator.

$$Incidence\ rate = \frac{new\ surgical\ cases}{person - years\ at\ risk}$$

bias

- change in surgeons' selection criteria
- change in classification / coding practice
- misclassification
- coverage not nationwide
- increased use of abdominal radiology

bias

- lack of information on surgical history
- incomplete information on migration

Several aspects improved the numerator: The DVR has nationwide coverage, and no change in coding practice occurred during the study period. To identify "first-time" index cases, we excluded patients with a AAA repair history before 1996 and cases that were registered as ReDo, assistance, or reoperations. The inclusion of a case required both a relevant operation code and a clear indication for surgery. The numerator of our study is prone to selection bias in three aspects. One, in around 2010, the female AAA diameter threshold was lowered from 5.5 to 5.0. However, as most AAAs are unknown at rupture, this would mostly affect the intact AAA repair incidence, which varied over time in both sexes with no significant overall trend. Moreover, a rather stable female incidence in both ruptured and intact AAA repair point to that this may have had limited significance. Two, surgeons' "fit for surgery" gestalt have likely changed since the 1990s due to advances in perioperative care and the introduction of the less invasive endovascular repair. Thus, older and more frail and comorbid patients were more likely to be invited for surgery at the end of the study. This may explain some of the 81 % IR increase in the octogenarians, but it is unlikely to explain the 53-62 % IR reduction in persons aged below 70 years because an expanding AAA cannot be treated conservatively. Intermittent claudication, on the contrary, is increasingly managed conservatively in some countries.[52,53] Three, increased use of abdominal radiography may have disclosed more AAAs and promoted a transition from

ruptured to elective AAA repair, but this would not change our key finding, that there is a net decrease in incident AAA repairs.

The precision of the denominator, person-years, may be improved in our study, as we did not use historical data on residency. Residents of 2019 that have lived abroad sometime between 1996 and the date the CPR dataset was updated, 2019-10-31, have not been removed from the risk time. This may overestimate person-year risk time in the beginning of the study period, and consequently underestimate both the IR in the beginning of the study period and the IR decrease over calendar time.

The CCI was based on Charlson's comorbidity items registered in the DNPR. The majority of these diagnoses require hospitalization and there are reimbursement-driven incentives for hospitals for coding diagnoses into the DNPR. However, diagnoses made in primary care, e.g., diabetes, may have been missing in the DNPR. The positive predictive value (PPV) of CCI diagnoses recorded in the DNPR is 90 %.[54]

Statistics

IRs and incidence rate ratios were established by direct standardization and Poisson regression modelling, resp. Standardization has the advantage of presenting the absolute risk for AAA surgery in the population. Poisson regression provides relative values and aids covariate interaction analyses.

We were curious to know how the comorbidity had developed in patients undergoing AAA repair. Reporting crude CCI numbers over calendar time would likely be confounded by increasing age. This was solved by standardizing the patients' CCI to the CCI of the Danish population in the year 2000 by age (integer) and sex. We established that the age and sex standardized CCI increased significantly during the study period. Although the propensity to record CCI diagnoses into the DNPR may have increased and is a possible source of bias, we believe the data suggest, that today's AAA patient are either more comorbid or that surgeons have lowered their "fit for surgery" threshold.

Deliberately, we did not report the incidence of AAA *diagnoses* as this would overflood the manuscript with information, but it may provide valuable information on the AAA epidemiology because AAA development and expansion carry separate risk factors. A validation study of the DNPR data have demonstrated a high PPV of 100 % (95 % CI = 93 to 100 %) for AAA diagnoses.[55] DNPR data on LEAD are apparently poorer with a PPV of 70-90 %.[55,56]

In support of the validity of the methods used in study I, most of our key findings have been demonstrated in studies from other Western populations: the age-shift in the AAA incidence to an older population is also demonstrated in data from the UK, Australia and the US.[27,57,58] Sex heterogeneity in rupture AAA repair incidence demonstrated is shown in Sweden.[59] Age heterogeneity in AAA survival was observed in UK data.[24]

13.2.2 Study II-III

Design

In many aspects, we did our utmost to minimize bias in the design of the TV trial and enrich the trial population. An enrichment strategy seeks to identify and confine the trial population to patients in whom the intervention effect can be demonstrated.[60] Patient underwent individual randomization using a web-based system; the protocol was written adhering to the SPIRIT guideline and published along with the SAP in a peer-reviewed journal. We blinded patients-, statistician-, and the outcome assessor (ECG and chest x-rays blindly assessed). We used intention to treat analysis,[61] and reported trial results according to the CONSORT guidelines. Limitations include the limited trial sample, which increases the risk of a chance finding, albeit a feasibility trial, and the single centre design, limiting external validity.

Why a feasibility trial

We were not confident that a protocol aiming to restrict RBC transfusion, compared with liberal transfusion, would have clinical equipoise among anaesthesiologists and surgeons, or whether such a protocol would effectively separate Hb levels and units of RBCs transfused. Critics say that the question of whether groups would separate is trivial. However, the trial aim was not to determine efficacy (explanatory approach) of RBC transfusions on the Hb; clinicians observe this on an everyday basis, that RBC transfusions increase the Hb level. The aim was to determine the effectiveness (pragmatic approach) of such a protocol by adhering to the intention to treat principle. Other trials that have intervened during surgery have not been effective in separating Hb levels.[40,62-64] So, it is not a foregone conclusion whether restricting RBCs during bleeding in vascular surgery effectively lowers Hb levels compared with a liberal transfusion strategy.

Patient Selection

We used a two-step procedure for inclusion. Patients who gave consent were first registered, then randomized, but only if the Hb decreased below 9.7 g/dL. This enriched the population with patients at risk of blood transfusion or ischaemic events secondary to anaemia, which should be

the target population of any RBC trigger trial; like patients with postoperative pain should be the target population of a pain killer trial. In trials where allcomers are randomized preoperatively, heterogeneity and noise are introduced from patients who were never near receiving a transfusion due to high preoperative Hb or "dry surgery", which may obscure a true treatment effect of either intervention arm.[62,65,66] Excluding non-anaemic patients after randomization may both introduce imbalance in patient risk factors and bias clinicians' co-interventions before the transfusion threshold is reached. Postoperative randomization, or delaying the trial intervention implementation,[67] leaves the question of how blood loss should be replaced during surgery unanswered, is prone to selection bias, and introduces un-protocolized RBC transfusion in 25 % of patients ahead of randomization.[67-69] A perioperative enrichment strategy is currently used in the ongoing multicentre LIBERAL trial[70] that shares various features with the TV trial, but the method has otherwise not been described in transfusion RCTs.[71,72]

We also provided data on patients that underwent surgery but were missed for screening and the reasons for that, see top of flow chart. Such data are not trivial as they contain information on possible selection bias. For example, in the FOCUS trial it was demonstrated that RBC transfusion at an Hb of 8 g/dL was as safe as 10 g/dL following hip-fracture surgery.[69] However, despite a preceding pilot trial, several alterations were made during trial conduct: *i)* inclusion criteria were widened from patients with cardiovascular disease (CVD) to also include patients with risk factors for CVD, *ii)* the sample size was changed from 2,600 to 2,016, *iii)* participating centres from 25 to 47, and *iv)* the enrolment period was expanded to 5 years. 14,438 patients were screened before the stipulated sample size of 2,016 was met. It follows that a large proportion of eligible patient have likely been "missed" in the screening process, but that information is not contained in a classic CONSORT flow chart. It raises a concern that patients with overt or severe CVD were omitted in the screening process due to a lack of equipoise among surgeon or trial personnel. The FOCUS trial was initiated when liberal transfusion was recommended in CVD patients, and very few trials had been conducted in this population, which we will return to in the next section.

Statistics

Continuous outcome variables were analyzed using analysis of covariance (ANCOVA), as recommended by Cochrane, where the baseline value of the outcome is a covariate in the linear regression.[73] Binary outcomes were analyzed using logistic regression. A downside with the

logistic regression is, that is provides odds ratios, and odds ratios are often misinterpreted as risk ratios or relative risks. An interpretation that is hardly fair if the probability of occurrence of the outcome is high.[74] Due to this, we converted the odds ratios to relative risks using the delta-method, where probabilities were derived from the coefficients of the logistic regression using the mean age (73 years) and operation type (0.43). Then, the standard error was used to calculate 95% CI. There is no closed formula for such a conversion, and it is important to remember that the resulted relative risk is based on a fixed age and arbitrary operation type.

We also performed complication-free survival analysis using Cox regression. When using complication-free-survival, there are no competing risks. An advantage with survival analysis is that the power of the analysis is increased because observation time at risk is included in the outcome (events per time unit, i.e., event rate). Right censoring was 90 days after the last randomized patient. Thus, patients included at the beginning of the trial contributed with the most information. Critics may say that events occurring more than a year after the intervention are unlikely to be correlated to the intervention, but we don't know this. In theory, an ischaemic event may likely have long-term consequences, e.g., acute myocardial infarction -> congestive heart failure -> immobilisation -> increased frailty and care dependency etc.

Analysis of count data was adjusted for the stratum (operation type), as were all regression models, which may minimize variance and increase the power of the analysis.[49]

Missing Data

The primary outcome of study II, postoperative Hb, was analysed between day 0 and 15 because one patient was randomized on day 15. The median duration of hospitalization was around 8 days, which naturally introduces missing data from discharge date to day 15. This was handled using the mixed model, but because GEE was the model mentioned in the SAP, we had to report this as the primary analysis. However, GEE handles missing data with last observation caried forward which is likely biased in this case. Confining randomization to Hb drop occurring within a more limited time frame following surgery could minimize this problem.

14 Current evidence; clinical and methodological implications

14.1.1 Study I

A decreasing rate of AAA incidence and mortality has been described in several studies from Western populations.[24,25,27,59] Smoking is the principal risk factor for AAA development and expansion to rupture, which makes Danish trends in smoking patterns, as described in the background, a plausible explanation for both the age- and sex heterogeneity we observed.

Clinical implications

From a vascular surgeon's point of view, this shift in age at presentation is rather ill-timed in light of the recent and wide-spread implementation of AAA screening programs. Given the increasing life expectancy in the population and the delayed onset of AAA, future screening trials could consider expanding the study population to an even older age group, such as 65-80 years. Given the marked mortality reduction following AAA repair and the reduced AAA repair incidence rate, a revaluation of the current AAA diameter thresholds for pre-emptive AAA repair seems reasonable. However, an RCT comparing AAA repair with watchful waiting was never setup due to lack of equipoise among surgeons.[75]

Methodological implications

Both age, sex, and comorbidity were identified as mortality risk factors and are potentially relevant stratification variables in future trials. Especially age, as the mortality rate increased by 5-6 % per year increase in age in all LEAD and AAA populations studied. Stratification variables may aid in balancing risk factors between trial groups and adjusting for these strata may enhance the power of the analyses of the trial outcome data as described above.[49]

Methodological implications are further debated in section 14.2 Thoughts on a future trial.

14.1.2 Study II-III

This discussion will focus on RBC transfusion in patients with CVD.

Until the late 1990s, no RCTs on transfusion triggers in surgical patients or patients with CVD were available to guide clinicians.[40,72] Transfusion practice was informed by the physiologic rationale that patients with CVD have limited capacity to increase CO and to compensate for anaemia, and therefore should have their Hb maintained above 10 g/dL.[40] This was supported by a retrospective observational study by Carson and colleagues.[3] The study included 1,958 patients

undergoing surgery who refused RBC transfusions for religious reasons. They found that CVD was associated with increased mortality if the preoperative Hb level was below 10 g/dl or if significant blood loss occurred (volume not specified), compared to patients without CVD. At preoperative Hb levels above 10 g/dL, the presence of CVD did not associate to increased mortality. Two central question remains unanswered, *i)* does low Hb levels simply reflect severe chronic comorbidity, a confounder, which in a joint effect (interaction) with CVD explains the high mortality in anaemic CVD patients (i.e., there is not necessarily a causal link between low Hb and mortality). *ii)* can the adverse effects associated with a low Hb in patients with CVD be mitigated by RBC transfusion? So far, the latter has not been substantiated in RCTs[62,67,69,76], and the debate continues.[72,77-79]

A potential limitation with the available evidence is that restrictive RBC transfusion has not been adequately tested in patients with significant surgical haemorrhage (>500 ml) due to inadequate separation of trial group.[40,62-64] Blood loss of this magnitude has been identified as a critical threshold. Wu and colleagues established that intraoperative blood transfusion associated to a lower mortality in noncardiac surgery patients when blood loss was above 500 ml, compared to a propensity matched control group that did not receive RBCs; regardless of the preoperative Hb levels.[80]

Both cardiac disease, preoperative anaemia and major haemorrhage are frequent in vascular surgery, and cardiac disease is more likely to aggravate following vascular surgery as opposed to cardiac surgery.[2,4,8] These factors justified the initiation of the TV feasibility trial, which provided important novel information on transfusion RCT methodology: Recruitment can be enriched, and significant trial group separation can be achieved already during surgery, in terms of Hb levels and units of RBC transfused. The TV trial cannot be used to infer on the treatment effect on patient-important outcomes. However, the results formed the basis for a timely pause and reflection. Was it reasonable to extrapolate results from RCTs in critical care and postoperative stable patients and universally adopt a restrictive transfusion trigger? Quote from the Blood Commentary "*As the field has moved toward restrictive transfusion thresholds for an expanding number of patients, this study creates pause. This study highlights that there may be patient populations who benefit from liberal thresholds and that end points other than mortality should be considered*".[81]

An adequately powered trial is essential to confirm or refute the exploratory outcome finding. One concern is that some doctors may question the equipoise of the two

proposed allocations. Nonetheless, both vascular surgery- and anaesthesiology societies presently advocate a restrictive approach to transfusion.[18,82,83] The design of a future trial is discussed in the next section. Until then, three ongoing trials may help inform practice in a shorter time frame:

Ongoing trials in patients with CVD:

Acronym / title / sample size / NCT / Estimated Study Completion Date:

- MINT / Myocardial Ischemia and Transfusion / 3,500 / NCT02981407 / October 2021
- LIBERAL / Liberal Transfusion Strategy in Elderly Patients / 2,470 / NCT03369210 / December 2021
- TOP / Transfusion Trigger After Operations in High Cardiac Risk Patients / 3,070 / NCT03229941 / February 2022

LIBERAL and TOP trial include vascular surgical patients among others.

To reflect on study III, I will briefly return to the physiologic rationale for liberal transfusion in CVD patients. Recommendations have proposed that RBC transfusions should be provided to increase DO_2, and transfusion should be guided by the DO_2 instead of arbitrary Hb levels.[84,85] Study III adds the following to our knowledge in this context. Despite CVD, vascular surgery patients exposed to restrictive RBC transfusion can increase CO and maintain the global DO_2 at levels comparable to a liberal strategy; contrary to previous assumptions.[3,40,85,86] The findings indicate that the previously established increased mortality risk in patients with CVD is not necessarily due to limitations in *global* physiologic reserve. In support, not only the DO_2 but also SmO_2 was also similar in the two trial groups. Instead, the results indicate that the lower ScO_2 in response to haemodilution in the low trigger group is a regional phenomenon, as supported by human and animal experiments.[45,87] Namely that in states of anaemic hypoxia (low CaO_2 caused by haemodilution) the increase in cerebral blood flow is insufficient to maintain the cerebral oxygen delivery, as opposed to states of hypoxaemic hypoxia (low CaO_2 caused by low O_2 Hb saturation).[45] The Hb molecule works as a potent vasodilator as nitric oxide is released when oxy-Hb transitions to deoxy-Hb.[44,45] The theoretical explanation may be that this signal transduction originating from the RBCs is impaired by haemodilution.[45] The regional cerebral blood flow regulation may also be affected by anaesthesia (sympathetic tone),[86] or be blunted by reduced shear stress as haemodilution lowers blood viscosity, affecting the nitric oxide release from the vascular endothelium.[45] However, the brain can double the oxygen extraction,[45] and the clinical relevance of the post-hoc analysis is unknown.

14.2 Thought on the design of a future trial

14.2.1 PICO

Population:

1) Scheduled for open repair of either
    a) Intact AAA
    b) LEAD
    c) Eventual: ruptured AAA
2) Age > 40

Experimental Intervention: RBC transfusion $\leq$ 10 g/dL

Control Intervention: RBC transfusion $\leq$ 8 g/dL

Note, experimental and control groups are reversed compared to the TV trial, because current anaesthesiologic and vascular surgical societies recommend restrictive transfusion which would be considered "control intervention".[18,82,83] Also, given the hypothesis is that liberal RBC transfusion reduces death or complications during vascular surgery, it would be more ethically sound to use liberal transfusion as the experimental intervention and test whether it would improve outcome. An Hb threshold of 10 g/dL (6.2 mmol/L) for the high trigger is chosen to have an even number for international collaboration.

14.2.2 Primary outcome measure and sample size

Given the low 90-day mortality of less than 5 % in elective AAA and LEAD-surgery, mortality is not a feasible endpoint. In the TV trial, we saw a remarkably high proportion of patients having death or a major vascular event, which could be used as composite primary outcome measure. Alternatively, days alive out of hospital < 90 days; or neurologic outcome such as postoperative cognitive dysfunction or covert stroke[88], given the results of the NIRS data showing lower cerebral oxygen saturation in the low trigger group.

Example, sample size calculation using R:

Assuming the incredible, as in difficult to believe, absolute risk difference in death or major vascular complication observed in the TV trial of 38 % (66 % *vs.* 28 %), 2 x 25 patient would be enough with a significance level of 0.05 and power of 0.80. However, such treatment effects are dubious, and the TV-Trial's observed treatment effect is most possibly a "random high". A more conservative and realistic approach would be to assume a 20 % relative reduction and a baseline

risk (p1) corresponding to all patients randomized: p1 = 27/58 = 0.46, p2 = 0.46 x (1 – 0.2) = 0.37. With an alfa of 0.05 and power of 0.8, 2 x 469 patient are required to show a reduction in death or major vascular complication from 46 % to 37 %. With a power of 0.9, the total sample would be 1254.

In the TV trial, we obtained a randomization rate of 58 randomized/17 months = 3.4 /month/centre. With 20 participating centres in northern Europe, 1,000 patients could be randomized in

1000 x (3.4 randomized/month/centre x 20 centres)$^{-1}$ =

1000 x (68 randomized / month)$^{-1}$ = 15 months.

A lot of uncertainty remains in the assumption: the baseline risk in other centres and in the years to come, the surgical caseload of open repairs, see table below, blood loss, and the incidence of AAA and LEAD surgery in the population, which we have established is declining.

Frequent interim analysis would be scheduled as this would likely increase the trial acceptance among vascular surgeons who do not see equipoise in the proposed treatment arms.

The annual caseload in Denmark (data source: DVR):

Surgery	2018	2019
Intact AAA, open*	283	300
Intact AAA, endovascular	246	220
Ruptured AAA	140	117
Aorto-iliac bypass*	65	80
Lower-limb bypass*	343	342
Lower-limb TEA	379	366
TOTAL	1456	1425

*included in the TV trial. Around 1/10 ruptured AAAs are managed by endovascular repair.

14.2.3 Blinding

Patients, statistician, and ideally, blinded outcome adjudicators. A fully “quadruple” blinded RBC transfusion RCT is hard, but not impossible to design. It could be achieved by partial blinding of the Hb laboratory results, where only the gap between the measured Hb level and the allocated trigger was displayed in the laboratory result.

For instance, a Hb of 7, 9 and 11 g/dL would appear as

“Hb: trigger – 1”, “Hb: trigger + 1”, “Hb: trigger + 3” in the low trigger group (< 8 g/dL), and as

“Hb: trigger – 3”, “Hb: trigger – 2”, “Hb: trigger + 1” in the high trigger group (< 10 g/dL).

A minus "–" would trigger protocolized RBC transfusion. Such design could be difficult to accept by physicians and surgeons and would likely require another pilot trial to demonstrate feasibility.

14.2.4 Potential stratification variables

1) Surgical Centre/Site
2) Surgery type (intact AAA vs. LEAD vs. rupture)
3) Age >70 years, or > 75 years

The survival analysis of the registry study also identified a main effect of sex and comorbidity on mortality rate and are thus other potential stratification variables.

14.2.5 Further prerequisites

We have recently received data on allogeneic transfusions from the Danish Transfusion Database. This will improve the estimate of the p1, as we may compute a transfusion specific baseline risks of death or major vascular events in vascular surgery. Also, a systematic review and meta-analysis incorporating the results of the ongoing LIBERAL and TOP trial seems prudent ahead of a definitive trial.

15 Conclusion and perspectives

This thesis illustrates the importance and feasibility of a trial on RBC transfusions strategies in vascular surgery and proposes a future trial design. Of relevance to a future trial, the results of the registry study identified several strong predictors of mortality which could be important stratification variables. The incidence of both AAA and LEAD surgery is decreasing, and unless the population continues to grow, the hospital caseload of vascular surgery may likely decrease despite an increasing incidence of surgery in the elderly, which could complicate recruitment in a future trial. Nevertheless, a trial on transfusion strategies in vascular surgery is urgently needed, as it has the potential to improve outcome following surgery of the vulnerable vascular surgery patients, in which the current evidence to guide transfusion is very limited.

16 REFERENCES

1. Feringa HH, Karagiannis SE, Vidakovic R, et al. The prevalence and prognosis of unrecognized myocardial infarction and silent myocardial ischemia in patients undergoing major vascular surgery. *Coron Artery Dis* 2007;18(7):571-6.
2. Hertzer NR, Beven EG, Young JR, et al. Coronary artery disease in peripheral vascular patients. A classification of 1000 coronary angiograms and results of surgical management. *Ann Surg* 1984;199(2):223-33.
3. Carson JL, Duff A, Poses RM, et al. Effect of anaemia and cardiovascular disease on surgical mortality and morbidity. *Lancet* 1996;348(9034):1055-60.
4. Kougias P, Sharath S, Barshes NR, Chen M, Mills JL, Sr. Effect of postoperative anemia and baseline cardiac risk on serious adverse outcomes after major vascular interventions. *J Vasc Surg* 2017;66(6):1836-1843.
5. Duceppe E, Parlow J, MacDonald P, et al. Canadian Cardiovascular Society Guidelines on Perioperative Cardiac Risk Assessment and Management for Patients Who Undergo Noncardiac Surgery. *Can J Cardiol* 2017;33(1):17-32.
6. Schwarze ML, Barnato AE, Rathouz PJ, et al. Development of a list of high-risk operations for patients 65 years and older. *JAMA Surg* 2015;150(4):325-31.
7. Bihl F, Castelli D, Marincola F, Dodd RY, Brander C. Transfusion-transmitted infections. *J Transl Med* 2007;5:25.
8. Moller A, Nielsen HB, Wetterslev J, et al. Low vs high hemoglobin trigger for transfusion in vascular surgery: a randomized clinical feasibility trial. *Blood* 2019;133(25):2639-2650.
9. Desborough M, Sandu R, Brunskill SJ, et al. Fresh frozen plasma for cardiovascular surgery. *Cochrane Database Syst Rev* 2015(7):CD007614.
10. Curley GF, Shehata N, Mazer CD, Hare GM, Friedrich JO. Transfusion triggers for guiding RBC transfusion for cardiovascular surgery: a systematic review and meta-analysis*. *Crit Care Med* 2014;42(12):2611-24.
11. Dua A, Lee CJ. Epidemiology of Peripheral Arterial Disease and Critical Limb Ischemia. *Tech Vasc Interv Radiol* 2016;19(2):91-5.
12. AABB. Highlights of Transfusion Medicine History. https://www.aabb.org/news-resources/resources/transfusion-medicine/highlights-of-transfusion-medicine-history Accessed 2021-01-11.
13. Friedman SG. *A history of vascular surgery*. 2nd ed. Malden, Mass.: Blackwell Futura, 2005.
14. Vamvakas EC. The abandoned controversy surrounding universal white blood cell reduction. *Blood Transfus* 2014;12(2):143-5.
15. Kent KC, Zwolak RM, Egorova NN, et al. Analysis of risk factors for abdominal aortic aneurysm in a cohort of more than 3 million individuals. *J Vasc Surg* 2010;52(3):539-48.
16. Norman PE, Curci JA. Understanding the effects of tobacco smoke on the pathogenesis of aortic aneurysm. *Arterioscler Thromb Vasc Biol* 2013;33(7):1473-7.
17. Brown LC, Powell JT. Risk factors for aneurysm rupture in patients kept under ultrasound surveillance. UK Small Aneurysm Trial Participants. *Ann Surg* 1999;230(3):289-96; discussion 296-7.
18. Chaikof EL, Dalman RL, Eskandari MK, et al. The Society for Vascular Surgery practice guidelines on the care of patients with an abdominal aortic aneurysm. *J Vasc Surg* 2018;67(1):2-77 e2.

19. Cronenwett JL, Murphy TF, Zelenock GB, et al. Actuarial analysis of variables associated with rupture of small abdominal aortic aneurysms. *Surgery* 1985;98(3):472-83.
20. Pisinger C, Jorgensen T, Toft U. A multifactorial approach to explaining the stagnation in national smoking rates. *Dan Med J* 2018;65(2).
21. Brady AR, Thompson SG, Fowkes FG, et al. Abdominal aortic aneurysm expansion: risk factors and time intervals for surveillance. *Circulation* 2004;110(1):16-21.
22. Eickhoff JH. Incidence of diagnosis, operation and death from abdominal aortic aneurysms in Danish hospitals: results from a nation-wide survey, 1977-1990. *Eur J Surg* 1993;159(11-12):619-23.
23. Fowkes FG, Macintyre CC, Ruckley CV. Increasing incidence of aortic aneurysms in England and Wales. *BMJ* 1989;298(6665):33-5.
24. Anjum A, Powell JT. Is the incidence of abdominal aortic aneurysm declining in the 21st century? Mortality and hospital admissions for England & Wales and Scotland. *Eur J Vasc Endovasc Surg* 2012;43(2):161-6.
25. Sidloff D, Stather P, Dattani N, et al. Aneurysm global epidemiology study: public health measures can further reduce abdominal aortic aneurysm mortality. *Circulation* 2014;129(7):747-53.
26. Lederle FA. The rise and fall of abdominal aortic aneurysm. *Circulation* 2011;124(10):1097-9.
27. Choke E, Vijaynagar B, Thompson J, et al. Changing epidemiology of abdominal aortic aneurysms in England and Wales: older and more benign? *Circulation* 2012;125(13):1617-25.
28. Svensjo S, Bjorck M, Gurtelschmid M, et al. Low prevalence of abdominal aortic aneurysm among 65-year-old Swedish men indicates a change in the epidemiology of the disease. *Circulation* 2011;124(10):1118-23.
29. Oliver-Williams C, Sweeting MJ, Turton G, et al. Lessons learned about prevalence and growth rates of abdominal aortic aneurysms from a 25-year ultrasound population screening programme. *Br J Surg* 2018;105(1):68-74.
30. Lindholt JS, Juul S, Fasting H, Henneberg EW. Hospital costs and benefits of screening for abdominal aortic aneurysms. Results from a randomised population screening trial. *Eur J Vasc Endovasc Surg* 2002;23(1):55-60.
31. Lindholt JS, Rasmussen LM, Sogaard R, et al. Baseline findings of the population-based, randomized, multifaceted Danish cardiovascular screening trial (DANCAVAS) of men aged 65-74 years. *Br J Surg* 2019;106(7):862-871.
32. Lindholt JS, Sogaard R. Population screening and intervention for vascular disease in Danish men (VIVA): a randomised controlled trial. *Lancet* 2017;390(10109):2256-2265.
33. Lederle FA, Kyriakides TC, Stroupe KT, et al. Open versus Endovascular Repair of Abdominal Aortic Aneurysm. *N Engl J Med* 2019;380(22):2126-2135.
34. Bahia SS, Holt PJ, Jackson D, et al. Systematic Review and Meta-analysis of Long-term survival After Elective Infrarenal Abdominal Aortic Aneurysm Repair 1969-2011: 5 Year Survival Remains Poor Despite Advances in Medical Care and Treatment Strategies. *Eur J Vasc Endovasc Surg* 2015;50(3):320-30.
35. Eldrup N, Cerqueira C, de la Motte L, Rathenborg LK, Hansen AK. The Danish Vascular Registry, Karbase. *Clin Epidemiol* 2016;8:713-718.
36. Altreuther M, Menyhei G. International Validation of the Danish Vascular Registry Karbase: A Vascunet Report. *Eur J Vasc Endovasc Surg* 2019;58(4):609-613.

37. Laustsen J, Jensen LP, Hansen AK, Danish National Vascular R. Accuracy of clinical data in a population based vascular registry. *Eur J Vasc Endovasc Surg* 2004;27(2):216-9.
38. Bodewes TCF, Pothof AB, Darling JD, et al. Preoperative anemia associated with adverse outcomes after infrainguinal bypass surgery in patients with chronic limb-threatening ischemia. *J Vasc Surg* 2017;66(6):1775-1785 e2.
39. Bisgaard J, Gilsaa T, Ronholm E, Toft P. Optimising stroke volume and oxygen delivery in abdominal aortic surgery: a randomised controlled trial. *Acta Anaesthesiol Scand* 2013;57(2):178-88.
40. Bush RL, Pevec WC, Holcroft JW. A prospective, randomized trial limiting perioperative red blood cell transfusions in vascular patients. *Am J Surg* 1997;174(2):143-8.
41. Prinssen M, Verhoeven EL, Buth J, et al. A randomized trial comparing conventional and endovascular repair of abdominal aortic aneurysms. *N Engl J Med* 2004;351(16):1607-18.
42. Calvo-Vecino JM, Ripolles-Melchor J, Mythen MG, et al. Effect of goal-directed haemodynamic therapy on postoperative complications in low-moderate risk surgical patients: a multicentre randomised controlled trial (FEDORA trial). *Br J Anaesth* 2018;120(4):734-744.
43. Pape A, Stein P, Horn O, Habler O. Clinical evidence of blood transfusion effectiveness. *Blood Transfus* 2009;7(4):250-8.
44. Helms CC, Gladwin MT, Kim-Shapiro DB. Erythrocytes and Vascular Function: Oxygen and Nitric Oxide. *Front Physiol* 2018;9:125.
45. Hoiland RL, Bain AR, Rieger MG, Bailey DM, Ainslie PN. Hypoxemia, oxygen content, and the regulation of cerebral blood flow. *Am J Physiol Regul Integr Comp Physiol* 2016;310(5):R398-413.
46. Murkin JM, Arango M. Near-infrared spectroscopy as an index of brain and tissue oxygenation. *Br J Anaesth* 2009;103 Suppl 1:i3-13.
47. Vlaar AP, Juffermans NP. Transfusion-related acute lung injury: a clinical review. *Lancet* 2013;382(9896):984-94.
48. Moller A, Nielsen HB, Wetterslev J, et al. Low vs. high haemoglobin trigger for transfusion in vascular surgery: protocol for a randomised trial. *Acta Anaesthesiol Scand* 2017;61(8):952-961.
49. Kahan BC, Morris TP. Improper analysis of trials randomised using stratified blocks or minimisation. *Stat Med* 2012;31(4):328-40.
50. Schmidt M, Schmidt SA, Sandegaard JL, et al. The Danish National Patient Registry: a review of content, data quality, and research potential. *Clin Epidemiol* 2015;7:449-90.
51. Sidloff DA, Saratzis A, Sweeting MJ, et al. Sex differences in mortality after abdominal aortic aneurysm repair in the UK. *Br J Surg* 2017;104(12):1656-1664.
52. Aboyans V, Ricco JB, Bartelink MEL, et al. 2017 ESC Guidelines on the Diagnosis and Treatment of Peripheral Arterial Diseases, in collaboration with the European Society for Vascular Surgery (ESVS): Document covering atherosclerotic disease of extracranial carotid and vertebral, mesenteric, renal, upper and lower extremity arteriesEndorsed by: the European Stroke Organization (ESO)The Task Force for the Diagnosis and Treatment of Peripheral Arterial Diseases of the European Society of Cardiology (ESC) and of the European Society for Vascular Surgery (ESVS). *Eur Heart J* 2018;39(9):763-816.
53. Behrendt CA, Sigvant B, Kuchenbecker J, et al. Editor's Choice - International Variations and Sex Disparities in the Treatment of Peripheral Arterial Occlusive Disease: A Report

from VASCUNET and the International Consortium of Vascular Registries. *Eur J Vasc Endovasc Surg* 2020;60(6):873-880.
54. Thygesen SK, Christiansen CF, Christensen S, Lash TL, Sorensen HT. The predictive value of ICD-10 diagnostic coding used to assess Charlson comorbidity index conditions in the population-based Danish National Registry of Patients. *BMC Med Res Methodol* 2011;11:83.
55. Sundboll J, Adelborg K, Munch T, et al. Positive predictive value of cardiovascular diagnoses in the Danish National Patient Registry: a validation study. *BMJ Open* 2016;6(11):e012832.
56. Lasota AN, Overvad K, Eriksen HH, et al. Validity of Peripheral Arterial Disease Diagnoses in the Danish National Patient Registry. *Eur J Vasc Endovasc Surg* 2017;53(5):679-685.
57. Norman PE, Spilsbury K, Semmens JB. Falling rates of hospitalization and mortality from abdominal aortic aneurysms in Australia. *J Vasc Surg* 2011;53(2):274-7.
58. Schwarze ML, Shen Y, Hemmerich J, Dale W. Age-related trends in utilization and outcome of open and endovascular repair for abdominal aortic aneurysm in the United States, 2001-2006. *J Vasc Surg* 2009;50(4):722-729 e2.
59. Lilja F, Mani K, Wanhainen A. Editor's Choice - Trend-break in Abdominal Aortic Aneurysm Repair With Decreasing Surgical Workload. *Eur J Vasc Endovasc Surg* 2017;53(6):811-819.
60. Temple R. Enrichment of clinical study populations. *Clin Pharmacol Ther* 2010;88(6):774-8.
61. Fergusson D, Aaron SD, Guyatt G, Hebert P. Post-randomisation exclusions: the intention to treat principle and excluding patients from analysis. *BMJ* 2002;325(7365):652-4.
62. Mazer CD, Whitlock RP, Fergusson DA, et al. Restrictive or Liberal Red-Cell Transfusion for Cardiac Surgery. *N Engl J Med* 2017;377(22):2133-2144.
63. Nielsen K, Dahl B, Johansson PI, Henneberg SW, Rasmussen LS. Intraoperative transfusion threshold and tissue oxygenation: a randomised trial. *Transfus Med* 2012;22(6):418-25.
64. Palmieri TL, Holmes JHt, Arnoldo B, et al. Transfusion Requirement in Burn Care Evaluation (TRIBE): A Multicenter Randomized Prospective Trial of Blood Transfusion in Major Burn Injury. *Ann Surg* 2017;266(4):595-602.
65. Gillies MA, Ghaffar S, Moppett IK, et al. A restrictive versus liberal transfusion strategy to prevent myocardial injury in patients undergoing surgery for fractured neck of femur: a feasibility randomised trial (RESULT-NOF). *Br J Anaesth* 2021;126(1):77-86.
66. Hajjar LA, Vincent JL, Galas FR, et al. Transfusion requirements after cardiac surgery: the TRACS randomized controlled trial. *JAMA* 2010;304(14):1559-67.
67. Murphy GJ, Pike K, Rogers CA, et al. Liberal or restrictive transfusion after cardiac surgery. *N Engl J Med* 2015;372(11):997-1008.
68. de Almeida JP, Vincent JL, Galas FR, et al. Transfusion requirements in surgical oncology patients: a prospective, randomized controlled trial. *Anesthesiology* 2015;122(1):29-38.
69. Carson JL, Terrin ML, Noveck H, et al. Liberal or restrictive transfusion in high-risk patients after hip surgery. *N Engl J Med* 2011;365(26):2453-62.
70. Meybohm P, Lindau S, Treskatsch S, et al. Liberal transfusion strategy to prevent mortality and anaemia-associated, ischaemic events in elderly non-cardiac surgical patients - the study design of the LIBERAL-Trial. *Trials* 2019;20(1):101.

71. Fominskiy E, Putzu A, Monaco F, et al. Liberal transfusion strategy improves survival in perioperative but not in critically ill patients. A meta-analysis of randomised trials. *Br J Anaesth* 2015;115(4):511-9.
72. Hovaguimian F, Myles PS. Restrictive versus Liberal Transfusion Strategy in the Perioperative and Acute Care Settings: A Context-specific Systematic Review and Meta-analysis of Randomized Controlled Trials. *Anesthesiology* 2016;125(1):46-61.
73. Higgins J. 10.5.2 Meta-analysis of change scores. *Cochrane Handbook for Systematic Reviews of Interventions*, Version 6.1, 2020.
74. Zhang J, Yu KF. What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. *JAMA* 1998;280(19):1690-1.
75. Oliver-Williams C, Sweeting MJ, Jacomelli J, et al. Safety of Men With Small and Medium Abdominal Aortic Aneurysms Under Surveillance in the NAAASP. *Circulation* 2019;139(11):1371-1380.
76. Steg PG. REALITY - A Trial of Transfusion Strategies for Myocardial Infarction and Anemia. https://www.acc.org/latest-in-cardiology/clinical-trials/2020/08/29/13/09/reality?utm_medium=social&utm_source=twitter_post&utm_campaign=esc20&utm_content= Accessed 2021-01-26.
77. Docherty AB, O'Donnell R, Brunskill S, et al. Effect of restrictive versus liberal transfusion strategies on outcomes in patients with cardiovascular disease in a non-cardiac surgery setting: systematic review and meta-analysis. *BMJ* 2016;352:i1351.
78. Shah A, Stanworth SJ, Docherty AB. Restrictive blood transfusion - is less really more? *Anaesthesia* 2020;75(4):433-437.
79. Jentzer JC, Lawler PR, Katz JN, et al. Red blood cell transfusion threshold and mortality in cardiac intensive care unit patients. *Am Heart J* 2021.
80. Wu WC, Smith TS, Henderson WG, et al. Operative blood loss, blood transfusion, and 30-day mortality in older patients after major noncardiac surgery. *Ann Surg* 2010;252(1):11-7.
81. Shaz BH. Red blood cells: beyond the transfusion. *Blood* 2019;133(25):2627-2628.
82. American Society of Anesthesiologists Task Force on Perioperative Blood M. Practice guidelines for perioperative blood management: an updated report by the American Society of Anesthesiologists Task Force on Perioperative Blood Management*. *Anesthesiology* 2015;122(2):241-75.
83. Kozek-Langenecker SA, Ahmed AB, Afshari A, et al. Management of severe perioperative bleeding: guidelines from the European Society of Anaesthesiology: First update 2016. *Eur J Anaesthesiol* 2017;34(6):332-395.
84. Hahn RG. The transfusion trigger in major surgery. *Acta Anaesthesiol Scand* 2018;62(2):270.
85. Vallet B, Adamczyk S, Barreau O, Lebuffe G. Physiologic transfusion triggers. *Best Pract Res Clin Anaesthesiol* 2007;21(2):173-81.
86. Hebert PC, Van der Linden P, Biro G, Hu LQ. Physiologic aspects of anemia. *Crit Care Clin* 2004;20(2):187-212.
87. Kei T, Mistry N, Tsui AKY, et al. Experimental assessment of oxygen homeostasis during acute hemodilution: the integrated role of hemoglobin concentration and blood pressure. *Intensive Care Med Exp* 2017;5(1):12.
88. Neuro VI. Perioperative covert stroke in patients undergoing non-cardiac surgery (NeuroVISION): a prospective cohort study. *Lancet* 2019;394(10203):1022-1029.

Paper I

Title page

Trends in abdominal aortic aneurysm surgery incidence, comorbidity, and mortality: a Danish nationwide cohort, 1996-2018

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Running head: AAA surgery, incidence, mortality trend

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Abstract

Background: Reports from Western populations have shown significant changes in the abdominal aortic aneurysm (AAA) epidemiology following the introduction of screening and endovascular AAA repair. We examined trends in the incidence, comorbidity, and mortality of AAA repair in Denmark, where AAA screening is not implemented.

Methods: Nationwide cohort study of prospectively collected data from population-based Danish registries covering 1996-2018. We identified 15,395 patients undergoing first-time AAA repair using the nationwide Danish Vascular Registry. Comorbidity was assessed by Charlson's Comorbidity Index (CCI). Multivariable Poisson and Cox regression were used to estimate incidence rate (IR) ratios and mortality rate ratios (MRR).

Results:

The overall AAA repair IR decreased by 24% from 1996 through 2018, mainly driven by a 53% IR reduction in ruptured AAA repairs in men. IRs decreased 52-63 % in age groups less than 70 years and increased by 81% among octogenarians. Ruptured AAA repair IR was highest in the population with moderate comorbidity, whereas intact AAA repair IR was highest in persons registered with very severe comorbidity. The CCI increased independently of age and sex by 0.9% annually for both surgery types. The adjusted five-year MRR in 2016-2018 *vs.* 1996-2000 was 0.46 (95% confidence interval (CI): 0.39-0.54) following ruptured and 0.51 (95% CI: 0.44-0.59) following intact AAA repair.

Conclusion:

AAA repair incidence decreases due to a reduction among males and a shift to an older population requiring intervention, suggestive of a cohort effect. Regardless of age and comorbidity, AAA repair mortality decreased markedly during the study period.

Key Words: vascular surgery, cohort, Danish Vascular Registry, survival.

Introduction

Abdominal aortic aneurysm (AAA) is a frequent and potentially fatal condition. AAA predominately affects males and is prevalent in 1.3-5 % of men aged 65-75 years. Recent studies reports decreasing prevalence in UK and Sweden,[1-3] in contrast to stable or even increasing prevalence in Denmark.[4-6] The United States (US) Centers for Disease Control ranked AAA as one of the top 15 causes of death in persons aged 85-89 years. Only a minority of ruptured AAA patients reach the hospital alive, and those who undergo emergency repair have a 30-50% mortality risk within 30 days.[7-9] Preemptive measures such as open- or endovascular AAA repair are effective. Number needed to treat with elective AAA repair to avoid one death is three.[10,11] However, open AAA repair carries risks of both death and ischemic events, especially cardiac, and therefore classified as high-risk surgery.[12,13] Consequently, AAA repair is recommended to AAA diameters above 5.5 cm in men and 5.0 cm in women for which the associated 1-year rupture risks equal the 30 day mortality associated with surgery. In the past 10-15 years, endovascular repair was introduced on a large scale to treat AAA with anatomy allowing for proximal and distal landing zones.[7,9] The perioperative mortality associated with endovascular AAA repair is lower than for open repair, however, the five-year mortality rate is comparable for the two techniques. The optimal surgical approach is thus unsettled.[14]

Considering the shift in treatment strategies of AAA and because AAA repair remains a high-risk procedure, it is imperative to provide data on trends in AAA- incidence, comorbidity, and mortality[15,16] to inform screening programs, trials, and observational studies seeking to identify treatments that may improve outcome.

By use of Danish nationwide data, we describe the changes in AAA repair incidence and mortality by sex, age, and Charlson's comorbidity index (CCI).

Methods

Study design and setting

This is a nationwide, longitudinal cohort study on prospectively collected data from the Danish Vascular Registry (DVR) and population-based medical and administrative registries.[17] The Danish National Health Service ensures tax-supported healthcare to all Danish citizens. The Danish Civil Registration System (CPR), established in 1968, assigns each citizen a unique ten-digit personal identifier that enables individual-level linkage across registries.[18] The Danish population was 5,781,190 persons in 2018.

Study population

We used the DVR to identify Danish patients undergoing first-time "index" aortoiliac aneurysm repair from January 1st, 1996 to December 31st, 2019. The DVR is the first nationwide Danish surgical database. It was established for quality improvement and research purposes and contains prospective data on all vascular procedures since January 1st, 1996.[19] Patient data are registered prospectively by the vascular surgeon with either direct data entry into the DVR, or into a paper chart which is later transferred to the DVR. Validation of the DVR has shown an external validity of 98.4% for AAAs and a high data quality superior to local administrative data.[20,21] The CPR was used to add information on sex, age, vital status, and migration, and was last updated on October 31st, 2019. From the Danish National Patient registry (DNPR), we gathered information on all somatic inpatient hospitalizations since 1977 and all hospital outpatient and emergency visits since 1995, including discharge diagnoses according to the International Classification of Diseases [Eighth Revision (ICD-8) until 1993 and Tenth Revision (ICD-10) hereafter].[22] The DNPR data set was updated December 31st, 2018. We also present smoking prevalence data extracted from a Danish Cancer Society review on smoking surveys in Denmark since 1953.[23]

Inclusion was based on the DVR and the criteria were *i)* operation code for aortoiliac reconstruction (Scandinavian coding system: PDG10-99, PDH10-35, PDQ10-30), *ii)* indication being either ruptured AAA or intact AAA (symptomatic or elective), *iii)* age above 40 and below 100 years. The age cut off has been used as inclusion criteria in randomized trials and was chosen to fit the general vascular surgery target population.[24] To ensure all patients were AAA repair naive at our study base, we excluded patients with AAA surgery before 1996 using the DNPR.

Comorbidity

At baseline, patients were described by CCI score using both in- and outpatient records in the DNPR since 1977. We exclusively used DNPR records concluded before the date of surgery. We grouped patients with a CCI score of 0, 1, 2, or 3 and above, as having normal, moderate, severe, or very severe comorbidity, respectively. We chose moderate CCI as the reference group because an AAA diagnosis accounts for one CCI point. Baseline characteristics disaggregated by calendar period are available in the supplemental digital content.

Mortality

We obtained vital status from the CPR and report 5-year survival following AAA surgery. Five-year survival was the primary analysis, as endovascular and open repair have a different short-term risk of death, but similar long-term.

Statistical analyses

Ruptured and intact AAA patients were analyzed separately, and only aggregated for the incidence reporting. We described the population by sex, age group (of six decades from 41 to 99 years), CCI group, and calendar period (5 intervals from 1996 to 2018). We exclusively included year 2019 in the age-and sex-standardized incidence plots.

Based on the CPR, we identified residents aged above 40 and below 100 years as of January 1st from 1996 to 2019. Using the DNPR, we computed each resident's annual accrued CCI score. We then calculated *i)* person-years at risk for AAA operation in the population as follow-up time resident in Denmark by age, sex, calendar year and CCI group; *ii)* crude incidence rates (IRs) by calendar-period, age, sex and CCI, *iii)* annual IRs standardized to the year 2000 age and sex composition of the Danish population by means of direct standardization; *iv*) and finally, incidence rate ratios (IRRs) using a log-linear Poisson regression model including the covariates: calendar-period, age-category, sex, and CCI group, offset by the logarithm of person-years at risk.

To compare the CCI of AAA patients to the Danish population, we calculated a mean observed CCI by each sex-, age-, and calendar year-strata and standardized this to the Danish population's corresponding strata of year 2000, as reference. We then used Poisson regression to estimate CCI ratios with 95 % confidence intervals. The same method was applied to compare CCI ratios across age groups with persons aged 71-75 years as reference.

We used the Kaplan-Meier estimator to compute mortality risks for each calendar-period. Patients were censored five years following surgery, on migration, or on October 31st, 2019, at the latest. Using Cox regression, we compared the five-year mortality rate ratio (MRR, via hazard ratios for death) of each calendar-period. We present the crude MRR, and MRRs adjusted for age, sex, CCI group, health care region, and stratified for surgical technique (open vs. endovascular repair). Visual inspection of Schoenfeld residuals indicated no gross violation of the proportional-hazards assumption for any of the analyses. In our secondary analysis, we also adjusted for smoking history, alcohol abuse, and care dependency. There were only missing data in the secondary analysis's discrete variables, and missingness was included as separate levels in these covariates.

The adequacy of the models for estimating incidence and mortality were examined by tentatively adding an interaction term between calendar year, as a linear predictor, and each of the remaining covariates. The effect of adding an interaction term was assessed by the likelihood ratio test.

Ethics

The study was approved by the Danish Data Protection Agency (record no: REG-144-2017) and by Statens Seruminstitut compliance. We did not need to obtain permission from the Danish Ethical Committee as this study did not involve contact to study participants.

Results

Incidence

We identified 15,291 first-time incident AAA repairs. Overall, there was 24 % decline in the sex- and age-standardized IR of AAA surgery from 25.1 (95 % CI, 23.2–27.1) per 100,000 person-years in 1996 to 19.1 (95 % CI, 17.4–20.9) per 100,000 person-years in 2018, figure 1. This was primarily attributable to a reduction of ruptured AAA surgery from 8.1 to 4.0 per 100,000 person-years (IRR, 0.48; 95 % CI, 0.39–0.60), whereas the intact AAA surgery IR was reduced from 17.0 to 15.1 per 100,000 person-years (IRR, 0.89; 95 % CI, 0.78–1.01). When adjusting the IR for sex, age and CCI group, a 20 % IR reduction was demonstrated for intact AAAs from 1996-1999 to 2015-2018 (IRR, 0.80; 95 % CI, 0.75–0.85; table 1). Intact AAA repair demonstrated a transient increase in IR from 2004 to 2011 which coincided with the introduction of endovascular AAA repair and an IR increase in persons aged 71-90, eFig.s 1 and 3.

Incidence by age and sex

Figure 1 panel b shows the trends in age-specific IRs. IRs were highest in the population aged 71-80 years for both types of surgery and was unaltered from 1996 to 2018 (IRR, 0.95; 95 % CI, 0.81 − 1.12). For persons aged 81-90 years the incidence increased by 78% (IRR, 1.81, 95% CI, 1.25 − 2.55) and was more than halved for persons aged 51-60 years (IRR, 0.37; 95% CI, 0.23 − 0.58) and 61-70 years (IRR, 0.48; 95% CI, 0.40 − 0.58). Variation in age-specific IR by calendar time was heterogeneous for both intact and ruptured AAA repair (p interaction < 0.001, eTable 1).

Females had 81% lower incidence of intact and 88% lower incidence of ruptured AAA repair compared with males, table 1. Overall, the average annual IR reduction throughout the study period was most pronounced in men ([annual IRR_{males}, 0.99; 95% CI, 0.99–0.99] *vs.* [annual $IRR_{females}$, 1.00; 95% CI, 1.00–1.01]; p interaction=0.001, fig. 2). We could not discern any sex heterogeneity in the IR trend for intact AAA repair (p interaction=0.45). For ruptured AAA repair, the IR decrease was most pronounced in men ([annual IRR_{males}, 0.96; 95% CI, 0.96–0.97] *vs.* [annual $IRR_{females}$, 0.98; 95% CI, 0.97–1.00]; p interaction<0.001).

Incidence by comorbidity

Intact AAA surgery was rare among persons registered with normal CCI (IRR, 0.04; 95% CI, 0.04–0.05), and most frequent in the population with very severe CCI (IRR, 1.29; 95% CI, 1.22–1.34), compared to moderate CCI. The IR of ruptured AAA repair was most frequent in the population with moderate CCI. The IR reduction over time was most pronounced for persons registered with normal CCI, eTable 1.

Patient Characteristics

Table 2 shows the baseline characteristics of patients operated for ruptured (n=4,508) and intact (n=10,887) AAA. Notably, more ruptured AAA patients had a normal CCI-score 38 % vs. 5.4 % of intact AAA patients. History of cardio- or cerebro-vascular (50 %) and chronic pulmonary (23 %) disease was common among AAA patients. Endovascular repair was a rare technique for ruptured (2 %) compared to intact (25 %) AAAs.

From 1996 to 2018 the mean age increased by 3.4 years in intact and 2.4 years in ruptured AAA patients. Compared to the Danish population, the age- and sex-adjusted CCI-score was 2.10-fold higher in intact (95% CI, 2.08−2.13) and 1.21-fold higher in ruptured (95 % CI, 1.18−1.25) AAA patients. The annual CCI increase was by 0.9 % among intact (95% CI, 0.7-1.1%) and by 0.9 % in ruptured (95% CI, 0.5-1.3%) AAAs, set against 2.4 % in the Danish population (CI 95%, 2.4-2.4%), figure 3a. The CCI score peaked in the octogenarians, for both AAA patients and the Danish population, followed by a declining trend in patients aged above 90 years, figure 3b. The CCI varied less across age groups in AAA-patients compared to the Danish population.

Mortality

Comparing 2015-2018 with 1996-1999, the adjusted 5-year MRR was 0.51 for intact (95% CI, 0.44-0.59) and 0.46 for ruptured (95 % CI, 0.39–0.54) AAA repair, table 3. Increasing age and CCI explained the difference between crude and adjusted MRR for both ruptured and intact AAA. Adjusting for smoking, alcohol abuse, care dependency or priority at time of surgery did not explain the mortality reduction, and even when we replaced missing parameters of these covariates with best- or worst-case scenarios (e.g., non-smoker or smoker) the MRR changed by 0.01 or less. And due to frequent missing data in the beginning of the study period we did not include these covariates in the primary analysis.

The 5-year mortality rate was also reduced in the Danish population aged 41-99 during the same period ([sex, age and CCI adjusted MRR, 0.56; 95 % CI, 0.56-0.56]; [sex and age adjusted MRR 0.72; 95% CI, 0.72-0.72]).

Mortality by age and sex

The five-year mortality rate increase per year in age was 5.7% following intact (95% CI, 5.1-6.3 %) and 5.3% following ruptured (95% CI, 4.7-5.9 %) AAA repair. The decrease in mortality rate over calendar time was consistent across age groups for intact AAA repair (p interaction=0.43). Following ruptured AAA repair, the MRR was higher in the last calendar periods in patients aged 81-90 years (MRR, 0.72; 95% CI, 0.51-1.00) than in age group 61-70 years (MRR, 0.40; 95% CI, 0.28-0.57) and 71-80 years (MRR, 0.44; 95% CI, 0.35-0.55); Figure 4; p interaction=0.006 (p interaction=0.02 with calendar year as linear predictor).

The adjusted five-year MRR in women *vs.* men was 1.06 in intact (95% CI, 0.96–1.17) (90-day MRR: 1.29; 95% CI, 1.06–1.57) and 1.03 in ruptured (95% CI, 0.92–1.15) AAA surgery (90-day MRR: 1.03; 95% CI, 0.90–1.18), eTable3. The crude five-year MRR indicated a higher mortality in women ([intact: 1.11; 95% CI, 1.01–1.22], [ruptured: 1.17; 95% CI, 1.05–1.31]), but adjusting for age alone explained the sex difference. The adjusted five-year MRR decreased over calendar time for both sexes ([annual MRR_{males}, 0.96; 95% CI, 0.96–0.97] *vs.* [annual $MRR_{females}$, 0.95; 95% CI, 0.93–0.96]; p interaction=0.08).

Mortality by comorbidity

Compared with moderate comorbidity, severe comorbidity associated to an adjusted five-year MRR of 1.29 (95 % CI, 1.16-1.42) following intact and 1.20 (95% CI, 1.10-1.39) following ruptured AAA repair; and very severe comorbidity associated to an adjusted MRR of 1.90 (95% CI, 1.74-2.08) in intact and 1.52 (95% CI, 1.35-1.70) in ruptured AAA repair, eTable 3.

Discussion

We found a 24% decline in the overall AAA repair IR in Denmark from 1996 to 2018, mostly attributable to a marked IR reduction of ruptured AAA repair in men. The IR of intact AAA repair was highest in persons with very severe comorbidity, whereas ruptured AAA repair incidence was highest in the population with moderate comorbidity. We observed considerable age heterogeneity in the incidence change over calendar time with a decrease among persons aged below 75 years and an increase in those aged above 75 years. Comorbidity, defined according to CCI, increased independently of age and sex during the study period. The adjusted five-year mortality rate was halved following both intact and ruptured AAA repair. In ruptured AAAs, the largest mortality reduction was seen in the youngest age groups and was less pronounced in the octogenarians. CCI was strongly associated to 5-year mortality, while sex was not.

Incidence findings in relation to other studies

During the 20th century, AAA was a disease rising in the US, United Kingdom (UK), and Denmark, as demonstrated by a surge in AAA-related deaths.[25-27] A later report covering 1994 to 2010 suggested a decline in the AAA specific mortality in Western countries, but not Denmark.[27,28] Reduction on tobacco use was put forward as a possible explanation for these changes because smoking strongly associates to AAA development and accelerated AAA expansion.[29,30] In Denmark, 78 % of men and 40 % of women smoked daily in 1953. While male smoking prevalence decreased in the 1950-1960s, female prevalence increased through to 1970 before declining.[23] In the 1960s, Danish women had the highest recorded smoking prevalence in the world.[31] We interpret, similar to what has been observed for lung cancer[32], that a cohort effect of smoking contributes to the age and sex heterogeneity of the AAA repair incidence trends in this study. Specifically, a large proportion of persons born in the interwar

period (1920-1940) have been exposed to tobacco in the 1950-60s at the age of 20-40. It explains the high incidence within the youngest age bands in the 1990s, and as persons from that birth cohort move through time, the highest incidence follows along. At the end of our study period, the incidence was halved in the youngest age groups and doubled in those aged >80 years. The latter may also reflect that surgeons accept increasing age for surgery following advances in surgical technique and perioperative care. However, we believe that change in indications for surgery only explain little of the 52-63 % IR reduction in persons aged 50-70 years because an expanding AAA cannot be treated conservatively. This finding is supported by UK data on AAA hospital admission incidence, which has decreased in patients aged below 75 years and an increase in those above 75 years.[28]

While numerous risk factors for AAA development have been described,[33] few risk factors for AAA expansion and rupture have been identified, with smoking and female sex being the most significant.[30,34] This likely explains why we did not see a sex heterogeneity in the change of intact AAA repair incidence. Our finding is in agreement with UK AAA mortality statistics,[28,35] and trends in Swedish AAA repair incidence.[9] An alternative explanation may be that an increasing number of women entered the labor marked during the 20th century with a potentially increasing exposure to stress. The sex difference makes previously proposed environmental explanations for AAA development, such as carbon monoxide emission[35] less plausible, as this would likely affect both sexes equally. A transition from ruptured to intact AAA repair may have occurred due to increased use of ultrasound and computed tomography disclosing AAAs coincidentally before rupture. To our knowledge, use of abdominal radiography is not more common in men than in women, and is unlikely to explain the sex heterogeneity since this was seen for both ruptured and total AAA repairs.

Mortality findings in relation to other studies

UK mortality statistics have also shown a 50% reduction in AAA- and aortic dissection-related deaths in persons aged below 75 years and a 25 % reduction in those aged 75 years or above.[35] That mortality reduction was observed between 1997 and 2009, which coincides with the age-group separation we observed for ruptured AAAs. The reasons for the age heterogeneity are unclear.[35] The introduction of endovascular repair does not seem to explain our findings because this was an exceedingly rare technique in ruptured AAAs. It appears that ruptured AAA mortality has decreased in all age strata of our cohort, but there seems to be a time-shift between them. All age bands approach a 50% mortality rate reduction but occurs with a delay in the oldest patients undergoing ruptured AAA repair, which could be a cohort effect, e.g., of smoking or unknown patient risk factors such as social status. Another possible explanation is that AAA rupture is a surgical emergency where the patient often encounters recurrent episodes of hypovolemic shock that puts high demand on cardiovascular compensatory mechanisms to maintain tissue oxygenation. The physiological reserve decreases with age, which makes it harder to achieve a 50% mortality reduction given the higher absolute mortality in the octogenarians.

A WHO mortality database study 1994-2010 showed a global reduction in age-standardized AAA mortality, with a few exceptions, including Danish women. Our study shows that the mortality trend has now reversed in females too. EU countries, including the UK, demonstrate a higher female *vs.* male in-hospital mortality following elective AAA repair.[36,37] We also found a higher 90-day mortality rate in females undergoing intact AAA repair. However, with longer follow-up, female AAA repair survival approached that of male patients, possibly reflecting that life expectancy in general is longer among women than among men. Possible bias in the UK- and Swedish data is that these countries implemented

male AAA screening in 2006-2009. Thus, men scheduled for elective surgery may be otherwise healthy, whereas females are often referred for elective AAA surgery due to co-existing disease, e.g., cancer, that prompts the radiography disclosing the AAA. Notably, previous findings lack adjustment for comorbidity or have high missingness of comorbidity data. The impact of female gender warrants further study and follow-up to inform the decision of when to pursue AAA repair, and whether to screen for AAA in women.[10,16,38,39]

We performed a survival analysis of the general Danish population aged 41-99 that also demonstrated a marked mortality rate reduction during the study period. So, generally improved health in the Danish population may explain some, but not all, of the improved survival following AAA repair. Thus, there seems to be an excess improved survival unique to the AAA population that needs further exploration.

Strength and Limitations

Strengths include the longitudinal design covering 23 years of data from well-validated registries that enable individual-level linkage and estimation of comorbidity-specific incidences. AAA coding rules and classifications were unaltered during the study period. Our estimates are comparable to other studies. In addition, because screening is not implemented in Denmark, as opposed to the US, UK, and Sweden, our data provide unique information on the trends in incident AAAs requiring repair in a country with a historically high smoking prevalence where the incidence is not affected by screening programs.[31]

Around 2010, the female AAA diameter threshold for repair was lowered from 5.5 to 5.0 cm, which likely affected the incidence. However, a rather stable female incidence in both intact and ruptured AAA repairs suggests that it may have had limited significance. The temporal increase in CCI may partially be explained by a higher propensity to register

diagnoses on discharge, although reimbursement for registering has been a part of the Danish health care system throughout the study period.

Clinical implications and conclusion

AAAs also occur in non-smokers,[30] but smoking shortens time to AAA development in genetically predisposed.[28] Thus, AAAs requiring surgery will continue to occur, but will probably present at a later age and a reduced rate, especially in males. This may have important implications for the future hospital caseload, and the design and cost-effectiveness of screening programs. Given the marked mortality reduction we observed and the failure to show an all-cause mortality benefit from AAA screening in the previous Danish VIVA trial,[6] continuous reevaluation of the threshold for AAA repair seems prudent.[40] However, a proposed randomized trial assigning men with AAAs 5.5 to 6.5 cm to treatment or watchful waiting lacked equipoise among surgeons.[41]

Our results should not discourage repair of ruptured AAA in the octogenarians. Survival of ruptured AAA has improved in the elderly, but apparently with a delay/time-shift, indicating that mortality has decreased due to altered patients risk factors more than due to changes in surgical technique or proficiency.

In conclusion, Danish AAA repair incidence is decreasing due to a reduction in males undergoing repair and a shift toward treatment of an older population, which may be explained by a cohort effect of smoking. Both crude and adjusted mortality was markedly reduced over the study period. Thus, reasons for the mortality reduction are unclear.

Tables

Table 1. Incidence rate of Intact (top) and Ruptured (bottom) AAA repair

Intact AAA	Cases	Person–Years	Crude IR per 100,000	IRR (95% CI)
Calendar year				
1996 – 1999	1,529	9,800,052	15.6 (14.8–16.4)	Reference
2000 – 2004	1,824	12,697,911	14.4 (13.7–15.0)	0.85 (0.79–0.91)
2005 – 2009	2,499	13,392,843	18.7 (17.9–19.4)	0.99 (0.93–1.06)
2010 – 2014	2,792	14,075,863	19.8 (19.1–20.6)	0.93 (0.88–0.99)
2015 – 2018	2,243	11,712,246	19.2 (18.4–19.9)	0.80 (0.75–0.85)
Age, years				
41 – 50	75	18,192,142	0.4 (0.3–0.5)	0.03 (0.03–0.04)
51 – 60	849	16,844,751	5.0 (4.7–5.4)	0.26 (0.25–0.28)
61 – 70	3,961	13,398,250	29.6 (28.6–30.5)	Reference
71 – 80	5,076	8,678,501	58.5 (56.9–60.1)	1.49 (1.43–1.55)
81 – 90	918	3,947,749	23.3 (21.7–24.8)	0.56 (0.52–0.60)
91 – 99	8	617,522	1.3 (0.4–2.2)	0.04 (0.02–0.08)
Sex				
Male	9,072	29,731,151	30.5 (29.9–31.1)	Reference
Female	1,815	31,947,764	5.7 (5.4–5.9)	0.19 (0.18–0.20)
CCI group				
Normal	589	43,644,149	1.3 (1.2–1.5)	0.04 (0.04–0.05)
Moderate	3,972	8,162,109	48.7 (47.2–50.2)	Reference
Severe	2,687	5,332,614	50.4 (48.5–52.3)	0.97 (0.92–1.02)
Very Severe	3,639	4,540,043	80.2 (77.5–82.8)	1.29 (1.23–1.35)

Ruptured AAA	Cases	Person–Years	Crude IR per 100,000	IRR (95% CI)
Calendar year				
1996 – 1999	880	9,800,052	9.0 (8.4–9.6)	Reference
2000 – 2004	1,114	12,697,911	8.8 (8.3–9.3)	0.96 (0.88–1.05)
2005 – 2009	1,000	13,392,843	7.5 (7.0–7.9)	0.78 (0.71–0.85)
2010 – 2014	907	14,075,863	6.4 (6.0–6.9)	0.63 (0.57–0.69)
2015 – 2018	607	11,712,246	5.2 (4.8–5.6)	0.46 (0.42–0.51)
Age, years				
41 – 50	26	18,192,142	0.1 (0.1–0.2)	0.02 (0.01–0.02)
51 – 60	293	16,844,751	1.7 (1.5–1.9)	0.17 (0.15–0.20)
61 – 70	1,395	13,398,250	10.4 (9.9–11.0)	Reference
71 – 80	2,088	8,678,501	24.1 (23.0–25.1)	2.24 (2.09–2.40)
81 – 90	692	3,947,749	17.5 (16.2–18.8)	1.80 (1.64–1.97)
91 – 99	14	617,522	2.3 (1.1–3.5)	0.31 (0.18–0.52)
Sex				
Male	3,923	29,731,151	13.2 (12.8–13.6)	Reference
Female	585	31,947,764	1.8 (1.7–2.0)	0.12 (0.11–0.13)
CCI group				
Normal	1,727	43,644,149	4.0 (3.8–4.1)	0.46 (0.43–0.49)
Moderate	1,278	8,162,109	15.7 (14.8–16.5)	Reference
Severe	746	5,332,614	14.0 (13.0–15.0)	0.81 (0.74–0.89)
Very Severe	757	4,540,043	16.7 (15.5–17.9)	0.77 (0.71–0.85)

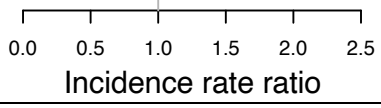


Table 2. Baseline characteristics of patients undergoing surgery for ruptured and intact AAA surgery in Denmark 1996-2018.

	Intact AAA (n=10,887)	Ruptured AAA (n=4,508)	Total (n=15,395)
Age – mean (sd)	71.5 (7.3)	73.0 (7.9)	71.9 (7.5)
Male sex – no. (%)	9,072 (83)	3,923 (87)	12,995 (84)
Charlson's comobidity index group – no. (%)			
Normal	589 (5)	1,727 (38)	2,316 (15)
Moderate	3972 (36)	1278 (28)	5,250 (34)
Severe	2687 (25)	746 (17)	3,433 (22)
Very Severe	3639 (33)	757 (17)	4,396 (29)
Chronic pulmonary disease – no. (%)	2,445 (22)	1,053 (23)	3,498 (23)
Diabetes – no. (%)	1,225 (11)	425 (9)	1,650 (11)
Renal disease – no. (%)	368 (3)	108 (2)	476 (3)
Cardiovascular disease – no. (%)			
Any	5,554 (51)	2,109 (47)	7,663 (50)
Angina history	2,680 (25)	823 (18)	3,503 (23)
Stroke or TCI	1,718 (16)	731 (16)	2,449 (16)
CABG or PCI	1,954 (18)	531 (12)	2,485 (16)
Congestive heart failure	870 (8)	355 (8)	1,225 (8)
Heart valve disease	548 (5)	170 (4)	718 (5)
Acute myocardial infarction	2,139 (20)	782 (17)	2,921 (19)
Pacemaker or ICD	446 (4)	119 (3)	565 (4)
Other	840 (8)	387 (9)	1,227 (8)
Cancer history – no. (%)	1,841 (17)	499 (11)	2,340 (15)
Care dependency – no. (%)			
Independent	10,131 (93)	3,572 (79)	13,703 (89)
Home care	547 (5)	317 (7)	864 (6)
Nursing home	28 (0)	88 (2)	116 (1)
missing	181 (2)	531 (12)	712 (5)
Tobacco use – no. (%)			
None	1,790 (16)	705 (16)	2,495 (16)
Previous (>6 weeks)	4225 (39)	907 (20)	5,132 (33)
Current smoker	4527 (42)	1514 (34)	6,041 (39)
Missing	345 (3)	1382 (31)	1,727 (11)
Alcohol abuse – no. (%)			
None	10,022 (92)	3,628 (80)	13,650 (89)
More than > 5 units/day	216 (2)	131 (3)	347 (2)
Missing	649 (6)	749 (17)	1,398 (9)
Endovascular repair – no. (%)	2669 (25)	106 (2)	2,775 (18)

TCI, transient cerebral ischaemia. CABG, Coronary artery bypass. PCI, Percutaneous coronary intervention. ICD, implantable cardioverter-defibrillator. Data source for Charlson's comorbidity index was the Danish National Patient Registry, DNPR. Other disease items were the union set of data in the DNPR and the Danish Vascular Registry, DVR. Dependency, tobacco and alcohol history was based on the DVR. Other cardiac disease included: cardiomyopathy (DNPR); unspecified cardiac surgery history with no current symptoms (DVR); AMI< 6 weeks, unstable angina or congestive heart failure (DVR); AMI> 6 weeks or asymptomatic arrythmia (DVR), stable angina or heart medication (DVR). Full list of International Classification of Diseases-codes uses from the DNPR is available in the supplementary digital content as well as a full baseline table of the 19 CCI items. When excluding non-melanoma skin cancer, the prevalence of patients with a history of any cancer was 14 % in intact and 8 % in ruptured AAA surgery. 2,020 (18 %) of intact AAA patients had symptomatic aneurisms. Less than 2 % were iliac repair without aortic involvement in both groups.

Table 3. Five-year mortality following AAA surgery in Denmark 1996-2018

Calendar period of surgery	No. of deaths	No. of patients	Mortality risk % (95 % CI)	Mortality rate ratio (95% CI)	
				Crude	Adjusted[a]
Intact AAA					
1996 - 1999	539	1,529	35.3 (32.8 - 37.6)	Reference	Reference
2000 - 2004	584	1,824	32.0 (29.8 - 34.1)	0.88 (0.79 - 0.99)	0.84 (0.74 - 0.94)
2005 - 2009	741	2,499	29.7 (27.8 - 31.4)	0.80 (0.72 - 0.90)	0.67 (0.60 - 0.75)
2010 - 2014	678	2,792	24.3 (22.7 - 25.9)	0.63 (0.56 - 0.71)	0.51 (0.46 - 0.58)
2015 - 2018	337	2,243	24.7 (22.4 - 27.0)	0.65 (0.56 - 0.74)	0.51 (0.44 - 0.59)
Ruptured AAA					
1996 - 1999	555	880	63.1 (59.7 - 66.1)	Reference	Reference
2000 - 2004	716	1,114	64.3 (61.3 - 67.0)	1.04 (0.93 - 1.16)	1.00 (0.90 - 1.12)
2005 - 2009	550	1,000	55.0 (51.8 - 58.0)	0.81 (0.72 - 0.91)	0.76 (0.67 - 0.86)
2010 - 2014	474	907	52.3 (48.9 - 55.4)	0.71 (0.63 - 0.81)	0.64 (0.57 - 0.73)
2015 - 2018	207	607	39.7 (35.4 - 43.8)	0.50 (0.43 - 0.59)	0.46 (0.39 - 0.54)

[a]Adjusted for age, sex, Charlson's comorbidity index, surgical center in a Cox regression stratified for open versus endovascular repair.

Figures

Figure 1. Standardized incidence rate (A) and age specific incidence (B) for AAA surgery in Denmark 1996-2019

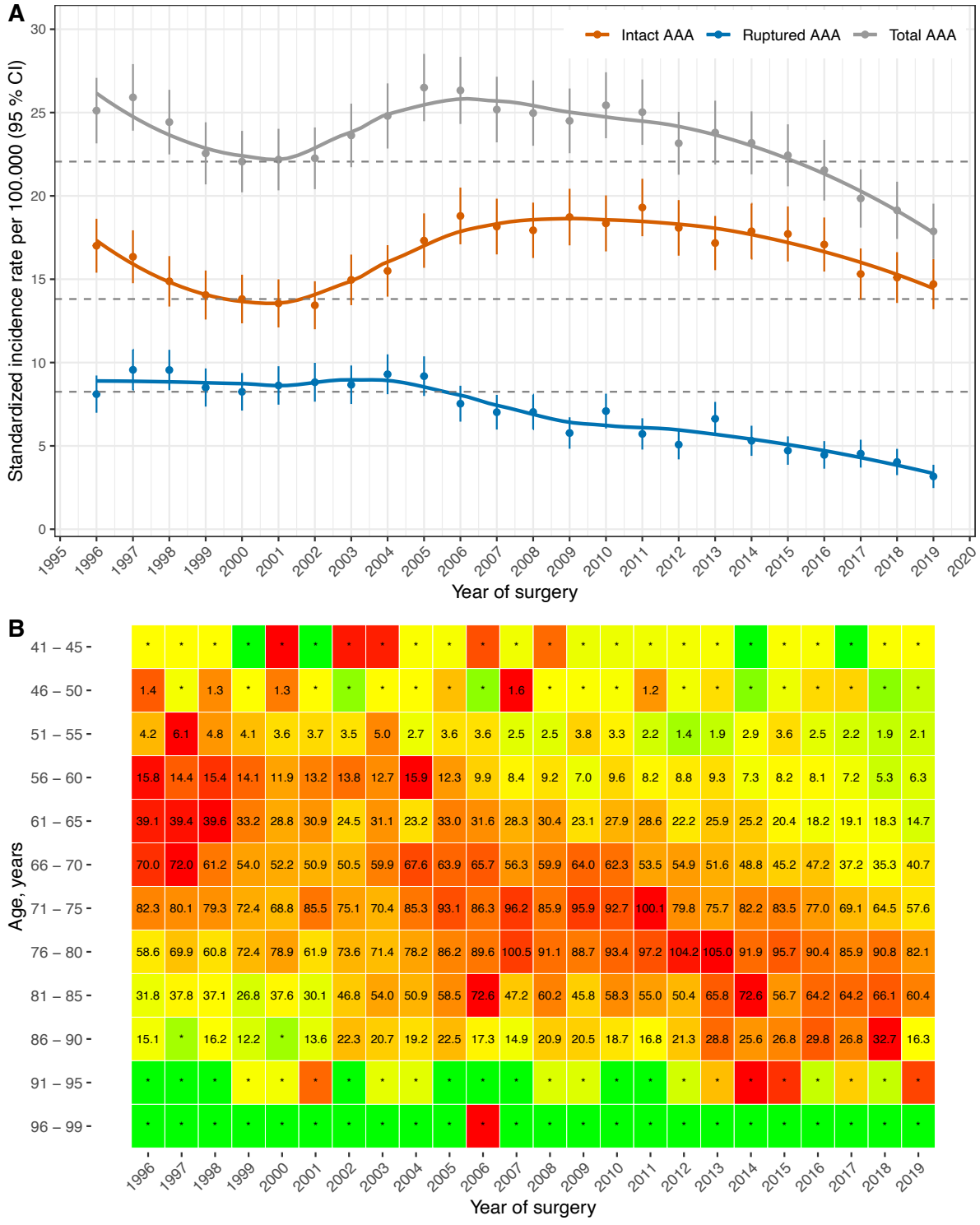


Figure 2. Sex-specific trends in age-standardized incidence rate of AAA repair in Denmark 1996-2019 (A) and smoking prevalence in Denmark 1953-2018 (B).

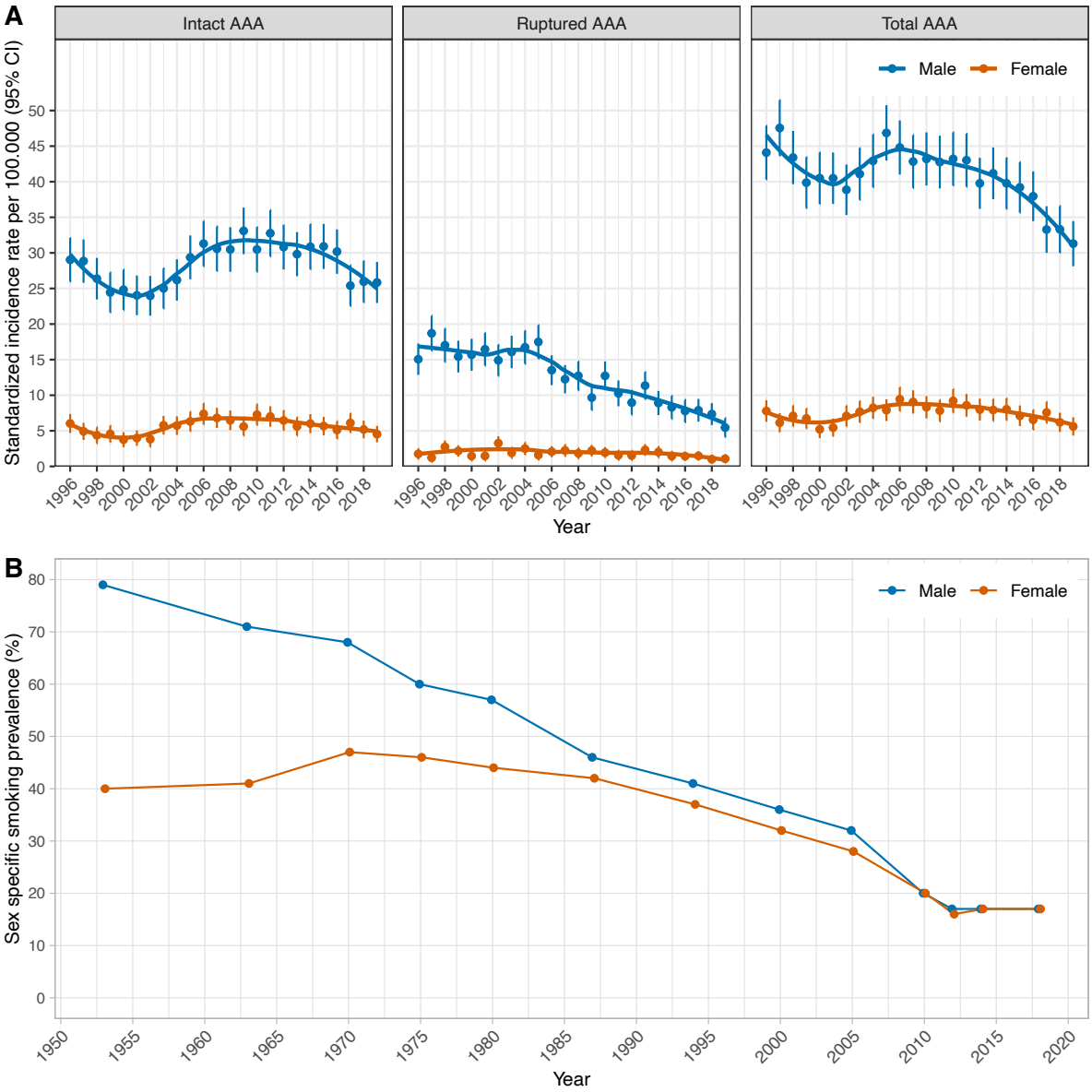


Figure 3. Trend in Charlson's comorbidity index score of AAA surgery relative to the Danish population in calendar year 2000 (A) and relative to age group 71-75 (B).

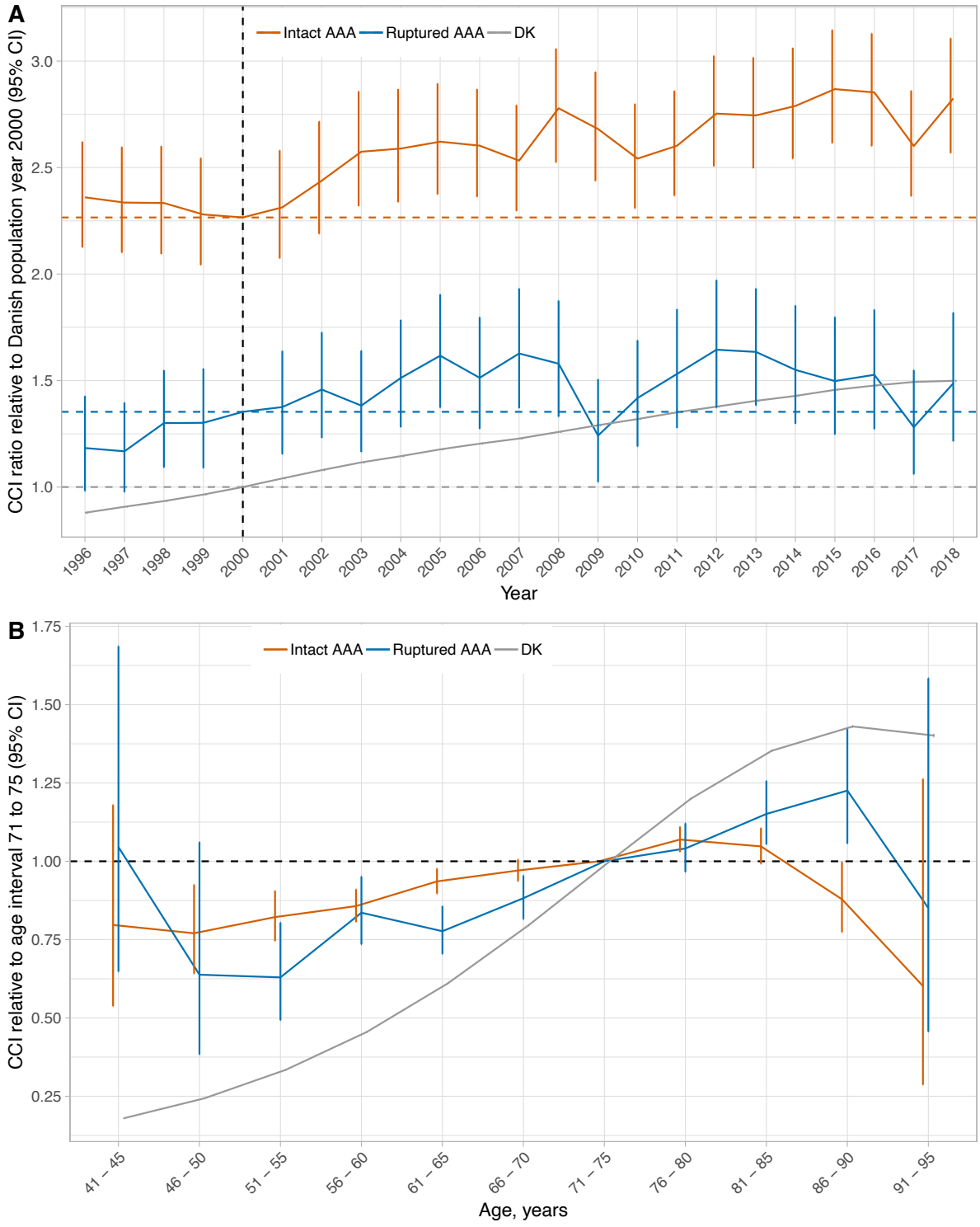


Figure 4. Trends in five-year mortality rate ratio following AAA repair stratified by age.

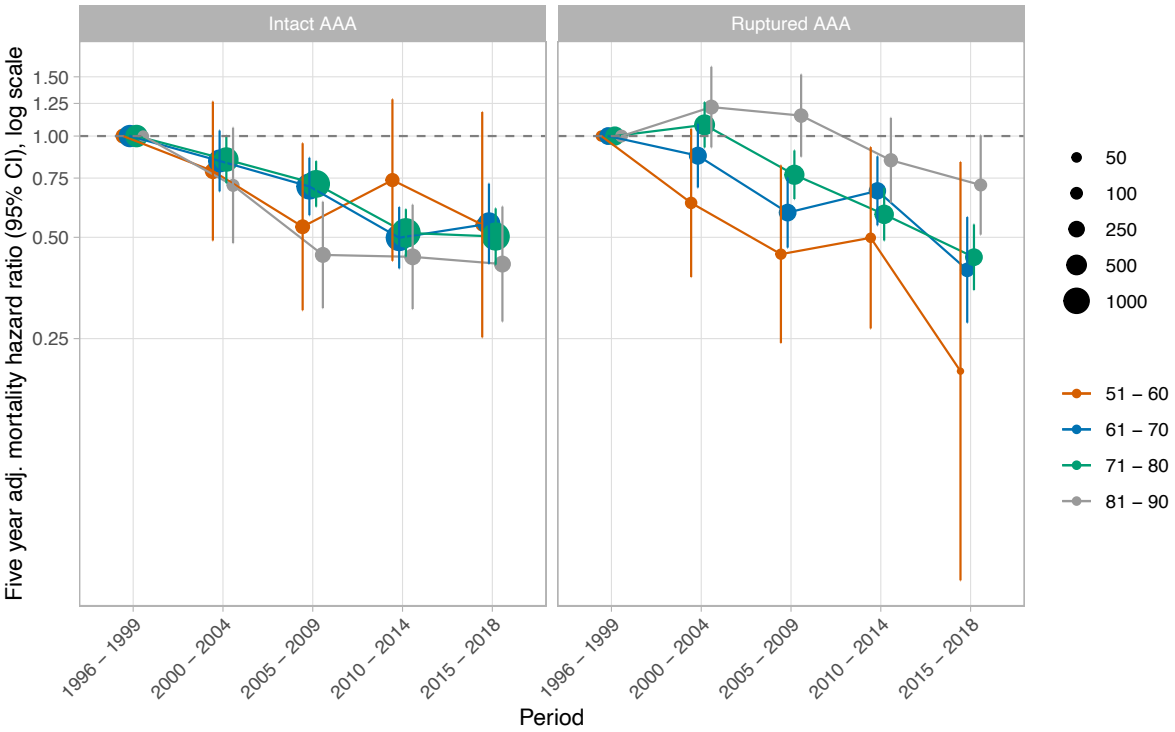


Figure Legends

Figure 1

Panel B: Incidence rate standardized to the age and sex distribution of the Danish population aged above 40 and below 100 years in 2000. Dashed lines indicate incidence rate in reference year 2000. **Panel B:** age specific incidence rates of total AAA surgery by calendar year. The crude incidence rates per 100.000 person-years are presented in each tile. Tiles are colored by each row (age band) in a red-yellow-green gradient from the highest value, red, to incidence rates of 50 % of the highest value, yellow, to incidence rate of 0 % percent of the highest value, green.

Figure 2

Panel a: Sex-specific trends in incident AAA repair, standardized to the age distribution of the Danish population in 2000. **Panel B**: displays the sex-specific trends in smoking

prevalence. Data were extracted from a Danish Cancer Society review on smoking surveys in Denmark since 1953.[23]

Figure 3

5-year mortality rate ratios by age decade and calendar period (reference year 1996-2000); adjusted for age, sex, Charlson's comorbidity index, and surgical center in a Cox regression stratified for open versus endovascular repair. Points are sized by number of surgeries performed.

Figure 4.

Panel A: Trends in Charlson's comorbidity index standardized to the age- and sex distribution of the Danish population in year 2000. **Panel B**: Charlson's comorbidity index standardized to calendar year and sex distribution of persons aged 71-75 years within each population (intact and ruptured abdominal aortic repair, and the Danish population).

References

1. Heather BP, Poskitt KR, Earnshaw JJ, Whyman M, Shaw E. Population screening reduces mortality rate from aortic aneurysm in men. *Br J Surg* 2000;87(6):750-3.
2. Oliver-Williams C, Sweeting MJ, Turton G, et al. Lessons learned about prevalence and growth rates of abdominal aortic aneurysms from a 25-year ultrasound population screening programme. *Br J Surg* 2018;105(1):68-74.
3. Svensjo S, Bjorck M, Gurtelschmid M, et al. Low prevalence of abdominal aortic aneurysm among 65-year-old Swedish men indicates a change in the epidemiology of the disease. *Circulation* 2011;124(10):1118-23.
4. Lindholt JS, Juul S, Fasting H, Henneberg EW. Hospital costs and benefits of screening for abdominal aortic aneurysms. Results from a randomised population screening trial. *Eur J Vasc Endovasc Surg* 2002;23(1):55-60.
5. Lindholt JS, Rasmussen LM, Sogaard R, et al. Baseline findings of the population-based, randomized, multifaceted Danish cardiovascular screening trial (DANCAVAS) of men aged 65-74 years. *Br J Surg* 2019;106(7):862-871.
6. Lindholt JS, Sogaard R. Population screening and intervention for vascular disease in Danish men (VIVA): a randomised controlled trial. *Lancet* 2017;390(10109):2256-2265.
7. Kent KC. Clinical practice. Abdominal aortic aneurysms. *N Engl J Med* 2014;371(22):2101-8.
8. Brown LC, Powell JT. Risk factors for aneurysm rupture in patients kept under ultrasound surveillance. UK Small Aneurysm Trial Participants. *Ann Surg* 1999;230(3):289-96; discussion 296-7.
9. Lilja F, Mani K, Wanhainen A. Editor's Choice - Trend-break in Abdominal Aortic Aneurysm Repair With Decreasing Surgical Workload. *Eur J Vasc Endovasc Surg* 2017;53(6):811-819.
10. Bergqvist D, Bjorck M, Wanhainen A. Abdominal aortic aneurysm--to screen or not to screen. *Eur J Vasc Endovasc Surg* 2008;35(1):13-8.
11. Wilmink AB, Quick CR, Hubbard CS, Day NE. Effectiveness and cost of screening for abdominal aortic aneurysm: results of a population screening program. *J Vasc Surg* 2003;38(1):72-7.
12. Duceppe E, Parlow J, MacDonald P, et al. Canadian Cardiovascular Society Guidelines on Perioperative Cardiac Risk Assessment and Management for Patients Who Undergo Noncardiac Surgery. *Can J Cardiol* 2017;33(1):17-32.
13. Schwarze ML, Barnato AE, Rathouz PJ, et al. Development of a list of high-risk operations for patients 65 years and older. *JAMA Surg* 2015;150(4):325-31.
14. Lederle FA, Kyriakides TC, Stroupe KT, et al. Open versus Endovascular Repair of Abdominal Aortic Aneurysm. *N Engl J Med* 2019;380(22):2126-2135.
15. Bahia SS, Holt PJ, Jackson D, et al. Systematic Review and Meta-analysis of Long-term survival After Elective Infrarenal Abdominal Aortic Aneurysm Repair 1969-2011: 5 Year Survival Remains Poor Despite Advances in Medical Care and Treatment Strategies. *Eur J Vasc Endovasc Surg* 2015;50(3):320-30.
16. O'Donnell TFX, Landon BE, Schermerhorn ML. AAA Screening Should Be Expanded. *Circulation* 2019;140(11):889-890.
17. Sorensen HT. Regional administrative health registries as a resource in clinical epidemiology: A study of options, strengths, limitations and data quality provided with examples of use. *Int J Risk Saf Med* 1997;10(1):1-22.

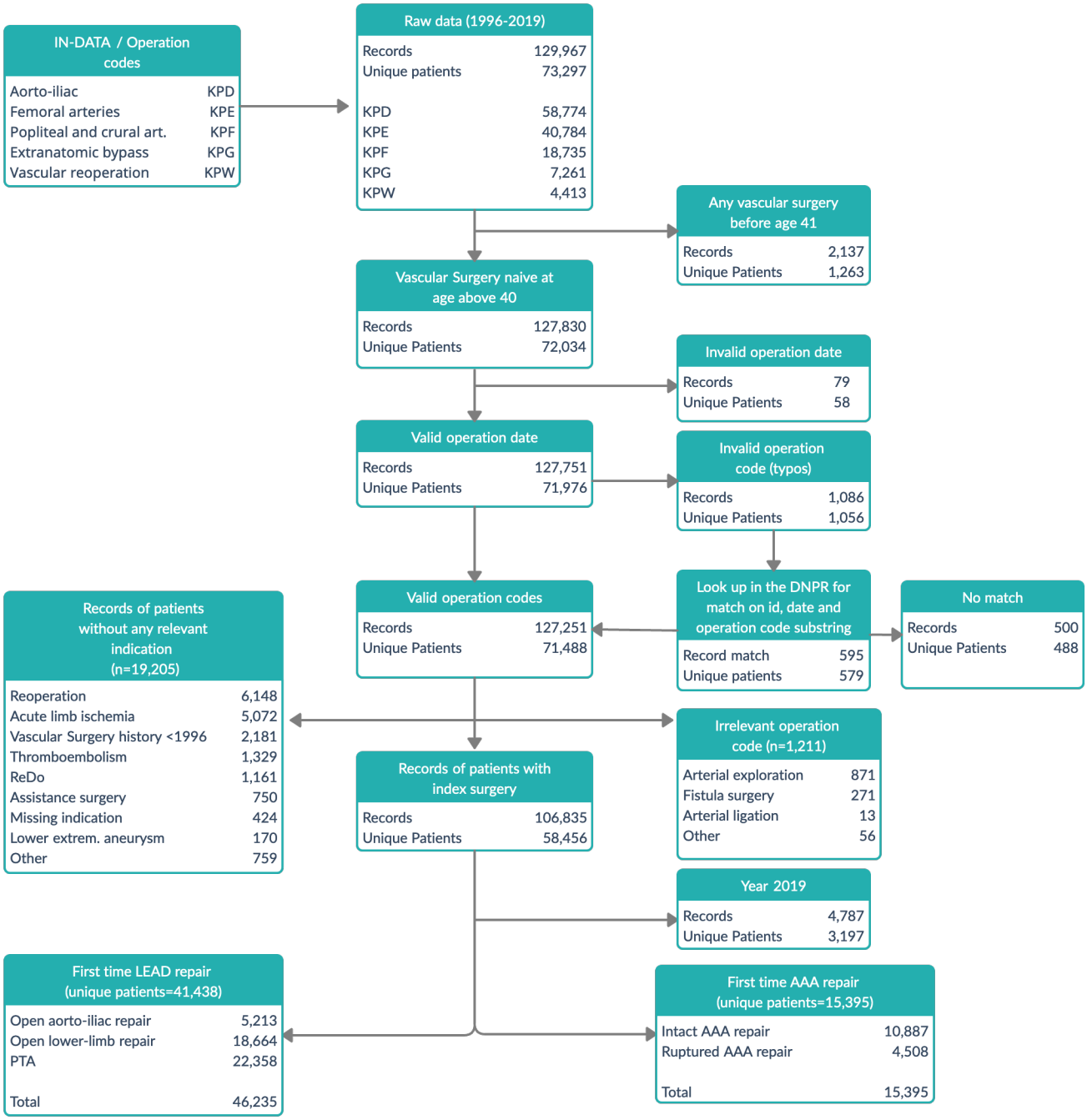
18. Schmidt M, Pedersen L, Sorensen HT. The Danish Civil Registration System as a tool in epidemiology. *Eur J Epidemiol* 2014;29(8):541-9.
19. Eldrup N, Cerqueira C, de la Motte L, Rathenborg LK, Hansen AK. The Danish Vascular Registry, Karbase. *Clin Epidemiol* 2016;8:713-718.
20. Altreuther M, Menyhei G. International Validation of the Danish Vascular Registry Karbase: A Vascunet Report. *Eur J Vasc Endovasc Surg* 2019;58(4):609-613.
21. Laustsen J, Jensen LP, Hansen AK, Danish National Vascular R. Accuracy of clinical data in a population based vascular registry. *Eur J Vasc Endovasc Surg* 2004;27(2):216-9.
22. Schmidt M, Schmidt SA, Sandegaard JL, et al. The Danish National Patient Registry: a review of content, data quality, and research potential. *Clin Epidemiol* 2015;7:449-90.
23. Clemmensen KK, Lynge E, Clemmensen IH. Nationwide tobacco surveys and sales data in Denmark from 1920 to 2010. *Dan Med J* 2012;59(6):A4448.
24. Moller A, Nielsen HB, Wetterslev J, et al. Low vs high hemoglobin trigger for transfusion in vascular surgery: a randomized clinical feasibility trial. *Blood* 2019;133(25):2639-2650.
25. Eickhoff JH. Incidence of diagnosis, operation and death from abdominal aortic aneurysms in Danish hospitals: results from a nation-wide survey, 1977-1990. *Eur J Surg* 1993;159(11-12):619-23.
26. Fowkes FG, Macintyre CC, Ruckley CV. Increasing incidence of aortic aneurysms in England and Wales. *BMJ* 1989;298(6665):33-5.
27. Sidloff D, Stather P, Dattani N, et al. Aneurysm global epidemiology study: public health measures can further reduce abdominal aortic aneurysm mortality. *Circulation* 2014;129(7):747-53.
28. Choke E, Vijaynagar B, Thompson J, et al. Changing epidemiology of abdominal aortic aneurysms in England and Wales: older and more benign? *Circulation* 2012;125(13):1617-25.
29. Lederle FA, Nelson DB, Joseph AM. Smokers' relative risk for aortic aneurysm compared with other smoking-related diseases: a systematic review. *J Vasc Surg* 2003;38(2):329-34.
30. Norman PE, Curci JA. Understanding the effects of tobacco smoke on the pathogenesis of aortic aneurysm. *Arterioscler Thromb Vasc Biol* 2013;33(7):1473-7.
31. Pisinger C, Jorgensen T, Toft U. A multifactorial approach to explaining the stagnation in national smoking rates. *Dan Med J* 2018;65(2).
32. Skuladottir H, Olsen JH, Hirsch FR. Incidence of lung cancer in Denmark: historical and actual status. *Lung Cancer* 2000;27(2):107-18.
33. Kent KC, Zwolak RM, Egorova NN, et al. Analysis of risk factors for abdominal aortic aneurysm in a cohort of more than 3 million individuals. *J Vasc Surg* 2010;52(3):539-48.
34. Chaikof EL, Dalman RL, Eskandari MK, et al. The Society for Vascular Surgery practice guidelines on the care of patients with an abdominal aortic aneurysm. *J Vasc Surg* 2018;67(1):2-77 e2.
35. Anjum A, Powell JT. Is the incidence of abdominal aortic aneurysm declining in the 21st century? Mortality and hospital admissions for England & Wales and Scotland. *Eur J Vasc Endovasc Surg* 2012;43(2):161-6.

36. Budtz-Lilly J, Venermo M, Debus S, et al. Editor's Choice - Assessment of International Outcomes of Intact Abdominal Aortic Aneurysm Repair over 9 Years. *Eur J Vasc Endovasc Surg* 2017;54(1):13-20.
37. Sidloff DA, Saratzis A, Sweeting MJ, et al. Sex differences in mortality after abdominal aortic aneurysm repair in the UK. *Br J Surg* 2017;104(12):1656-1664.
38. LeFevre ML, Force USPST. Screening for abdominal aortic aneurysm: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2014;161(4):281-90.
39. Sweeting MJ, Masconi KL, Jones E, et al. Analysis of clinical benefit, harms, and cost-effectiveness of screening women for abdominal aortic aneurysm. *Lancet* 2018;392(10146):487-495.
40. Earnshaw JJ. The Indication for Elective Repair of Abdominal Aortic Aneurysm Should Be Reviewed. *Eur J Vasc Endovasc Surg* 2021;61(1):7-8.
41. Oliver-Williams C, Sweeting MJ, Jacomelli J, et al. Safety of Men With Small and Medium Abdominal Aortic Aneurysms Under Surveillance in the NAAASP. *Circulation* 2019;139(11):1371-1380.

Table of content – Study I

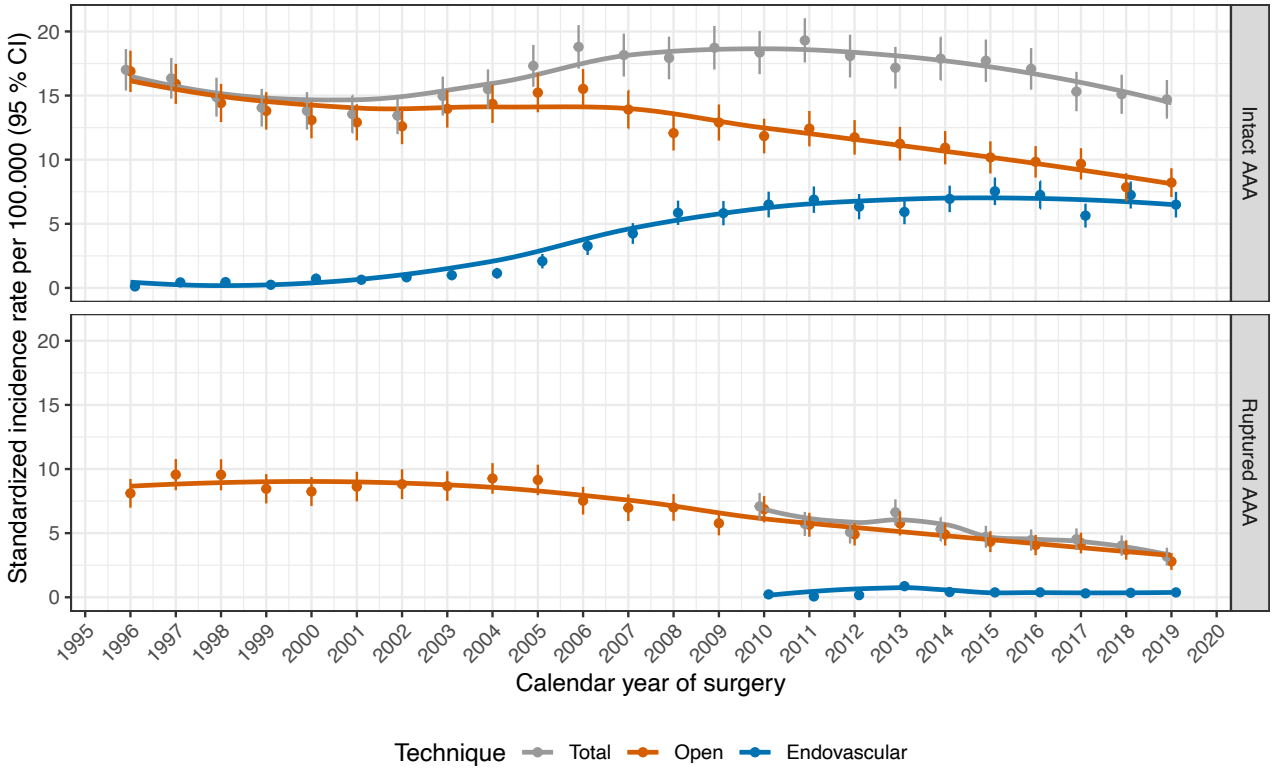
Supplemental eFigures

eFigure 0. Study flow chart

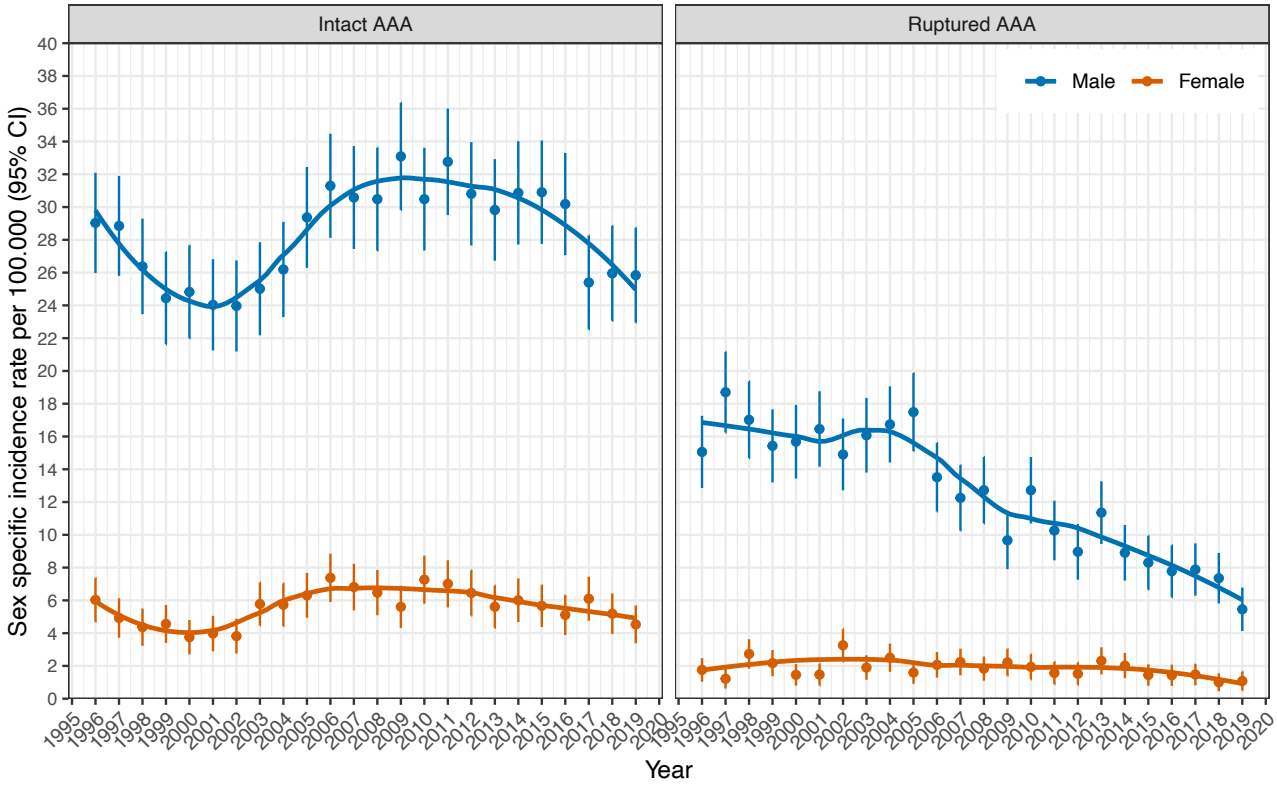


AAA, abdominal aortic aneurysm.
LEAD, lower extremity artery disease.

eFigure 1. Standardized incidence rate by indication and technique

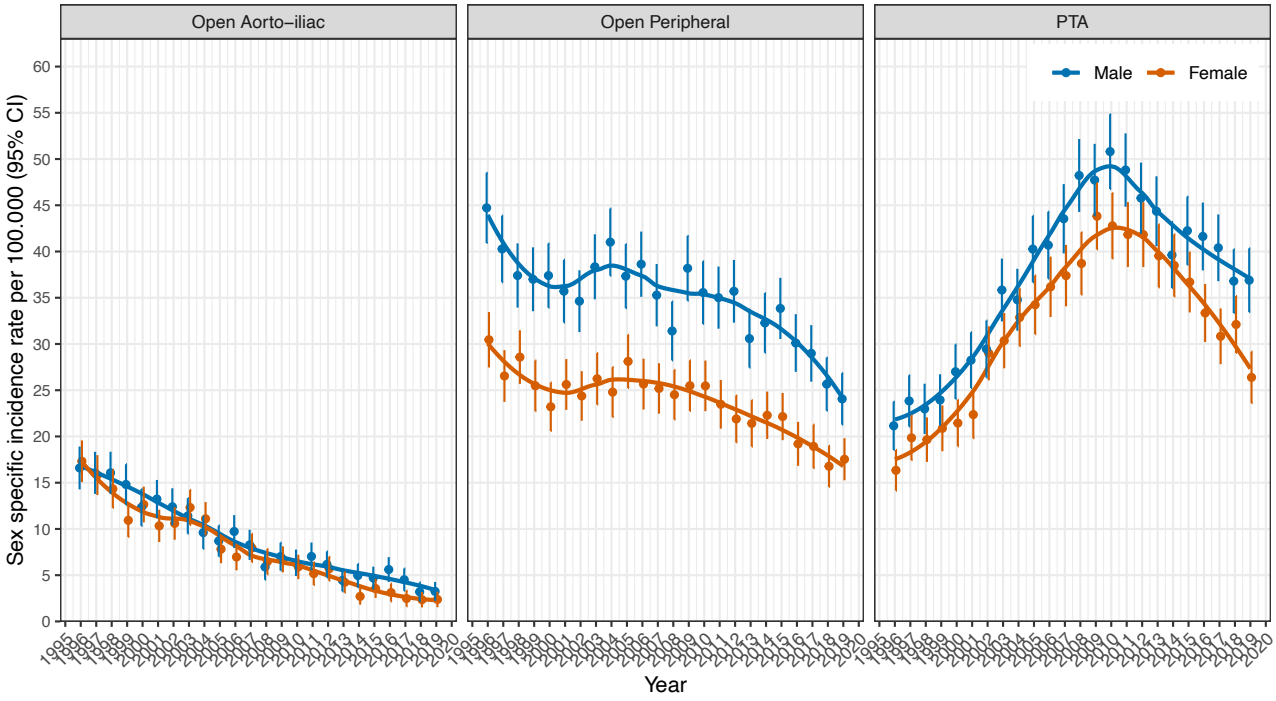


eFigure 2a. Sex specific incidence in AAA repair

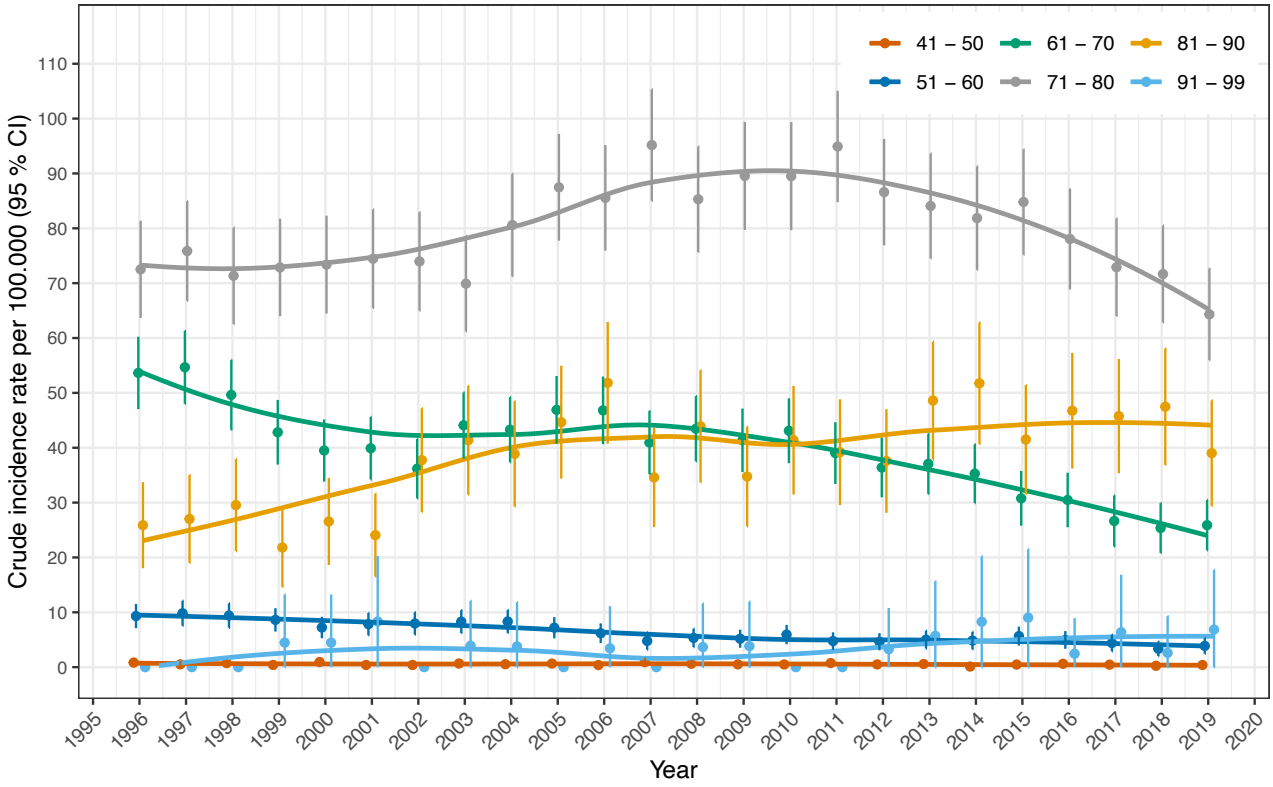


Se table of incidence rates on the next page

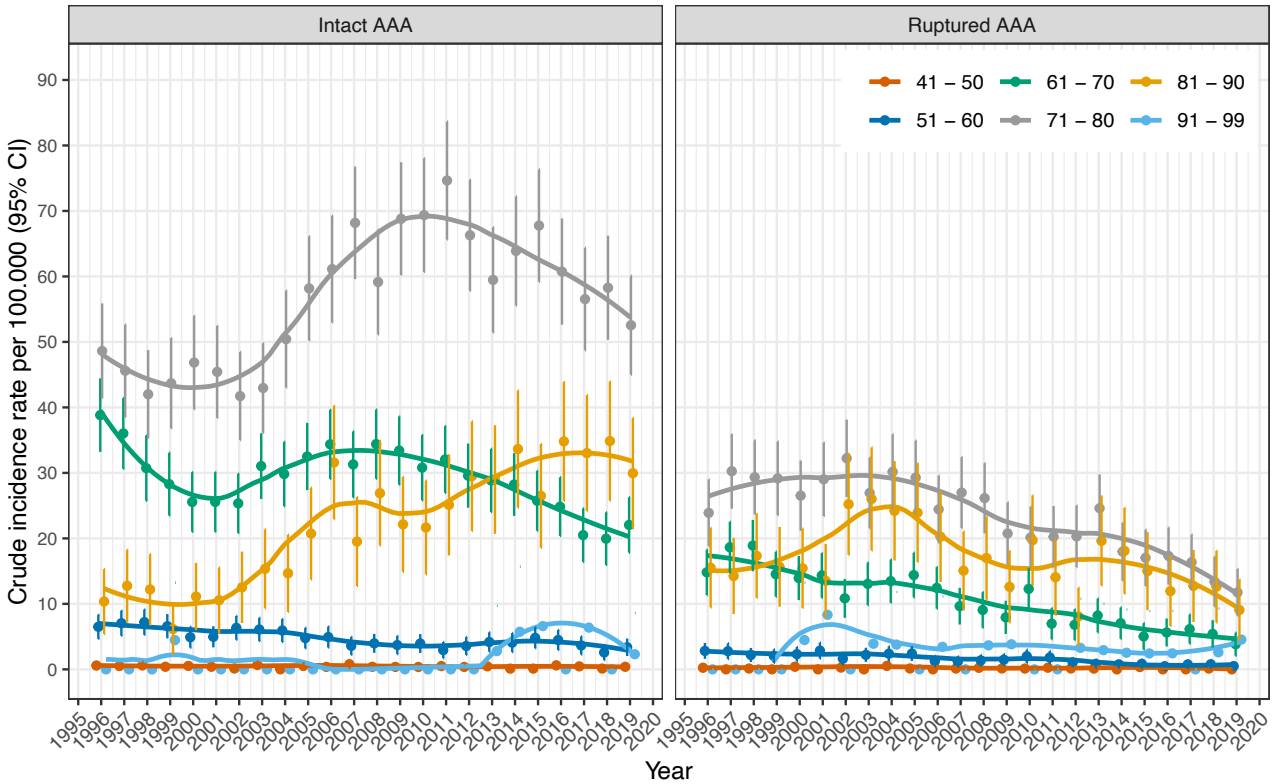
eFigure 2b. Sex specific incidence in LEAD surgery



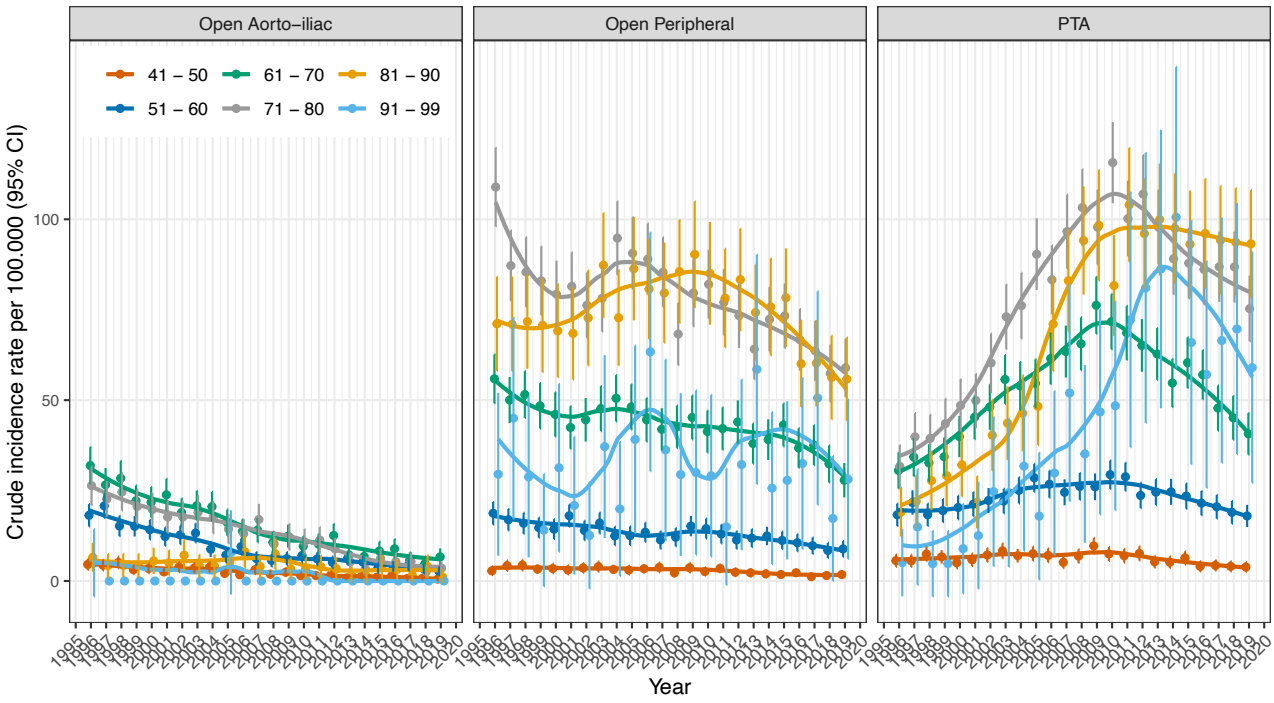
eFigure 3a. Trend in age specific AAA incidence rates



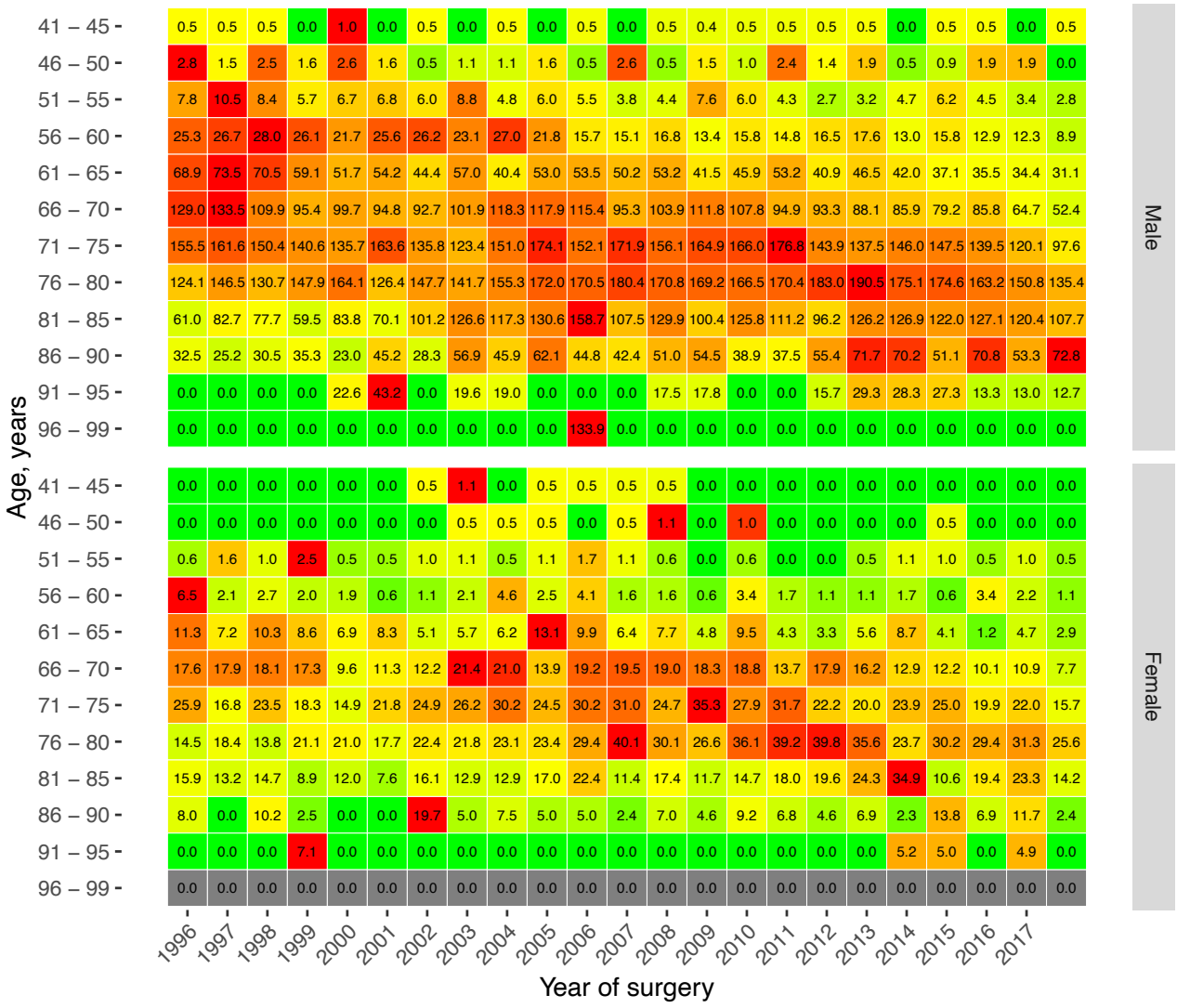
eFigure 3b. Trend in age specific incidence rates for intact and ruptured AAA



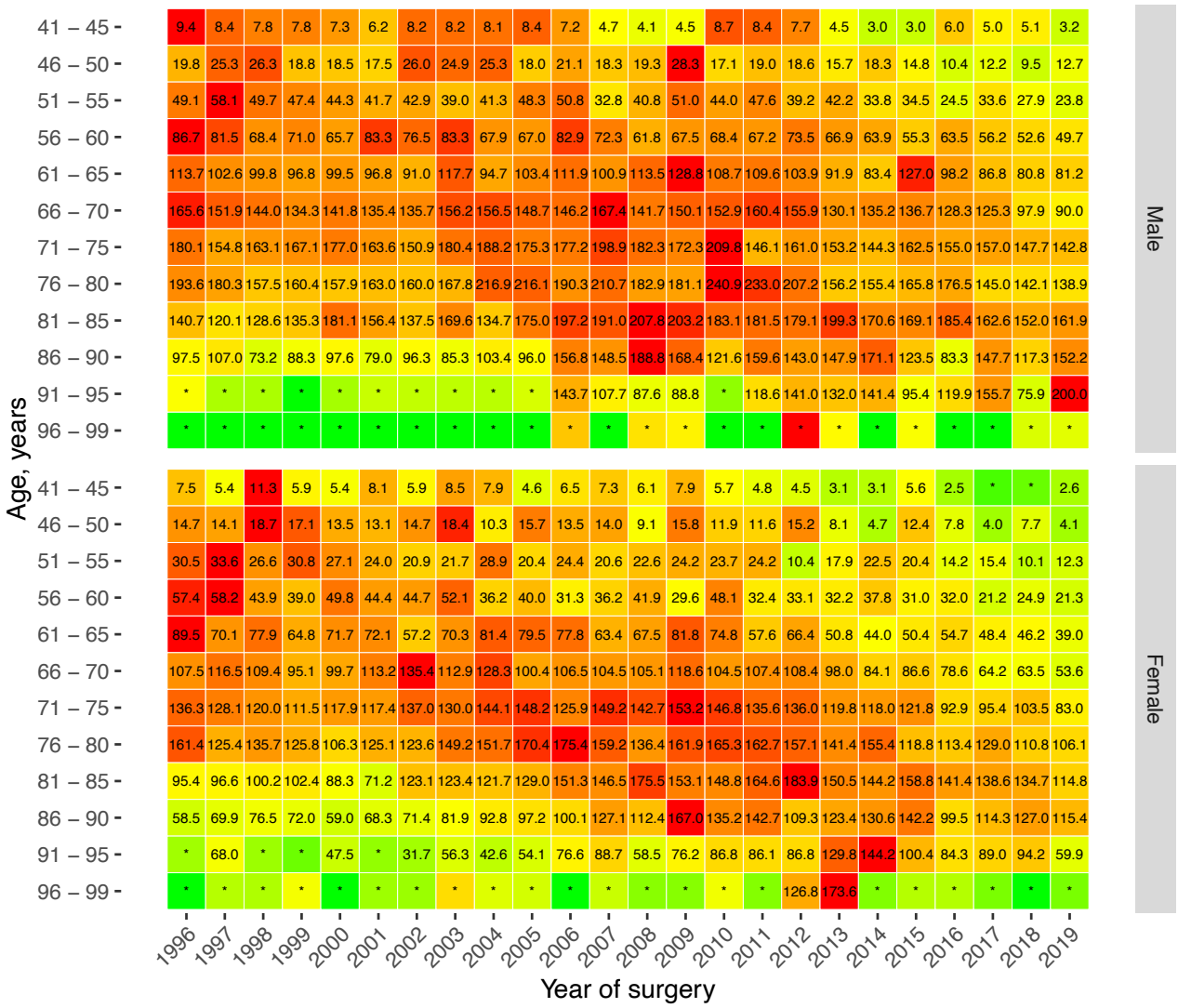
eFigure 3c. Trend in age specific incidence rates for LEAD repair



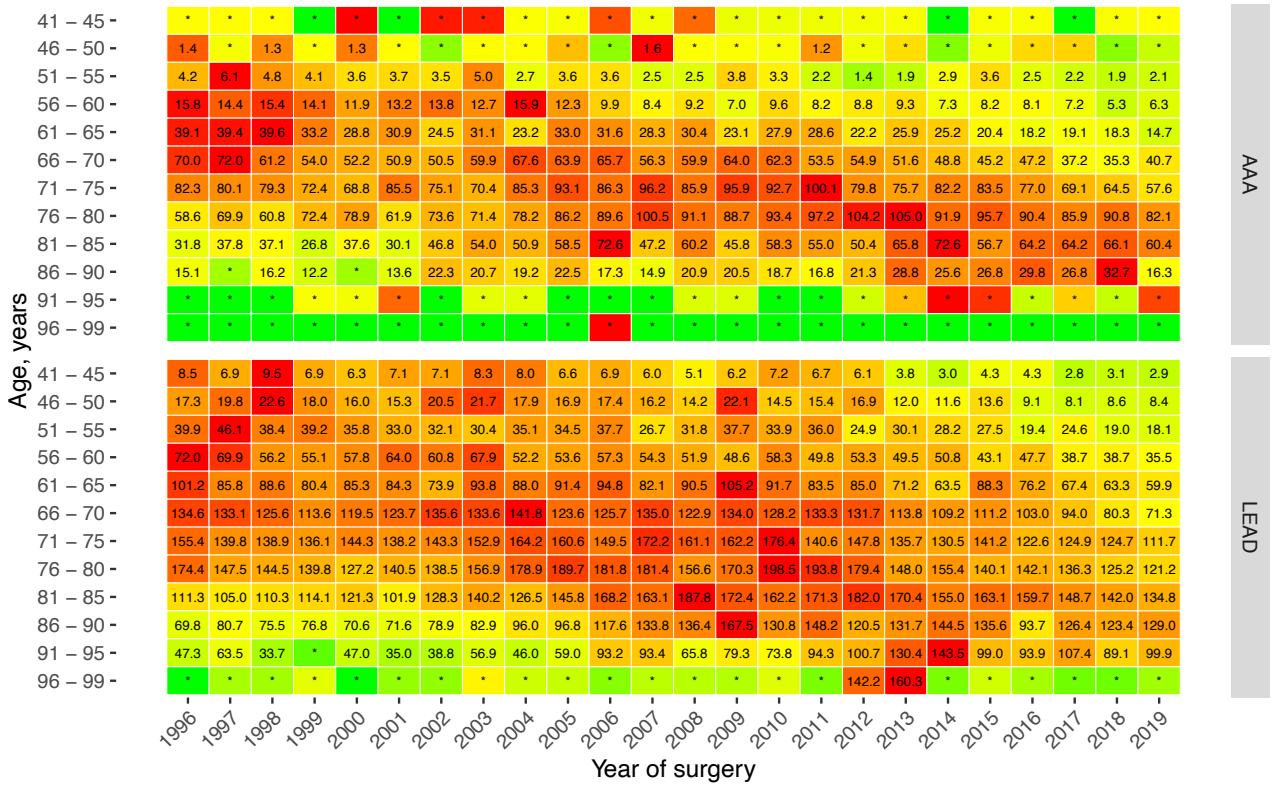
eFigure 4a. Heatmap of age specific AAA incidence by sex



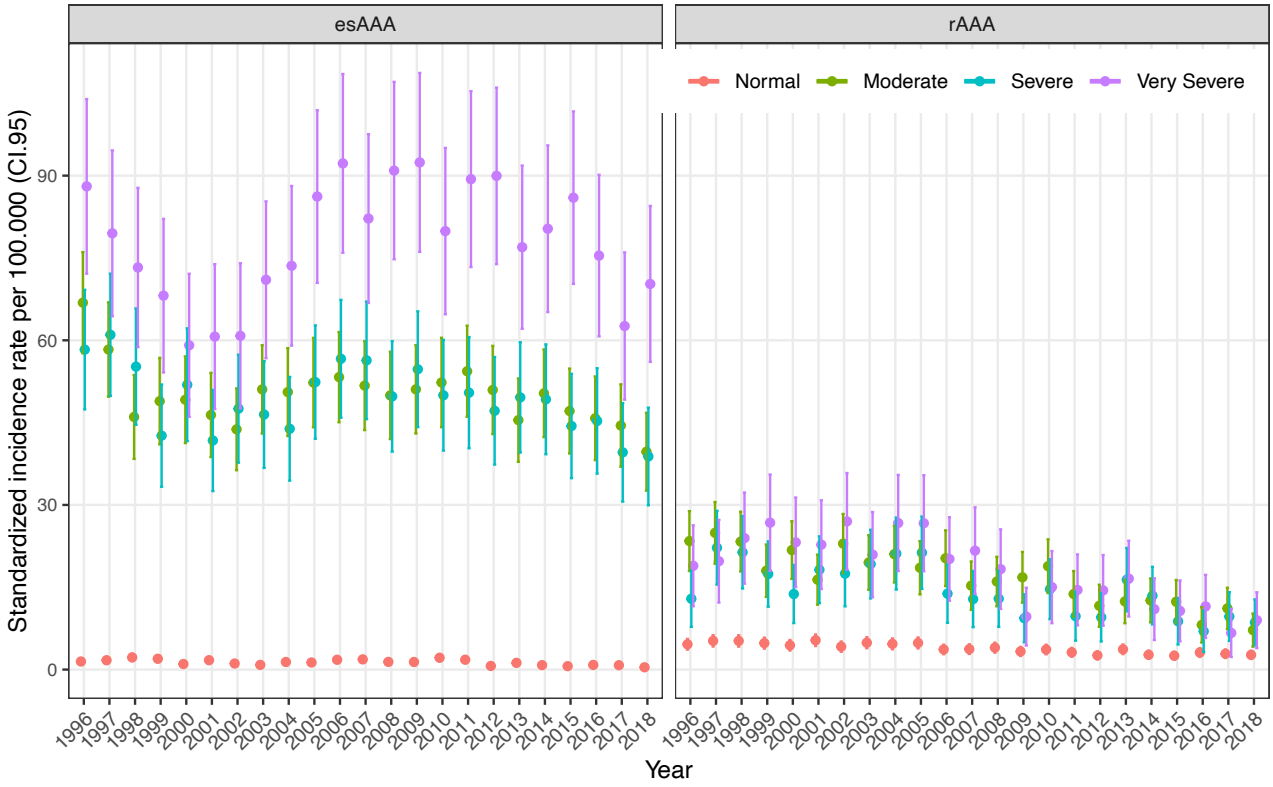
eFigure 4b. Heatmap of age specific LEAD incidence by sex



eFigure 4c. Heatmap of age specific AAA and LEAD incidence



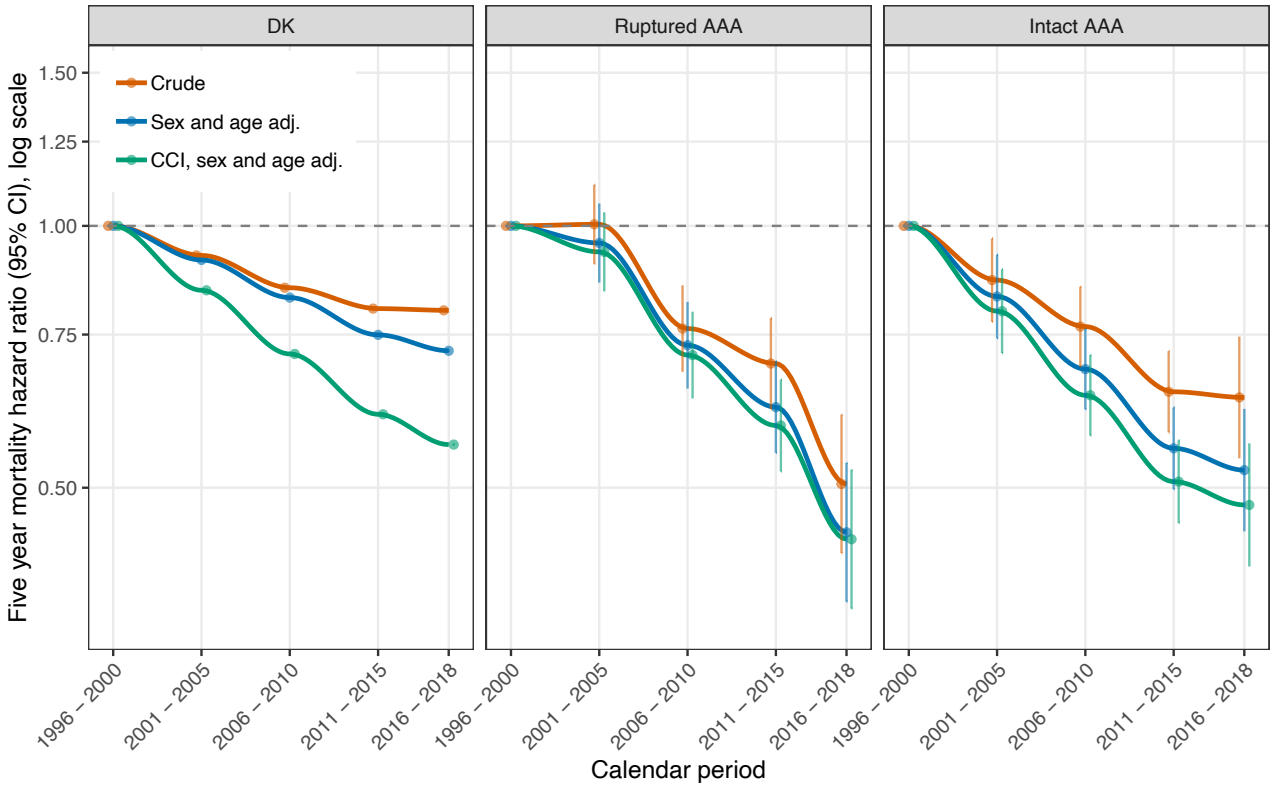
eFigure 4. Comorbidity-specific incidence, age- and sex standardized



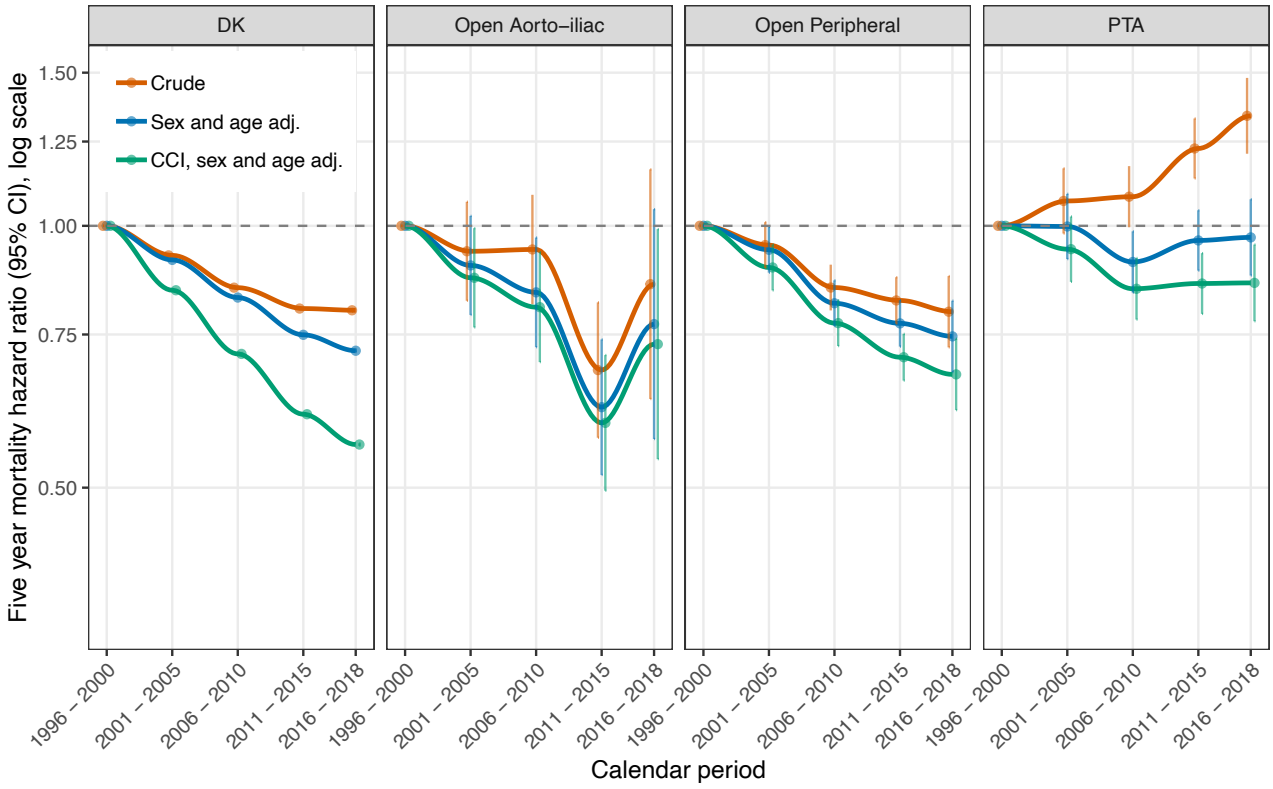
esAAA, elective and symptomatic AAA (intact AAA). rAAA, ruptured AAA.

eFigure 6. Plot of 5-year mortality HRs following surgery compared to Danish residents

eFigure 6a. 5-year mortality HR following AAA repair vs. Danish population aged 41-99



eFigure 6b. 5-year mortality HR following LEAD surgery vs. Danish population aged 41-99



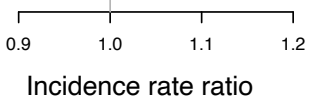
Supplemental eTables

eTables 1: Incidence rate subgroup analyses, calendar year:covariate

LYear, Calendar year as linear predictor.
IRR, annual incidence rate ratio.
CCI, Charlson's comorbidity index.

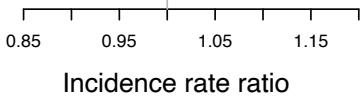
eTable 1a. Total AAA repair incidence

Total AAA	CCI adjusted	IRR (95% CI)	Change (%)	p of interaction
LYear: Sex				
Male	Yes	0.98 (0.98–0.98)	–1.9 %	
Female	Yes	0.99 (0.98–1.00)	–1.0 %	0.0066
Male	No	0.99 (0.99–0.99)	–0.8 %	
Female	No	1.00 (1.00–1.01)	+0.3 %	0.0014
LYear: Age_index				
41 – 50	Yes	0.97 (0.94–1.00)	–3.1 %	
51 – 60	Yes	0.95 (0.94–0.96)	–4.7 %	
61 – 70	Yes	0.97 (0.96–0.97)	–3.2 %	
71 – 80	Yes	0.99 (0.99–1.00)	–0.8 %	
81 – 90	Yes	1.01 (1.01–1.02)	+1.3 %	
91 – 99	Yes	1.04 (0.97–1.11)	+3.5 %	<0.001
41 – 50	No	0.98 (0.95–1.01)	–2.0 %	
51 – 60	No	0.96 (0.96–0.97)	–3.6 %	
61 – 70	No	0.98 (0.97–0.98)	–2.2 %	
71 – 80	No	1.00 (1.00–1.01)	+0.3 %	
81 – 90	No	1.02 (1.02–1.03)	+2.5 %	
91 – 99	No	1.05 (0.98–1.12)	+4.8 %	<0.001
LYear: CCI_group				
Normal	Yes	0.97 (0.96–0.97)	–3.4 %	
Moderate	Yes	0.98 (0.98–0.99)	–1.7 %	
Severe	Yes	0.98 (0.98–0.99)	–1.7 %	
Very Severe	Yes	0.99 (0.99–1.00)	–0.8 %	<0.001



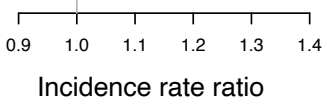
eTable 1b. Ruptured AAA repair incidence

Ruptured AAA	CCI adjusted	IRR (95% CI)	Change (%)	p of interaction
LYear: Sex				
Male	Yes	0.96 (0.95–0.96)	–4.19 %	
Female	Yes	0.98 (0.97–0.99)	–2.03 %	<0.001
Male	No	0.96 (0.96–0.97)	–3.89 %	
Female	No	0.98 (0.97–1.00)	–1.65 %	<0.001
LYear: Age_index				
41 – 50	Yes	0.98 (0.93–1.04)	–1.88 %	
51 – 60	Yes	0.94 (0.92–0.96)	–5.84 %	
61 – 70	Yes	0.95 (0.94–0.95)	–5.42 %	
71 – 80	Yes	0.97 (0.96–0.97)	–3.39 %	
81 – 90	Yes	0.98 (0.97–0.99)	–1.94 %	
91 – 99	Yes	1.00 (0.93–1.09)	+0.33 %	<0.001
41 – 50	No	0.98 (0.93–1.04)	–1.63 %	
51 – 60	No	0.94 (0.93–0.96)	–5.54 %	
61 – 70	No	0.95 (0.94–0.96)	–5.14 %	
71 – 80	No	0.97 (0.96–0.98)	–3.08 %	
81 – 90	No	0.98 (0.97–1.00)	–1.59 %	
91 – 99	No	1.01 (0.93–1.09)	+0.69 %	<0.001
LYear: CCI_group				
Normal	Yes	0.97 (0.96–0.97)	–3.29 %	
Moderate	Yes	0.96 (0.95–0.97)	–4.17 %	
Severe	Yes	0.96 (0.95–0.97)	–3.88 %	
Very Severe	Yes	0.95 (0.94–0.96)	–4.92 %	0.0641



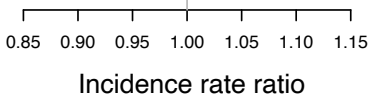
eTable 1c. Intact AAA repair incidence

Intact AAA	CCI adjusted	IRR (95% CI)	Change (%)	p of interaction
LYear: Sex				
Male	Yes	0.99 (0.99–1.00)	–0.79 %	
Female	Yes	0.99 (0.99–1.00)	–0.7 %	0.808
Male	No	1.01 (1.00–1.01)	+0.58 %	
Female	No	1.01 (1.00–1.02)	+0.87 %	0.447
LYear: Age_index				
41 – 50	Yes	0.96 (0.93–0.99)	–3.88 %	
51 – 60	Yes	0.95 (0.94–0.96)	–4.61 %	
61 – 70	Yes	0.97 (0.97–0.98)	–2.53 %	
71 – 80	Yes	1.00 (1.00–1.01)	+0.35 %	
81 – 90	Yes	1.04 (1.03–1.05)	+4.38 %	
91 – 99	Yes	1.13 (0.98–1.30)	+13.17 %	<0.001
41 – 50	No	0.98 (0.95–1.01)	–2.2 %	
51 – 60	No	0.97 (0.96–0.98)	–2.97 %	
61 – 70	No	0.99 (0.98–0.99)	–1.15 %	
71 – 80	No	1.02 (1.01–1.02)	+1.69 %	
81 – 90	No	1.06 (1.05–1.07)	+5.89 %	
91 – 99	No	1.15 (1.00–1.32)	+14.79 %	<0.001
LYear: CCI_group				
Normal	Yes	0.96 (0.95–0.98)	–3.59 %	
Moderate	Yes	0.99 (0.99–1.00)	–0.92 %	
Severe	Yes	0.99 (0.98–1.00)	–1.05 %	
Very Severe	Yes	1.00 (1.00–1.01)	+0.11 %	<0.001



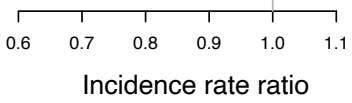
eTable 1d. Total “first-time” LEAD repair incidence

LEAD_1st	CCI adjusted	IRR (95% CI)	Change (%)	p of interaction
LYear: Sex				
Male	Yes	0.98 (0.97–0.98)	–2.3 %	
Female	Yes	0.97 (0.97–0.98)	–2.6 %	0.028
Male	No	0.99 (0.99–1.00)	–0.7 %	
Female	No	0.99 (0.99–0.99)	–0.8 %	0.407
LYear: Age_index				
41 – 50	Yes	0.95 (0.94–0.96)	–5.0 %	
51 – 60	Yes	0.96 (0.96–0.96)	–4.0 %	
61 – 70	Yes	0.97 (0.97–0.98)	–2.7 %	
71 – 80	Yes	0.98 (0.98–0.98)	–2.2 %	
81 – 90	Yes	1.00 (1.00–1.01)	+0.1 %	
91 – 99	Yes	1.03 (1.01–1.04)	+2.8 %	<0.001
41 – 50	No	0.97 (0.96–0.98)	–3.1 %	
51 – 60	No	0.98 (0.98–0.98)	–2.1 %	
61 – 70	No	0.99 (0.99–0.99)	–1.1 %	
71 – 80	No	1.00 (0.99–1.00)	–0.5 %	
81 – 90	No	1.02 (1.02–1.02)	+2.0 %	
91 – 99	No	1.05 (1.03–1.06)	+4.8 %	<0.001
LYear: CCI_group				
Normal	Yes	1.04 (1.03–1.04)	+3.6 %	
Moderate	Yes	0.96 (0.96–0.97)	–3.7 %	
Severe	Yes	0.98 (0.97–0.98)	–2.4 %	
Very Severe	Yes	0.98 (0.97–0.98)	–2.3 %	<0.001



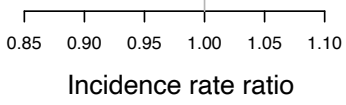
eTable 1e. Open aorto-iliac occlusive disease surgery incidence

Central LEAD	CCI adjusted	IRR (95% CI)	Change (%)	p of interaction
LYear: Sex				
Male	Yes	0.92 (0.91–0.92)	–8.12 %	
Female	Yes	0.90 (0.90–0.91)	–9.65 %	< 0.001
Male	No	0.93 (0.93–0.94)	–6.63 %	
Female	No	0.92 (0.92–0.93)	–7.84 %	0.00295
LYear: Age_index				
41 – 50	Yes	0.90 (0.89–0.92)	–9.51 %	
51 – 60	Yes	0.91 (0.90–0.91)	–9.34 %	
61 – 70	Yes	0.91 (0.91–0.92)	–8.57 %	
71 – 80	Yes	0.91 (0.90–0.92)	–9.04 %	
81 – 90	Yes	0.95 (0.93–0.97)	–5.07 %	
91 – 99	Yes	0.84 (0.68–1.04)	–16.01 %	0.00746
41 – 50	No	0.93 (0.91–0.94)	–7.25 %	
51 – 60	No	0.93 (0.92–0.93)	–7.4 %	
61 – 70	No	0.93 (0.92–0.93)	–7.17 %	
71 – 80	No	0.92 (0.92–0.93)	–7.58 %	
81 – 90	No	0.96 (0.94–0.99)	–3.54 %	
91 – 99	No	0.86 (0.69–1.05)	–14.47 %	0.02821
LYear: CCI_group				
Normal	Yes	0.92 (0.90–0.95)	–7.77 %	
Moderate	Yes	0.91 (0.91–0.92)	–8.92 %	
Severe	Yes	0.92 (0.91–0.93)	–8.28 %	
Very Severe	Yes	0.91 (0.90–0.91)	–9.36 %	0.22984



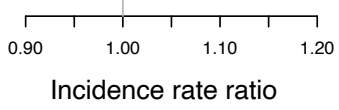
eTable 1f. Open peripheral surgery for arterial occlusive disease incidence

Peripheral LEAD	CCI adjusted	IRR (95% CI)	Change (%)	p of interaction
LYear: Sex				
Male	Yes	0.97 (0.97–0.97)	–3.2 %	
Female	Yes	0.96 (0.96–0.97)	–3.79 %	0.00660
Male	No	0.99 (0.98–0.99)	–1.38 %	
Female	No	0.98 (0.98–0.99)	–1.76 %	0.08208
LYear: Age_index				
41 – 50	Yes	0.94 (0.93–0.96)	–5.55 %	
51 – 60	Yes	0.96 (0.95–0.96)	–4.31 %	
61 – 70	Yes	0.97 (0.96–0.97)	–3.24 %	
71 – 80	Yes	0.96 (0.96–0.97)	–3.55 %	
81 – 90	Yes	0.97 (0.97–0.98)	–2.6 %	
91 – 99	Yes	0.98 (0.96–1.00)	–1.84 %	< 0.001
41 – 50	No	0.97 (0.95–0.98)	–3.33 %	
51 – 60	No	0.98 (0.97–0.98)	–2.2 %	
61 – 70	No	0.99 (0.98–0.99)	–1.46 %	
71 – 80	No	0.98 (0.98–0.99)	–1.73 %	
81 – 90	No	0.99 (0.99–1.00)	–0.51 %	
91 – 99	No	1.00 (0.98–1.03)	+0.39 %	< 0.001
LYear: CCI_group				
Normal	Yes	0.97 (0.96–0.98)	–2.91 %	
Moderate	Yes	0.96 (0.96–0.97)	–3.83 %	
Severe	Yes	0.97 (0.97–0.98)	–2.75 %	
Very Severe	Yes	0.96 (0.96–0.97)	–3.51 %	0.00316



eTable 1g. PTA Incidence

PTA	CCI adjusted	IRR (95% CI)	Change (%)	p of interaction
LYear: Sex				
Male	Yes	1.01 (1.01–1.01)	+1.02 %	
Female	Yes	1.01 (1.01–1.01)	+0.9 %	0.574
Male	No	1.03 (1.02–1.03)	+2.66 %	
Female	No	1.03 (1.02–1.03)	+2.7 %	0.849
LYear: Age_index				
41 – 50	Yes	0.97 (0.96–0.98)	−2.75 %	
51 – 60	Yes	0.99 (0.98–0.99)	−1.14 %	
61 – 70	Yes	1.00 (1.00–1.01)	+0.38 %	
71 – 80	Yes	1.02 (1.01–1.02)	+1.84 %	
81 – 90	Yes	1.05 (1.04–1.05)	+4.77 %	
91 – 99	Yes	1.08 (1.05–1.10)	+7.52 %	<0.001
41 – 50	No	0.99 (0.98–1.00)	−0.98 %	
51 – 60	No	1.01 (1.00–1.01)	+0.66 %	
61 – 70	No	1.02 (1.02–1.02)	+2 %	
71 – 80	No	1.03 (1.03–1.04)	+3.49 %	
81 – 90	No	1.07 (1.06–1.07)	+6.7 %	
91 – 99	No	1.10 (1.07–1.12)	+9.55 %	<0.001
LYear: CCI_group				
Normal	Yes	1.06 (1.06–1.07)	+6.43 %	
Moderate	Yes	0.99 (0.99–1.00)	−0.84 %	
Severe	Yes	1.01 (1.00–1.01)	+0.58 %	
Very Severe	Yes	1.02 (1.01–1.02)	+1.62 %	<0.001



eTable 2. Baseline characteristics by calendar period

eTable 2a. Ruptured AAA

Ruptured AAA	Level	1996 - 1999 (n=880)	2000 - 2004 (n=1114)	2005 - 2009 (n=1000)	2010 - 2014 (n=907)	2015 - 2018 (n=607)
Age	mean (sd)	72 (7)	73 (8)	73 (8)	73 (8)	74 (8)
Male_sex	Yes	777 (88)	976 (88)	868 (87)	777 (86)	525 (86)
CCI_group	Normal	379 (43)	437 (39)	367 (37)	315 (35)	229 (38)
	Moderate	260 (30)	317 (28)	291 (29)	253 (28)	157 (26)
	Severe	134 (15)	177 (16)	159 (16)	169 (19)	107 (18)
	Very Severe	107 (12)	183 (16)	183 (18)	170 (19)	114 (19)
Dependency	Independent	611 (69)	821 (74)	834 (83)	774 (85)	532 (88)
	Home care	65 (7)	89 (8)	82 (8)	61 (7)	20 (3)
	Nursing home	10 (1)	12 (1)	11 (1)	19 (2)	36 (6)
	missing	194 (22)	192 (17)	73 (7)	53 (6)	19 (3)
TOBACCO	None	81 (9)	102 (9)	200 (20)	206 (23)	116 (19)
	Previous	80 (9)	133 (12)	218 (22)	260 (29)	216 (36)
	Smoker	274 (31)	347 (31)	383 (38)	314 (35)	196 (32)
	missing	445 (51)	532 (48)	199 (20)	127 (14)	79 (13)
alcohol_abuse	No	468 (53)	868 (78)	905 (90)	826 (91)	561 (92)
	> 5 units/day	19 (2)	19 (2)	34 (3)	39 (4)	20 (3)
	missing	393 (45)	227 (20)	61 (6)	42 (5)	26 (4)
PRIORITY	Elective	14 (2)	10-15 (1)	15 (2)	9 (1)	5 (1)
	Subacute	82 (9)	123 (11)	79 (8)	42 (5)	15 (2)
	Acute	604 (69)	975 (88)	906 (91)	856 (94)	587 (97)
	missing	180 (20)	<5 (0)	0 (0)	0 (0)	0 (0)
Region	North	110 (12)	126 (11)	130 (13)	113 (12)	80 (13)
	Central	172 (20)	224 (20)	178 (18)	173 (19)	110 (18)
	South	237 (27)	293 (26)	266 (27)	321 (35)	263 (43)
	Capital	298 (34)	401 (36)	415 (42)	269 (30)	144 (24)
	Zealand	63 (7)	70 (6)	11 (1)	31 (3)	10 (2)
Technique	Open	875-885 (100)	1,105-1,114 (100)	990-999 (100)	853 (94)	560 (92)
	Endovascular	<5 (0)	<5 (0)	<5 (0)	54 (6)	47 (8)

eTable 2b. Intact AAA

Intact AAA	Level	1996 - 1999 (n=1529)	2000 - 2004 (n=1824)	2005 - 2009 (n=2499)	2010 - 2014 (n=2792)	2015 - 2018 (n=2243)
Age	mean (sd)	70 (7)	70 (8)	71 (7)	72 (7)	73 (7)
Male_sex	Yes	1,269 (83)	1,522 (83)	2,060 (82)	2,326 (83)	1,895 (84)
CCI_group	Normal	139 (9)	115 (6)	147 (6)	134 (5)	54 (2)
	Moderate	637 (42)	761 (42)	883 (35)	956 (34)	735 (33)
	Severe	389 (25)	456 (25)	610 (24)	676 (24)	556 (25)
	Very Severe	364 (24)	492 (27)	859 (34)	1026 (37)	898 (40)
Dependency	Independent	1,360 (89)	1,646 (90)	2,336 (93)	2,675 (96)	2,114 (94)
	Home care	121 (8)	129 (7)	129 (5)	79 (3)	89 (4)
	Nursing home	<5 (0)	<5 (0)	8 (0)	7 (0)	5 (0)
	missing	35-45 (3)	35-45 (2)	26 (1)	31 (1)	35 (2)
TOBACCO	None	207 (14)	270 (15)	386 (15)	506 (18)	421 (19)
	Previous	337 (22)	536 (29)	985 (39)	1261 (45)	1106 (49)
	Smoker	884 (58)	930 (51)	1078 (43)	971 (35)	664 (30)
	missing	101 (7)	88 (5)	50 (2)	54 (2)	52 (2)
alcohol_abuse	No	1,047 (68)	1,742 (96)	2,392 (96)	2,694 (96)	2,147 (96)
	> 5 units/day	20 (1)	40 (2)	70 (3)	55 (2)	31 (1)
	missing	462 (30)	42 (2)	37 (1)	43 (2)	65 (3)
PRIORITY	Elective	898 (59)	1,532 (84)	2,151 (86)	2,379 (85)	1,983 (88)
	Subacute	154 (10)	204 (11)	249 (10)	249 (9)	140 (6)
	Acute	101 (7)	88 (5)	99 (4)	164 (6)	110-120 (5)
	missing	376 (25)	0 (0)	0 (0)	0 (0)	<5 (0)
Region	North	173 (11)	179 (10)	204 (8)	260 (9)	228 (10)
	Central	359 (23)	398 (22)	536 (21)	707 (25)	454 (20)
	South	354 (23)	532 (29)	671 (27)	884 (32)	856 (38)
	Capital	516 (34)	560 (31)	968 (39)	749 (27)	571 (25)
	Zealand	127 (8)	155 (8)	120 (5)	192 (7)	134 (6)
Technique	Open	1,498 (98)	1,713 (94)	1,918 (77)	1,797 (64)	1,292 (58)
	Endovascular	31 (2)	111 (6)	581 (23)	995 (36)	951 (42)

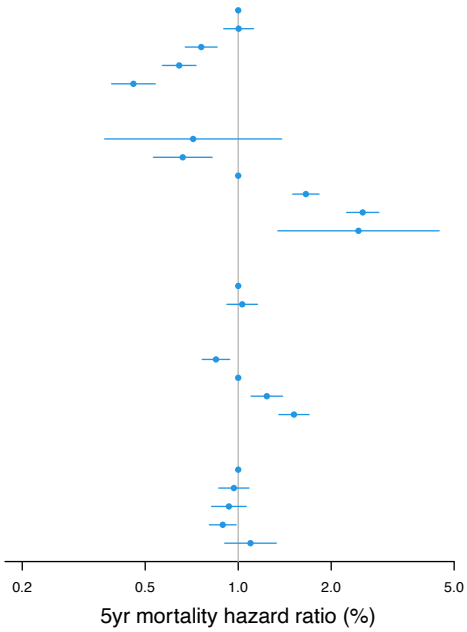
eTable 2c. First-time LEAD

First-time LEAD	Level	1996 - 1999 (n=6517)	2000 - 2004 (n=8369)	2005 - 2009 (n=9621)	2010 - 2014 (n=9823)	2015 - 2018 (n=7109)
Age	mean (sd)	68 (11)	68 (10)	69 (11)	70 (10)	71 (10)
Male_sex	Yes	3473 (53)	4523 (54)	5262 (55)	5440 (55)	4104 (58)
	No	3044 (47)	3846 (46)	4359 (45)	4383 (45)	3005 (42)
CCI_group	Normal	258 (4)	359 (4)	629 (7)	691 (7)	527 (7)
	Moderate	2900 (44)	3219 (38)	3318 (34)	2996 (30)	1912 (27)
	Severe	1322 (20)	1694 (20)	2060 (21)	1971 (20)	1403 (20)
	Very Severe	2037 (31)	3097 (37)	3614 (38)	4165 (42)	3267 (46)
Dependency	Independent	4973 (76)	6522 (78)	7674 (80)	8229 (84)	5999 (84)
	Home care	1275 (20)	1411 (17)	1450 (15)	1255 (13)	799 (11)
	Nursing home	102 (2)	159 (2)	235 (2)	228 (2)	138 (2)
	missing	167 (3)	277 (3)	262 (3)	111 (1)	173 (2)
TOBACCO	None	881 (14)	1083 (13)	1483 (15)	1323 (13)	1085 (15)
	Previous	1443 (22)	2375 (28)	3351 (35)	3955 (40)	2938 (41)
	Smoker	3889 (60)	4491 (54)	4466 (46)	4331 (44)	2825 (40)
	missing	304 (5)	420 (5)	321 (3)	214 (2)	261 (4)
alcohol_abuse	No	4541 (70)	7890 (94)	9079 (94)	9374 (95)	6603 (93)
	> 5 units/day	106 (2)	192 (2)	272 (3)	302 (3)	204 (3)
	missing	1870 (29)	287 (3)	270 (3)	147 (1)	302 (4)
PRIORITY	Elective	4483 (69)	7774 (93)	8650 (90)	8571 (87)	5962 (84)
	Subacute	314 (5)	487 (6)	910 (9)	1099 (11)	947 (13)
	Acute	100 (2)	100-110 (1)	61 (1)	145-155 (2)	191 (3)
	missing	1620 (25)	<5 (0)	0 (0)	<5 (0)	9 (0)
Region	North	642 (10)	798 (10)	994 (10)	1074 (11)	917 (13)
	Central	1343 (21)	1749 (21)	1983 (21)	2023 (21)	1456 (20)
	South	1741 (27)	2408 (29)	3005 (31)	3086 (31)	2179 (31)
	Capital	2208 (34)	2518 (30)	2549 (26)	2293 (23)	1602 (23)
	Zealand	583 (9)	896 (11)	1090 (11)	1347 (14)	955 (13)
Technique	Open	4417 (68)	4776 (57)	4299 (45)	3687 (38)	2455 (35)
	Endovascular	1984 (30)	3473 (41)	5196 (54)	5881 (60)	4267 (60)
	Hybrid	116 (2)	120 (1)	126 (1)	255 (3)	387 (5)

eTable 3. Regression table of survival analyses

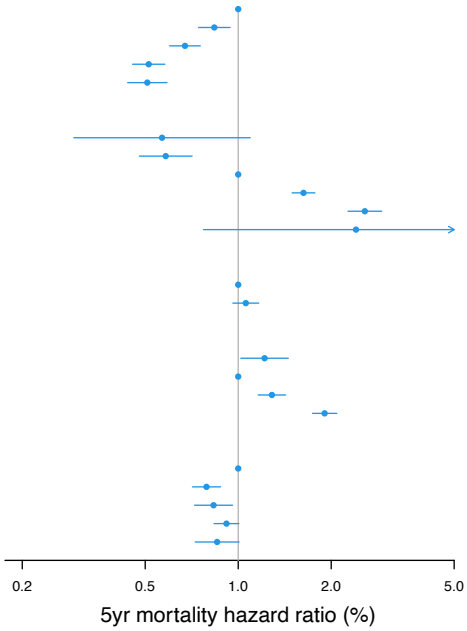
eTable 3a. Ruptured AAA

Ruptured AAA	No. Dead	No. Patients	Mortality risk (%)	Crude HR (95% CI)	Adj. HR (95% CI)
Year					
1996 – 1999	555	880	63.1 (59.7 – 66.1)	Reference	Reference
2000 – 2004	716	1,114	64.3 (61.3 – 67.0)	1.04 (0.93 – 1.16)	1.00 (0.90 – 1.12)
2005 – 2009	550	1,000	55.0 (51.8 – 58.0)	0.81 (0.72 – 0.91)	0.76 (0.67 – 0.86)
2010 – 2014	474	907	52.3 (48.9 – 55.4)	0.71 (0.63 – 0.81)	0.64 (0.57 – 0.73)
2015 – 2018	207	607	39.4 (34.0 – 44.4)	0.50 (0.43 – 0.59)	0.46 (0.39 – 0.54)
Age, years					
41 – 50	9	26	35.4 (13.8 – 51.6)	0.74 (0.38 – 1.42)	0.71 (0.37 – 1.38)
51 – 60	93	293	32.3 (26.7 – 37.5)	0.68 (0.55 – 0.85)	0.66 (0.53 – 0.82)
61 – 70	594	1,395	43.6 (40.9 – 46.2)	Reference	Reference
71 – 80	1,267	2,088	62.4 (60.2 – 64.4)	1.69 (1.53 – 1.86)	1.66 (1.50 – 1.83)
81 – 90	528	692	78.2 (74.7 – 81.2)	2.59 (2.30 – 2.91)	2.53 (2.24 – 2.85)
91 – 99	11	14	81.0 (39.7 – 94.0)	2.45 (1.35 – 4.45)	2.45 (1.34 – 4.47)
Sex					
Male	2,148	3,923	56.1 (54.4 – 57.6)	Reference	Reference
Female	354	585	62.1 (57.8 – 65.9)	1.17 (1.05 – 1.31)	1.03 (0.92 – 1.15)
CCI group					
Normal	791	1,727	46.9 (44.5 – 49.3)	0.80 (0.72 – 0.88)	0.85 (0.76 – 0.94)
Moderate	695	1,278	55.5 (52.6 – 58.2)	Reference	Reference
Severe	467	746	64.3 (60.6 – 67.7)	1.25 (1.11 – 1.41)	1.24 (1.10 – 1.39)
Very Severe	549	757	74.4 (70.9 – 77.4)	1.54 (1.38 – 1.72)	1.52 (1.35 – 1.70)
Region					
Capital	894	1,527	59.5 (56.9 – 61.9)	Reference	Reference
Central	463	857	55.1 (51.6 – 58.4)	0.91 (0.82 – 1.02)	0.97 (0.87 – 1.08)
Northern	315	559	57.8 (53.4 – 61.8)	0.95 (0.84 – 1.08)	0.93 (0.82 – 1.06)
Southern	710	1,380	53.4 (50.6 – 56.1)	0.83 (0.76 – 0.92)	0.89 (0.81 – 0.98)
Zealand	120	185	65.0 (57.4 – 71.2)	1.15 (0.95 – 1.40)	1.10 (0.90 – 1.33)



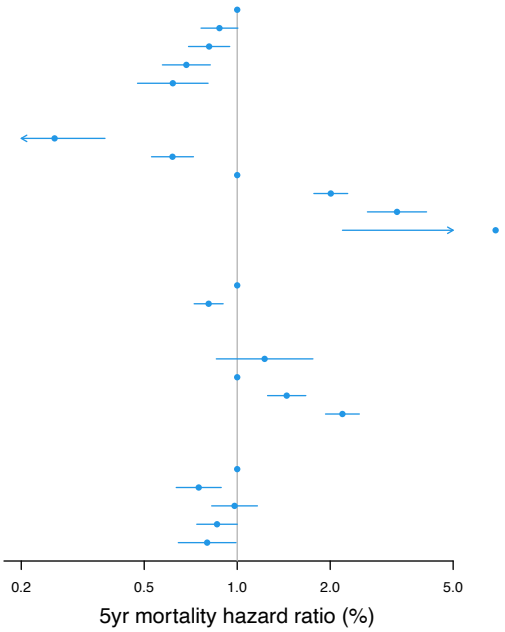
eTable 3b. Intact AAA.

Intact AAA	No. Dead	No. Patients	Mortality risk (%)	Crude HR (95% CI)	Adj. HR (95% CI)
Year					
1996 – 1999	539	1,529	35.3 (32.8 – 37.6)	Reference	Reference
2000 – 2004	584	1,824	32.0 (29.8 – 34.1)	0.88 (0.79 – 0.99)	0.84 (0.74 – 0.94)
2005 – 2009	741	2,499	29.7 (27.8 – 31.4)	0.80 (0.72 – 0.90)	0.67 (0.60 – 0.75)
2010 – 2014	678	2,792	24.3 (22.7 – 25.9)	0.63 (0.56 – 0.71)	0.51 (0.46 – 0.58)
2015 – 2018	337	2,243	23.3 (20.5 – 26.0)	0.65 (0.56 – 0.74)	0.51 (0.44 – 0.59)
Age, years					
41 – 50	<5	---	12.8 (4.6 – 20.2)	0.55 (0.28 – 1.06)	0.57 (0.29 – 1.09)
51 – 60	114	849	14.3 (11.8 – 16.7)	0.61 (0.50 – 0.74)	0.58 (0.48 – 0.71)
61 – 70	825	3,961	22.3 (20.9 – 23.6)	Reference	Reference
71 – 80	1,557	5,076	34.1 (32.6 – 35.4)	1.64 (1.51 – 1.78)	1.63 (1.49 – 1.77)
81 – 90	371	918	45.9 (42.2 – 49.3)	2.47 (2.18 – 2.79)	2.57 (2.27 – 2.91)
91 – 99	<5	---	53.3 (0.0 – 81.9)	2.24 (0.72 – 6.95)	2.41 (0.77 – 7.50)
Sex					
Male	2,366	9,072	28.6 (27.6 – 29.5)	Reference	Reference
Female	513	1,815	30.4 (28.1 – 32.6)	1.11 (1.01 – 1.22)	1.06 (0.96 – 1.17)
CCI group					
Normal	146	589	25.7 (22.0 – 29.2)	1.21 (1.01 – 1.44)	1.22 (1.02 – 1.45)
Moderate	808	3,972	22.1 (20.8 – 23.5)	Reference	Reference
Severe	677	2,687	27.6 (25.8 – 29.4)	1.30 (1.17 – 1.43)	1.29 (1.16 – 1.42)
Very Severe	1,248	3,639	37.9 (36.2 – 39.6)	1.92 (1.75 – 2.09)	1.90 (1.74 – 2.08)
Region					
Capital	1,013	3,364	32.4 (30.7 – 34.1)	Reference	Reference
Central	564	2,454	24.7 (22.9 – 26.5)	0.74 (0.67 – 0.82)	0.79 (0.71 – 0.88)
Northern	256	1,044	27.0 (24.1 – 29.8)	0.82 (0.72 – 0.94)	0.83 (0.72 – 0.96)
Southern	869	3,297	29.5 (27.8 – 31.2)	0.90 (0.82 – 0.98)	0.92 (0.84 – 1.01)
Zealand	177	728	26.1 (22.7 – 29.3)	0.80 (0.68 – 0.93)	0.85 (0.73 – 1.01)



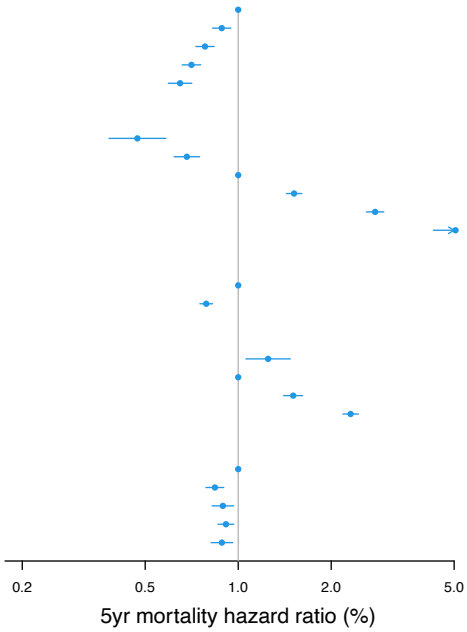
eTable 3c. Open Aorto-iliac LEAD

Open Aorto–iliac	No. Dead	No. Patients	Mortality risk (%)	Crude HR (95% CI)	Adj. HR (95% CI)
Year					
1996 – 1999	430	1,473	29.2 (26.8 – 31.5)	Reference	Reference
2000 – 2004	398	1,480	26.9 (24.6 – 29.1)	0.90 (0.79 – 1.04)	0.88 (0.76 – 1.01)
2005 – 2009	279	1,026	27.2 (24.4 – 29.9)	0.91 (0.78 – 1.06)	0.81 (0.70 – 0.95)
2010 – 2014	175	772	22.7 (19.7 – 25.6)	0.74 (0.62 – 0.88)	0.68 (0.57 – 0.82)
2015 – 2018	66	462	21.9 (15.2 – 28.1)	0.73 (0.57 – 0.95)	0.62 (0.48 – 0.81)
Age, years					
41 – 50	<5	---	6.7 (4.3 – 9.1)	0.23 (0.16 – 0.34)	0.26 (0.18 – 0.37)
51 – 60	240	1,534	16.2 (14.3 – 18.1)	0.60 (0.51 – 0.70)	0.62 (0.53 – 0.72)
61 – 70	473	1,928	25.5 (23.4 – 27.4)	Reference	Reference
71 – 80	506	1,131	46.1 (43.1 – 49.0)	2.09 (1.85 – 2.37)	2.01 (1.77 – 2.28)
81 – 90	97	171	59.1 (50.7 – 66.1)	3.08 (2.48 – 3.84)	3.29 (2.64 – 4.10)
91 – 99	<5	---	100.0 (NA – NA)	6.73 (2.16 – 20.97)	6.86 (2.19 – 21.48)
Sex					
Male	709	2,669	27.6 (25.9 – 29.4)	Reference	Reference
Female	639	2,544	25.8 (24.1 – 27.5)	0.92 (0.83 – 1.03)	0.81 (0.73 – 0.90)
CCI group					
Normal	32	147	22.7 (15.4 – 29.3)	1.24 (0.86 – 1.77)	1.23 (0.86 – 1.76)
Moderate	476	2,600	19.0 (17.4 – 20.5)	Reference	Reference
Severe	321	1,155	28.8 (26.1 – 31.4)	1.61 (1.40 – 1.85)	1.45 (1.25 – 1.67)
Very Severe	519	1,311	40.9 (38.1 – 43.6)	2.55 (2.25 – 2.89)	2.19 (1.93 – 2.49)
Region					
Capital	294	1,013	30.1 (27.1 – 32.9)	Reference	Reference
Central	259	1,141	23.2 (20.7 – 25.6)	0.74 (0.63 – 0.87)	0.75 (0.63 – 0.89)
Northern	247	853	30.3 (27.0 – 33.4)	1.02 (0.86 – 1.21)	0.98 (0.83 – 1.16)
Southern	430	1,707	26.2 (24.0 – 28.3)	0.85 (0.73 – 0.98)	0.86 (0.74 – 1.00)
Zealand	118	499	24.4 (20.4 – 28.1)	0.77 (0.63 – 0.96)	0.80 (0.64 – 0.99)



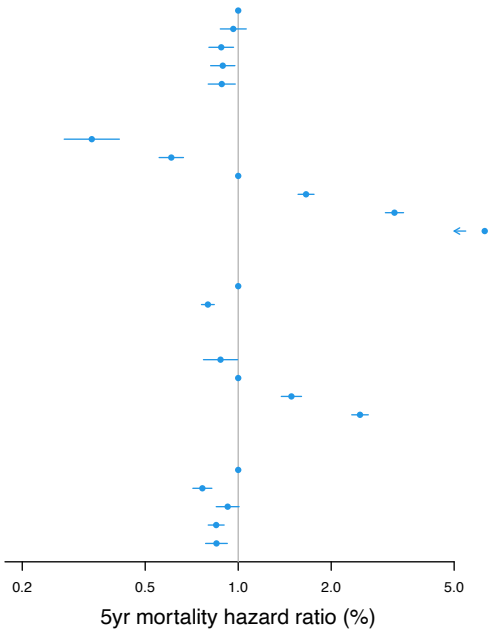
eTable 3c. Open Peripheral LEAD

Open Peripheral	No. Dead	No. Patients	Mortality risk (%)	Crude HR (95% CI)	Adj. HR (95% CI)
Year					
1996 – 1999	1,560	3,286	47.5 (45.8 – 49.2)	Reference	Reference
2000 – 2004	1,786	3,925	45.5 (43.9 – 47.0)	0.94 (0.88 – 1.01)	0.88 (0.83 – 0.95)
2005 – 2009	1,795	4,155	43.2 (41.7 – 44.7)	0.87 (0.82 – 0.93)	0.78 (0.73 – 0.84)
2010 – 2014	1,712	4,148	41.3 (39.8 – 42.8)	0.82 (0.76 – 0.88)	0.71 (0.66 – 0.76)
2015 – 2018	804	3,151	38.3 (34.7 – 41.6)	0.78 (0.71 – 0.85)	0.65 (0.59 – 0.71)
Age, years					
41 – 50	90	533	17.7 (14.3 – 21.0)	0.45 (0.36 – 0.55)	0.47 (0.38 – 0.58)
51 – 60	550	2,314	25.2 (23.3 – 27.0)	0.67 (0.61 – 0.73)	0.68 (0.62 – 0.75)
61 – 70	1,919	5,859	35.2 (33.9 – 36.4)	Reference	Reference
71 – 80	3,041	6,793	48.1 (46.9 – 49.4)	1.52 (1.43 – 1.61)	1.52 (1.43 – 1.61)
81 – 90	1,900	2,972	67.6 (65.7 – 69.3)	2.62 (2.46 – 2.79)	2.77 (2.60 – 2.96)
91 – 99	157	194	85.2 (78.7 – 89.7)	4.41 (3.75 – 5.19)	5.05 (4.28 – 5.96)
Sex					
Male	4,560	11,020	44.3 (43.3 – 45.3)	Reference	Reference
Female	3,097	7,645	42.9 (41.8 – 44.1)	0.97 (0.92 – 1.01)	0.79 (0.75 – 0.83)
CCI group					
Normal	154	454	35.1 (30.5 – 39.5)	1.26 (1.07 – 1.49)	1.25 (1.06 – 1.47)
Moderate	1,598	5,854	29.0 (27.8 – 30.2)	Reference	Reference
Severe	1,491	3,832	41.7 (40.1 – 43.4)	1.58 (1.47 – 1.69)	1.51 (1.40 – 1.62)
Very Severe	4,414	8,525	55.6 (54.5 – 56.7)	2.41 (2.27 – 2.55)	2.31 (2.18 – 2.45)
Region					
Capital	2,175	4,915	46.5 (45.0 – 47.9)	Reference	Reference
Central	1,522	3,993	41.0 (39.4 – 42.6)	0.84 (0.78 – 0.89)	0.84 (0.79 – 0.90)
Northern	831	1,985	44.5 (42.1 – 46.7)	0.95 (0.88 – 1.03)	0.89 (0.82 – 0.97)
Southern	2,302	5,610	44.0 (42.6 – 45.4)	0.93 (0.87 – 0.98)	0.91 (0.86 – 0.97)
Zealand	827	2,162	41.2 (38.9 – 43.3)	0.84 (0.77 – 0.91)	0.89 (0.82 – 0.96)



eTable 3c. PTA

PTA	No. Dead	No. Patients	Mortality risk (%)	Crude HR (95% CI)	Adj. HR (95% CI)
Year					
1996 – 1999	622	2,045	30.4 (28.4 – 32.4)	Reference	Reference
2000 – 2004	1,225	3,709	33.0 (31.5 – 34.5)	1.11 (1.00 – 1.22)	0.96 (0.87 – 1.06)
2005 – 2009	1,837	5,570	33.0 (31.7 – 34.2)	1.11 (1.02 – 1.22)	0.88 (0.80 – 0.97)
2010 – 2014	2,260	6,355	35.6 (34.4 – 36.8)	1.23 (1.13 – 1.35)	0.89 (0.81 – 0.98)
2015 – 2018	1,140	4,679	36.8 (33.5 – 39.9)	1.33 (1.21 – 1.47)	0.88 (0.80 – 0.98)
Age, years					
41 – 50	96	1,152	8.8 (7.1 – 10.4)	0.29 (0.24 – 0.36)	0.34 (0.27 – 0.41)
51 – 60	628	4,033	16.7 (15.5 – 17.9)	0.57 (0.52 – 0.62)	0.61 (0.55 – 0.67)
61 – 70	1,869	7,379	27.4 (26.3 – 28.5)	Reference	Reference
71 – 80	2,646	6,813	42.8 (41.5 – 44.1)	1.74 (1.64 – 1.85)	1.66 (1.56 – 1.76)
81 – 90	1,624	2,701	65.3 (63.3 – 67.2)	3.42 (3.20 – 3.65)	3.20 (2.99 – 3.43)
91 – 99	221	280	85.3 (79.9 – 89.3)	6.30 (5.47 – 7.24)	6.28 (5.45 – 7.23)
Sex					
Male	3,894	11,859	35.5 (34.6 – 36.4)	Reference	Reference
Female	3,190	10,499	32.7 (31.8 – 33.7)	0.90 (0.86 – 0.94)	0.80 (0.76 – 0.84)
CCI group					
Normal	289	1,864	17.2 (15.4 – 19.0)	0.81 (0.72 – 0.92)	0.88 (0.77 – 1.00)
Moderate	1,413	7,222	21.0 (20.0 – 22.0)	Reference	Reference
Severe	1,317	4,414	32.1 (30.7 – 33.5)	1.67 (1.55 – 1.80)	1.49 (1.38 – 1.60)
Very Severe	4,065	8,858	50.0 (48.9 – 51.1)	3.04 (2.86 – 3.23)	2.48 (2.33 – 2.64)
Region					
Capital	2,350	6,473	38.7 (37.4 – 39.9)	Reference	Reference
Central	1,146	4,321	28.8 (27.4 – 30.2)	0.69 (0.64 – 0.74)	0.77 (0.71 – 0.82)
Northern	706	2,118	37.0 (34.8 – 39.2)	0.96 (0.88 – 1.05)	0.92 (0.85 – 1.01)
Southern	2,073	6,700	33.4 (32.2 – 34.6)	0.84 (0.79 – 0.89)	0.85 (0.80 – 0.90)
Zealand	809	2,746	31.8 (30.0 – 33.6)	0.79 (0.73 – 0.85)	0.85 (0.78 – 0.92)

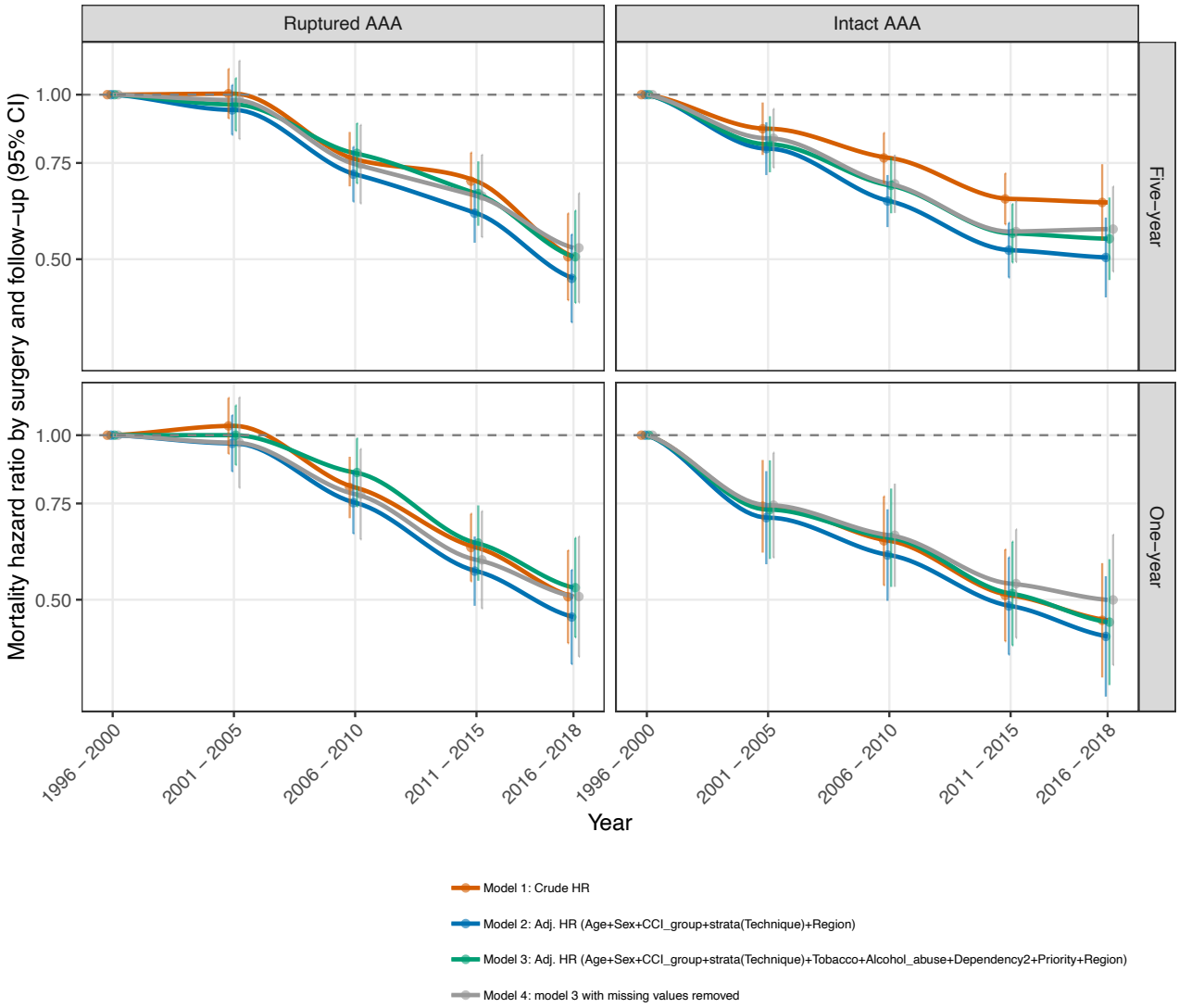


eTable 4. Mortality risk, crude HR and adjusted HR for by covariate and type of surgery at 30d, 90d, 1-yr, 2.5-yr and 5-yr following surgery

se enclosed excel-file

eTable 5. Sensitivity analyses.

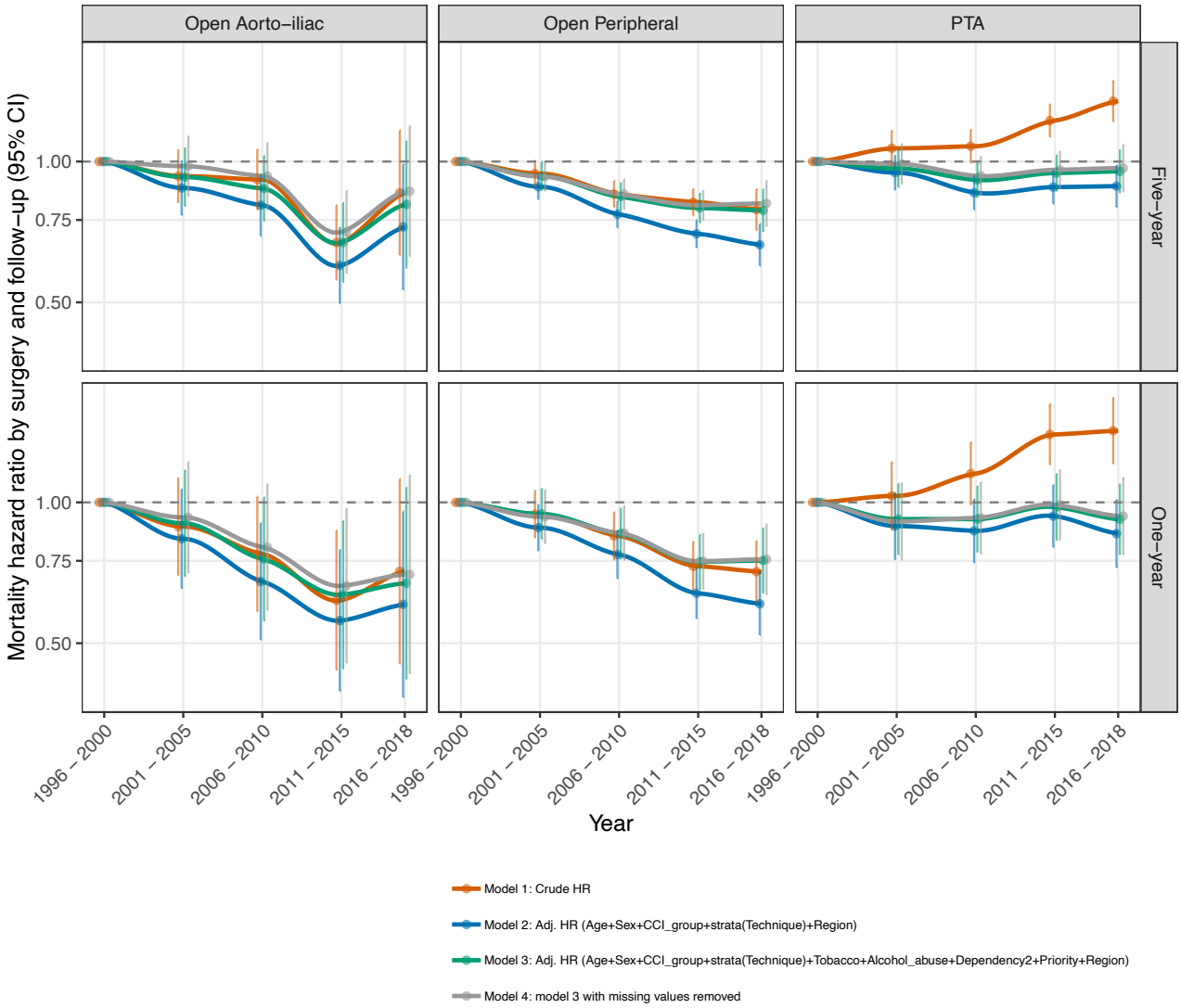
eTable+Figure 5a. AAA Five-year mortality hazard ratios, sensitivity analyses



follow_up	Surgery	Units	Model 1 crude	Model 2 Primary analysis	Model 3 Second. analysis	Model 4
Five-year	esAAA	1996 - 2000	Reference	Reference	Reference	Reference
Five-year	esAAA	2001 - 2005	0.87 (0.78-0.97)	0.80 (0.71-0.89)	0.81 (0.72-0.91)	0.83 (0.73-0.94)
Five-year	esAAA	2006 - 2010	0.77 (0.69-0.85)	0.64 (0.57-0.71)	0.68 (0.61-0.77)	0.69 (0.61-0.78)
Five-year	esAAA	2011 - 2015	0.64 (0.58-0.72)	0.52 (0.46-0.58)	0.56 (0.49-0.63)	0.56 (0.49-0.64)
Five-year	esAAA	2016 - 2018	0.63 (0.54-0.75)	0.50 (0.43-0.60)	0.54 (0.46-0.65)	0.57 (0.47-0.68)
Five-year	rAAA	1996 - 2000	Reference	Reference	Reference	Reference
Five-year	rAAA	2001 - 2005	1.00 (0.90-1.11)	0.94 (0.84-1.04)	0.96 (0.86-1.07)	0.98 (0.83-1.15)
Five-year	rAAA	2006 - 2010	0.76 (0.68-0.85)	0.72 (0.64-0.80)	0.78 (0.69-0.89)	0.75 (0.63-0.88)
Five-year	rAAA	2011 - 2015	0.69 (0.62-0.78)	0.61 (0.54-0.69)	0.66 (0.58-0.75)	0.65 (0.55-0.78)
Five-year	rAAA	2016 - 2018	0.50 (0.42-0.61)	0.46 (0.38-0.56)	0.51 (0.42-0.61)	0.52 (0.42-0.66)
One-year	esAAA	1996 - 2000	Reference	Reference	Reference	Reference

One-year	esAAA	2001 - 2005	0.74 (0.61-0.90)	0.71 (0.58-0.86)	0.73 (0.59-0.90)	0.74 (0.60-0.93)
One-year	esAAA	2006 - 2010	0.64 (0.53-0.77)	0.60 (0.50-0.73)	0.65 (0.53-0.80)	0.66 (0.53-0.82)
One-year	esAAA	2011 - 2015	0.51 (0.42-0.62)	0.49 (0.40-0.60)	0.51 (0.41-0.64)	0.54 (0.43-0.67)
One-year	esAAA	2016 - 2018	0.46 (0.36-0.58)	0.43 (0.33-0.55)	0.45 (0.35-0.59)	0.50 (0.38-0.66)
One-year	rAAA	1996 - 2000	Reference	Reference	Reference	Reference
One-year	rAAA	2001 - 2005	1.04 (0.92-1.17)	0.97 (0.86-1.09)	1.00 (0.88-1.13)	0.97 (0.80-1.17)
One-year	rAAA	2006 - 2010	0.80 (0.70-0.91)	0.75 (0.66-0.86)	0.85 (0.74-0.99)	0.78 (0.64-0.94)
One-year	rAAA	2011 - 2015	0.62 (0.54-0.72)	0.56 (0.49-0.65)	0.63 (0.54-0.74)	0.59 (0.48-0.73)
One-year	rAAA	2016 - 2018	0.51 (0.42-0.62)	0.46 (0.38-0.57)	0.53 (0.43-0.65)	0.51 (0.39-0.65)

eTable + eFigure 5b. LEAD Five-year mortality hazard ratios, sensitivity analyses



follow_up	Surgery	Units	Model 1	Model 2	Model 3	Model 4
Five-year	PTA	1996 - 2000	Reference	Reference	Reference	Reference
Five-year	PTA	2001 - 2005	1.07 (0.98-1.16)	0.95 (0.87-1.03)	0.97 (0.88-1.06)	0.99 (0.90-1.09)
Five-year	PTA	2006 - 2010	1.08 (0.99-1.17)	0.86 (0.79-0.93)	0.91 (0.84-1.00)	0.93 (0.85-1.02)
Five-year	PTA	2011 - 2015	1.22 (1.13-1.32)	0.88 (0.81-0.96)	0.94 (0.87-1.03)	0.96 (0.87-1.05)
Five-year	PTA	2016 - 2018	1.35 (1.22-1.49)	0.89 (0.80-0.98)	0.95 (0.86-1.06)	0.97 (0.86-1.09)
Five-year	cLEAD	1996 - 2000	Reference	Reference	Reference	Reference
Five-year	cLEAD	2001 - 2005	0.93 (0.81-1.06)	0.88 (0.77-1.00)	0.93 (0.80-1.07)	0.98 (0.84-1.13)
Five-year	cLEAD	2006 - 2010	0.91 (0.79-1.06)	0.81 (0.69-0.94)	0.88 (0.75-1.03)	0.93 (0.79-1.10)
Five-year	cLEAD	2011 - 2015	0.67 (0.56-0.81)	0.60 (0.50-0.72)	0.67 (0.55-0.82)	0.71 (0.58-0.87)
Five-year	cLEAD	2016 - 2018	0.86 (0.63-1.17)	0.72 (0.53-0.99)	0.81 (0.59-1.11)	0.86 (0.63-1.19)
Five-year	pLEAD	1996 - 2000	Reference	Reference	Reference	Reference
Five-year	pLEAD	2001 - 2005	0.94 (0.88-1.00)	0.88 (0.83-0.94)	0.93 (0.87-1.00)	0.93 (0.86-1.00)
Five-year	pLEAD	2006 - 2010	0.85 (0.80-0.91)	0.77 (0.72-0.82)	0.84 (0.78-0.90)	0.85 (0.79-0.92)

Five-year	pLEAD	2011 - 2015	0.82 (0.77-0.87)	0.70 (0.66-0.75)	0.80 (0.74-0.86)	0.81 (0.75-0.87)
Five-year	pLEAD	2016 - 2018	0.79 (0.71-0.87)	0.66 (0.60-0.74)	0.79 (0.71-0.87)	0.81 (0.73-0.91)
One-year	PTA	1996 - 2000	Reference	Reference	Reference	Reference
One-year	PTA	2001 - 2005	1.03 (0.87-1.22)	0.89 (0.75-1.05)	0.92 (0.77-1.10)	0.91 (0.75-1.10)
One-year	PTA	2006 - 2010	1.15 (0.99-1.34)	0.87 (0.74-1.02)	0.92 (0.78-1.08)	0.93 (0.78-1.10)
One-year	PTA	2011 - 2015	1.40 (1.20-1.62)	0.93 (0.80-1.09)	0.98 (0.83-1.15)	0.99 (0.83-1.18)
One-year	PTA	2016 - 2018	1.42 (1.21-1.67)	0.86 (0.73-1.01)	0.92 (0.77-1.10)	0.93 (0.77-1.13)
One-year	cLEAD	1996 - 2000	Reference	Reference	Reference	Reference
One-year	cLEAD	2001 - 2005	0.89 (0.70-1.13)	0.84 (0.66-1.07)	0.90 (0.69-1.17)	0.93 (0.71-1.22)
One-year	cLEAD	2006 - 2010	0.78 (0.58-1.03)	0.68 (0.51-0.90)	0.76 (0.56-1.03)	0.80 (0.59-1.09)
One-year	cLEAD	2011 - 2015	0.62 (0.44-0.87)	0.56 (0.40-0.79)	0.63 (0.44-0.91)	0.66 (0.45-0.97)
One-year	cLEAD	2016 - 2018	0.71 (0.45-1.12)	0.61 (0.38-0.96)	0.67 (0.42-1.08)	0.70 (0.43-1.14)
One-year	pLEAD	1996 - 2000	Reference	Reference	Reference	Reference
One-year	pLEAD	2001 - 2005	0.94 (0.84-1.06)	0.88 (0.79-0.99)	0.95 (0.84-1.07)	0.93 (0.82-1.06)
One-year	pLEAD	2006 - 2010	0.85 (0.75-0.95)	0.77 (0.69-0.87)	0.86 (0.76-0.97)	0.86 (0.75-0.98)
One-year	pLEAD	2011 - 2015	0.73 (0.65-0.82)	0.64 (0.57-0.72)	0.75 (0.65-0.85)	0.75 (0.65-0.86)
One-year	pLEAD	2016 - 2018	0.71 (0.61-0.83)	0.61 (0.52-0.71)	0.75 (0.64-0.88)	0.76 (0.64-0.90)

Miscellaneous

CCI item coding used

Disease categories and the International Classification of Diseases codes used to calculate Charlson's Comorbidity Index.

Disease category	ICD-8	ICD-10	Score
Myocardial infarction	410	I21, I23	1
Congestive heart failure	427.09; 427.10; 427.11; 427.19; 428.99; 782.49	I50; I11.0; I13.0; I13.2	1
Peripheral vascular disease	440; 441; 442; 443; 444; 445	I70- I74; I77	1
Cerebrovascular disease	430-438	I60-I69; G45; G46	1
Dementia	290.09-290.19; 293.09	F00-F03; F05.1; G30	1
Chronic pulmonary disease	490-493; 515-518	J40-J47; J60-J67; J68.4; J70.1; J70.3; J84.1; J92.0; J96.1; J98.2-J98.3	1
Connective tissue disease	712; 716; 734; 446; 135.99	M05; M06; M08; M09; M30-M36; D86	1
Ulcer disease	530.91; 530.98; 531-534	K22.1; K25-K28	1
Mild liver disease	571; 573.01; 573.04	B18; K70.0-K70.3; K70.9; K71; K73; K74; K76.0	1
Diabetes	249.00; 249.06; 249.07; 249.09; 250.00; 250.06; 250.07; 250.09	E10.0; E10.1; E10.9; E11.0; E11.1; E11.9	1
Hemiplegia	344	G81; G82	2
Moderate to severe renal disease	403; 404; 580-584; 590.09; 593.19; 753.10-753.19; 792	I12; I13; N00-N05; N07; N11; N14; N17-N19; Q61	2
Diabetes with end organ damage	249.01-249.05; 249.08; 250.01-250.05; 250.08	E10.2-E10.8; E11.2-E11.8	2
Any tumor	140-194	C00-C75	2
Leukemia	204-207	C91-C95	2
Lymphoma	200-203; 275.59	C81-C85; C88; C90; C96	2
Moderate to severe liver disease	070.00; 070.02; 070:04; 070.06; 070.08; 573.00; 456.00-456.09	B15.0; B16.0; B16.2; B19.0; K70.4; K72; K76.6; I85	3
Metastatic solid tumor	195-199	C76-C80	6
AIDS	079.83	B21-B24	6

ICD8-Criteria for excluding patients with AAA surgery prior to 1996:

1. Explicit operations codes for AAA surgery: ICD-8 codes 86550-5 and 86560
   OR
2. The combination of

a. ICD-8 codes for aorto-iliac bypass surgery:
   86740, 86760, 87049-55, 87060, 87063, 87064, 87069
   AND
b. A-diagnosis code consistent with AAA:
   ICD8: 44120-1, 44129
   ICD10: DI713, DI714

Criteria for imputing the operation code from the Danish National Patient Registry based on substring match between the DNPR and DVR

Imputation assumption / character match	N
char_1to5	25
letters_1to4	56
char_4to6	97
char_12356	72
char_5to6	56
last_3_char	12
SUM	318

Additional baseline characteristics coding

	DIAGNOSES ICD8	DIAGNOSES ICD10	SURGERY ICD8	SURGERY ICD10	Sks_ube
Angina history	413;	DI20			
Stroke or TIA	430; 431; 433; 434; 435; 43601; 43690	DI60-I64; DI69			
PM/ICD/loop recorder	99750	DT821; DT827I-P; DT828P; DZ450; DZ950	32122 32159 32199	KFPF-H	BFC
CABG/PCI			300;301; 30200; 30240; 30350	KFN	
Heart valve disease	394-6;39700; 424	DI05-8; DI091, DI091B DI098 DI34-7 DT820	303; 30600; 30620; 30640; 30729; 30780; 30799;	KFG; KFJE; KFJF; KFJW; KFK; KFM	

			30800; 30810; 30920; 30925; 30939; 30959; 30990; 311; 31268-9; Excl:30354, 30359		
Cardiomyopathy	42599	DI42-3			

Age trend

esAAA, elective and symptomatic AAA (intact AAA). rAAA, ruptured AAA.

Paper II

SPECIAL ARTICLE

Low vs. high haemoglobin trigger for transfusion in vascular surgery: protocol for a randomised trial

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*Principal investigator.

Conflicts of interest
None declared.

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Trial registration
ClinicalTrials.gov: NCT02465125.Registration date: 04/06/2015.URL: clinicaltrials.gov/ct2/show/NCT02465125.

Submitted 30 June 2017; accepted 7 July 2017; submission 5 July 2017.

Citation
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doi: 10.1111/aas.12953

Background: In patients with cardiovascular disease, guidelines for administration of red blood cells (RBC) are mainly based on studies outside the vascular surgical setting with the recommendation to use a haemoglobin (hb) trigger-level lower than by guidelines from The European Society for Vascular Surgery. Restricting RBC transfusion may affect blood O_2 transport with a risk for development of tissue ischaemia and postoperative complications.

Methods: In a single-centre, open-label, assessor blinded trial, 58 vascular surgical patients (> 40 years of age) awaiting open surgery of the infrarenal aorta or infrainguinal arterial bypass surgery undergo a web-based randomisation to one of two groups: perioperative RBC transfusion triggered by hb < 8 g/dl or hb < 9.7 g/dl. Administration of fluid follows an individualised strategy by optimising cardiac stroke volume and near-infrared spectroscopy determines tissue oxygenation. Serious adverse event rates are: myocardial injury (troponin-I ≥ 45 ng/l or ischaemic electrocardiographic findings at day 30), acute kidney injury, death, stroke and severe transfusion reactions. A follow-up visit takes place 30 days after surgery and a follow-up of serious adverse events in the Danish National Patient Register within 90 days is pending.

Discussion: This trial is expected to determine whether a RBC transfusion triggered by hb < 9.7 g/dl compared with hb < 8 g/dl results in adequate separation of postoperative hb levels, transfusion of more RBC units and maintains a higher tissue oxygenation. The results will inform the design of a multicentre trial for evaluation of important postoperative outcomes.

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An international journal of anaesthesiology and intensive care, pain and emergency medicine
SSAI The Scandinavian Society of Anaesthesiology and Intensive Care Medicine

Vascular surgical patients have high rates of comorbidity including coronary artery disease (CAD)[1–3] and during major surgery such as abdominal aortic aneurysm (AAA) repair, the subsequent blood loss may precipitate myocardial ischaemia, which increases mortality.[1,4,5] Thus, the central blood volume needs to be maintained through administration of intravenous fluids to aim for a normovolaemic condition.[6] When blood loss exceeds a certain level and especially with ongoing bleeding, tissue oxygenation may be compromised and red blood cell (RBC) transfusion is in need, however, the optimal haemoglobin (Hb) level is unknown. While RBC transfusion is necessary to maintain oxygen delivery,[7,8] use of blood products may be associated with increased mortality and morbidity during non-cardiac surgery,[9,10] including vascular surgery[11,12] and in patients with myocardial infarction.[13] It seems that perioperative use of RBC has shifted into a more conservative approach although prospective randomised clinical trials (RCTs) are unavailable to support such strategy as superior for patients with cardiovascular disease (CVD) including symptomatic CAD[14] and cardiac surgery.[15] Similar findings are reported for patients in upper gastrointestinal bleeding[16] and in hip fracture surgery patients.[17] In patients with CVD it is suggested that a conservative strategy for RBC administration is abandoned until further RCTs have been undertaken.[18]

The conflicting findings are reflected by that the European Society for Vascular Surgery recommends Hb to be maintained above 10 g/dl for patients undergoing open AAA repair[19] while The Danish Health Authority and The AABB recommend 8.0 g/dl in perioperative patients with CVD.[20,21] Therefore, a large multicentre trial to assess the optimal Hb trigger level for RBC administration during vascular surgery is warranted. Before a multicentre trial is planned, it needs to be addressed whether perioperative Hb levels as triggers for RBC transfusion separate postoperative Hb levels and use of blood product. This protocol presents the rationale, methods and detailed statistical analyses plan for such a trial.

Methods

Trial design

Single-centre, randomised, open-label trial.

Patients, outcome assessor and the statistician will be blinded to the allocated transfusion trigger. The anaesthetists will be blinded to the Near-infrared spectroscopy (NIRS) monitor. The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist and assessment figure of the trial can be found in Data S1: SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents.

Participant interventions and outcomes

Trial setting

University Hospital. One trial site: Regional Hospital Slagelse, Denmark.

Eligibility criteria

Inclusion.

1. Patients for planned open surgery of infrarenal AAA or infrainguinal arterial bypass AND
2. Age $\geq$ 40 years AND
3. Hb < 9.7 g/dl

Exclusion criteria.

1. Documented wish against transfusion OR
2. Previous serious adverse reaction with blood products OR
3. Unable to understand the benefits and risks of testing or sign written informed consent OR
4. Previous participation in the TV-trial

Interventions

After preoperative consent to join the trial, patients are randomised when the Hb is < 9.7 g/dl and allocated to an intervention or control group with the following RBC transfusion triggers:

1. intervention: Hb < 8 g/dl
2. control: Hb < 9.7 g/dl

Randomisation takes place no earlier than the day before operation and up until 30 days postoperatively. The intervention is given from randomisation and until 30 days after surgery, also in case of readmission. The triggers 8.0 and

9.7 g/dl correspond to the following haemoglobin units used in Denmark: 5 and 6 mmol/l, resp.

The attending anaesthetists will be in charge of the RBC transfusions during surgery and postoperative care. After discharge to the vascular surgery ward, the vascular surgeon will be responsible for protocol adherence.

Protocol suspension

The protocol may temporarily be suspended if the clinician in charge finds indication for additional RBC transfusions in case of clinical emergency:

1. severe hypotension (mean arterial pressure < 65 mmHg) unresponsive to fluid replacement OR
2. uncontrollable bleeding, defined as loss of surgical haemostasis resulting in overt or imminent haemodynamic instability with insufficient tissue oxygenation and increasing lactate production OR
3. stroke or ischaemia in extremities or intestines OR
4. RBC transfusion is omitted in case of suspected decompensated heart failure.

The protocol suspension will be discontinued when clinical stability is restored. Data collection will continue throughout periods of protocol suspension.

Criteria for discontinuing participation in trial—drop out

The patient can drop out of the trial by withdrawal of informed consent. In this case, the patient will be asked permission to continue the data registration and will be followed up in the intention-to-treat analysis.

Anaesthesia and surgery

Surgery is in accordance with local clinical guidelines. Fasting is 6 h for food and 2 h for clear fluids. All patients receive sevoflurane, fentanyl and rocuronium for general anaesthesia. For patients undergoing open AAA repair, epidural analgesia is used intra-operatively (1–3 ml bupivacaine 5 mg/ml repeated as needed) and for postoperative pain treatment (bupivacaine 2.5 mg/ml with fentanyl 2 μg/ml).

All patients are monitored with: invasive blood pressure (inserted in the arm with the highest non-invasively determined systolic blood pressure), 5-lead electrocardiogram, temperature, pulse oximetry and urinary output. In addition, AAA patients have central venous pressure monitoring. Heart rate is aimed for 40–90 min^{-1}, mean arterial pressure is maintained above 65 mmHg with noradrenaline if stroke volume is not affected by additional fluid, see below. Patients are mechanically ventilated (control mode) with a tidal volume ≥ 8 ml/kg to maintain end-tidal PCO_2 between 4.5 and 6.0 kPa, inspiratory oxygen fraction is set to 0.5 and positive end-expiratory pressure at 5 cm H_2O. Normothermia is ensured by forced-air warming and fluids and blood products are infused through blood warmers. The Hb level is measured every ½ h during ongoing bleeding.

Goal directed fluid therapy and tissue oxygenation

The primary i.v. fluid is Ringer's acetate solution. A cardiac output monitor, Edwards Flotrac 4.0®, guides fluid therapy by optimisation of cardiac stroke volume to aim for a normovolaemic condition. That is, stroke volume unaffected by fluid administration or an increase in stroke volume is below 10%.[22] Patients are also monitored by NIRS (Invos 5100c Cerebral Oximeter, Troy, MI, USA), with optodes is placed on the forehead and biceps, to assess changes in tissue oxygenation, which reflect the balance between O_2 supply and demand.[23]

Fluid strategy

Blood loss will be replaced by Ringer's Acetate in a 1 ml: 2–3 ml ratio in accordance with guidelines recommended by the Danish Society of Anaesthesia and Intensive Care.[24] When blood loss replacement exceeds 3 l of Ringer acetate, the bleeding will be replaced by 5% human albumin until the allocated transfusion threshold is reached. After 4–6 transfused RBC units, further transfusion will be supplemented with fresh frozen plasma (FFP) in a 1 : 1 RBC: FFP ratio. Bleeding exceeding 1.5 l prompts ROTEM analysis. In case of massive bleeding with deteriorated haemodynamics the protocol will be suspended and a balanced transfusion

strategy (RBC:FFP:trombocyte concentrate—balance of 4 : 4 : 1) commence.

Antithrombotic therapy

Practice for discontinuation of antithrombotic medicine adheres to the guideline of Danish Society of Thrombosis and Haemostasis.[25] All patients will intra-operatively receive a push dose of unfractionated heparin (50 IE/kg) intravenously 1 min before cross-clamp of the aorta or the common femoral artery.

Adherence to protocol

The principal investigator will follow-up on all RBC unit transfused during the trial period, register the reason for any non-adherence to the protocol and give feedback to the health care provider (doctor, nurse) to increase adherence.

Comcomitant care

All routine treatments including acute medical intervention are allowed during the trial. Use of synthetic colloids is prohibited.

Outcome measures

Primary outcome

Mean postoperative Hb day 0–15 (longitudinal outcome).

Secondary outcome

1. Units of RBCs transfused during hospital stay
2. Recruitment rate (number of randomised patients divided by number of eligible patients)
3. Proportion of patients having their protocol suspended
4. Adherence to Hb concentrations used for transfusion triggers
5. Intra-operative tissue oxygenation of the forehead (ScO_2) and biceps muscle (SmO_2) as determined by NIRS. Baseline is at the point where the patient is normovaemic according to the goal directed therapy principle after inducing anaesthesia before start of surgery. The outcome measure is defined as lowest value before a RBC transfusion. Area-under-baseline calculations are also applied; see statistical analysis plan in Data S4.
6. Coagulation competence determined by ROTEM at the end of surgery. Baseline sample drawn when placing arterial line.
7. Serious adverse events < 30 postoperative days
   a. myocardial injury as reflected by
      i. cardiac troponin-I ≥ 45 ng/l within two postoperative days OR
      ii. new onset of adverse electrocardiographic recordings on day 30 (bundle-branch block, Q-wave, inverted or flattened T-wave, ST-elevation or depression of 1 mm or more in two or more contiguous leads)
   b. acute kidney injury defined by the Kidney Disease: Improving Global Outcome criteria, see Data S2 for stage definition
      i. S-creatinine increment ≥ 26.5 μmol/l (≥ 0.3 mg/dl) within 48 h OR
      ii. S-creatinine increment × 1.5 times the preoperative level which has occurred within the prior 7 days OR
      iii. Urine production < 0.5 ml/kg/h within 6 h
   c. mortality
   d. ischaemic apoplexia
   e. severe adverse transfusion reactions, see definitions in Data S2

Explorative outcome measures at day 90 postoperative

1. Mortality
2. Major cardiovascular events (acute myocardial infarction, stroke, renal replacement therapy, vascular reoperation and amputation)
3. Days alive outside hospital within 90 days

Mortality data will be obtained from the CPR-registry (Danish Registry of National Identification Numbers) and major cardiovascular event data from The NPR (National Patient Registry).

For sub-analyses and bonferroni correction, see the detailed statistical analyses plan in Data S4.

For background literature related to the outcome measures see Data S2.

Participant timeline

Eligible patients will be approached consecutively and on the weekday before surgery, see Flow Chart in Data S5. For assessment of outcome, see Data S3—SPIRIT schedule of enrolment, assessments and interventions in the TV-trial.

Sample size

A review of 20 patient files at Slagelse Hospital in 2014 showed the following values for AAA repair patients, expressed as mean (standard deviation):

RBC transfusion: 750 ml (718 ml)
Postoperative Hb: 11.1 g/dl (1.45 g/dl)
ScO_2-standard deviation of 7%[26]

With a total of 50 randomised patients, a maximal type 1 error risk of 5%, and standard deviations as mentioned the trial has a power of:

1. 95% to show a difference in postoperative Hb of 1.6 g/dl with 44 patients.
2. 80% to show a difference of 600 ml of RBC volume transfused with 46 patients.
3. 80% to detect a difference in NIRS-determined ScO_2 of 6% during surgery with 44 patients.
4. And produces a 97.5% confidence interval (CI) equal to the sample adherence prevalence plus or minus 8% when the true prevalence of adherence is hypothesised to be 90% (as reported in Ref. 27).

Of note, we are not able to estimate the required sample size for area-under-baseline calculations due to lack of data for NIRS-monitored AAA repair patients.[28]

Recruitment

Based on a patient file review of 2014, where 120 bypass and 45 AAA operations were performed and anaemia (Hb < 9.7 g/dl) occurred at rates of 50% and 70%, resp, a 10 months trial period yields 76.25 recruitable patients: $(120 \times 0.50 + 45 \times 0.70) \times 10/12$. Assuming 1/3 of patients will not consent or be eligible, 50 recruited patients are expected.

Assignment of interventions

Adequate allocation sequence generation and concealment: computer-generated random numbers via a central web page provided by Copenhagen Trial Unit.

Implementation: Patients will be randomised and assigned by the investigator.

Stratification

Randomisation will be stratified for type of surgery: open AAA operation or infrainguinal arterial bypass.

Data collection, management and analysis

Data are continuously recorded in the paper-based case report form (CRF) and transferred with double entry into the trial database using OpenClinica®.

The trial has been accepted by The Danish Data Protection Agency (Region Zealand journal number 15-00024). Region Zealand and Copenhagen Trial Unit have signed an agreement on trial data processing (journal no. 2008-58-0020) including data management and statistical analyses.

It is possible to gain access to the trial protocol in accordance with The Law concerning Access to Public Records. Information obtained during the trial is confidential.

Baseline variables and paraclinical tests during hospital stay

See Data S3—SPIRIT schedule of enrolment, assessments and interventions in the TV trial.

Statistical analyses

It is assumed that the strategy with transfusion triggered by Hb < 9.7 g/dl compared to a transfusion triggered by Hb < 8 g/dl results in different postoperative Hb levels, units of RBC's transfused and tissue oxygenation. These hypotheses will thus be tested as in a superiority trial. Recruitment rate is presented with 95% confidence interval (CI). Furthermore, the secondary outcomes of proportion of patients having their protocol suspended and transfusions given adherent to Hb triggers will be calculated and tested. Our primary, secondary and explorative analyses will be based on the intention-

to-treat population and adjusted for age and the stratification variable. For all continuous outcomes, we will additionally adjust analyses for the baseline value. Missingness will be examined and in case of more than 5% of missing data and Littles test being statistical significant, we will perform best- and worst-case scenarios. If conclusions are different in the best- and worst-case scenarios, multiple imputation (MI) will be performed. Only two-sided tests will be used and $P < 0.05$ will be considered statistically significant.

1. Longitudinal data will be analysed using generalised estimating equations (GEE) or area under the curve (AUC) as appropriate according to the distribution of data and the assumptions for doing the analysis.
2. Count data will be analysed using van Elteren test.
3. Dichotomous outcomes will be analysed using generalised linear model (log link) or Fishers' exact test if there are few events.
4. Continuous outcomes will be analysed using linear regression.
5. Survival data will be analysed using both:
   a. Logistic regression presented with landmark serious adverse event forrest plot and odds ratios, 95% CI.
   b. Serious adverse event free days will be analysed with cox proportional hazards analysis of time to first event presented with Kaplan–Meier curve.

For further details regarding the statistical analyses plan, see Data S4.

Ethics and dissemination

Research ethical approval

The Scientific Ethical Committee of Region Zealand approved this protocol on May 12th 2015 (SJ-426).

Protocol amendments

1. The Scientific Ethical Committee of Region Zealand has approved extending the trial period to the end of 2016 due to an unanticipated 30% reduction in scheduled vascular surgery operations.
2. The Scientific Ethical Committee of Region Zealand approved extending the sample size to 58 patients and the trial period to 31 June 2017 to compensate for an unexpected high rate of patients randomised postoperatively and thus to secure a sufficient sample size ($n = 44$) of patients randomised intra-operatively.

Informed consent

The patient will be approached and presented for the trial when scheduled for surgery. On the day before the operation, the investigator will obtain informed consent.

Ethics

Patients receive the best treatment consistent with local and national recommendations. Participation in the trial will not delay diagnosis, surgery or discharge to the vascular surgery wards from the postoperative care. The Helsinki II declaration and national regulations will be respected.

No firm evidence exists from RCTs on the potential benefit or risk of RBC transfusion in vascular surgery. RBC transfusion is a part of current treatment of the majority of AAA operations and the triggers reflect practice and recommendations.

Due to the small sample size, no data monitoring committee, formal auditing or interim analysis has been implemented. Thus, the participants will not be exposed to known risks in the trial.

Injury and compensation

All trial participants are treated in the Danish public healthcare system and are thus lawfully entitled to get compensation through The Patient Compensation Association in case of injuries occurring in connection with the trial or any other injury during hospital stay not associated with the trial.

Dissemination policy

The results of the study are planned to be published as three different manuscripts. All manuscripts will be submitted for peer-reviewed publication and abstracts will be submitted for

presentation at national and international society meetings for anaesthesiology, vascular surgery and clinical immunology. The planned manuscripts are the main manuscript, a publication on ROTEM data, and a manuscript including observational data on bleeding, Hb levels and serious adverse events on enrolled, but excluded patients, due to sustained Hb ≥ 9.7 g/dl throughout the trial. A trial report is also provided to the funds of the trial and to those participants, who stated a wish for information in their consent form.

Discussion

This trial will assess postoperative Hb separation, use of blood product and adherence to protocol for perioperative RBC transfusion triggered by a Hb < 9.7 g/dl compared to a Hb < 8.0 g/dl. The trial is also expected to deliver information on recruitment rate and whether 9.7 g/dl results in a higher tissue oxygenation than 8.0 g/dl, though this cannot test or be extrapolated to differences in treatment effects on important patient centred outcomes. A large multicentre trial for evaluation of postoperative mortality in a high-risk surgical population is warranted.

Further work to prepare for the definitive trial includes a national registry study of 15 years of blood transfusion in vascular surgery to inform inclusion criteria and sample size estimation. We also plan a systematic review with meta-analyses and trial sequential analysis of randomised clinical trials testing conservative RBC use vs. a liberal transfusion strategy to inform the best design of a future large pragmatic trial.

Trial status

Enrolment started 13 July 2015 and the 58th patient was randomised on 8 December 2016, see "Fig. 1. Status of patients randomised in the TV trial". National patient registry follow-up on serious adverse events within 90 days is pending.

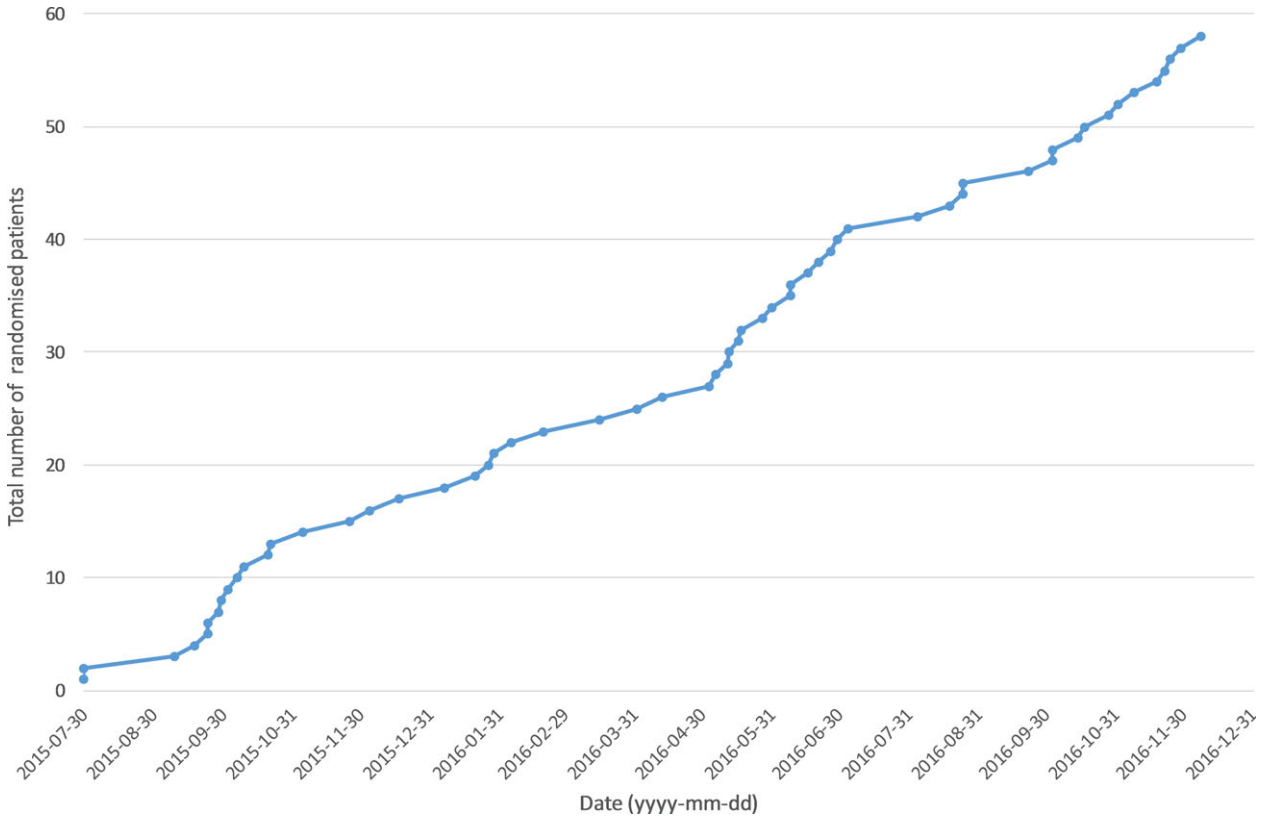

Fig. 1. Status of the TV trial. [Colour figure can be viewed at wileyonlinelibrary.com]

References

1. Hertzer NR, Beven EG, Young JR, O'Hara PJ, Ruschhaupt WF 3rd, Graor RA, Dewolfe VG, Maljovec LC. Coronary artery disease in peripheral vascular patients. A classification of 1000 coronary angiograms and results of surgical management. Ann Surg 1984; 199: 223–33.
2. Feringa HH, Karagiannis SE, Vidakovic R, Elhendy A, ten Cate FJ, Noordzij PG, van Domburg RT, Bax JJ, Poldermans D. The prevalence and prognosis of unrecognized myocardial infarction and silent myocardial ischemia in patients undergoing major vascular surgery. Coron Artery Dis 2007; 18: 571–6.
3. Ferro CR, de Oliveira DC, Guerra Fde F, de Lucena AJ, Nunes FP, Ortiz ST, Egito ES, de Sousa LC, Jatene AD, Piegas LS. Prevalence and risk factors for combined coronary artery disease and aortic aneurysm. Arq Bras Cardiol 2007; 88: 40–4.
4. Botto F, Alonso-Coello P, Chan MT, Villar JC, Xavier D, Srinathan S, Guyatt G, Cruz P, Graham M, Wang CY, Berwanger O, Pearse RM, Biccard BM, Abraham V, Malaga G, Hillis GS, Rodseth RN, Cook D, Polanczyk CA, Szczeklik W, Sessler DI, Sheth T, Ackland GL, Leuwer M, Garg AX, Lemanach Y, Pettit S, Heels-Ansdell D, Luratibuse G, Walsh M, Sapsford R, Schünemann HJ, Kurz A, Thomas S, Mrkobrada M, Thabane L, Gerstein H, Paniagua P, Nagele P, Raina P, Yusuf S, Devereaux PJ, Devereaux PJ, Sessler DI, Walsh M, Guyatt G, McQueen MJ, Bhandari M, Cook D, Bosch J, Buckley N, Yusuf S, Chow CK, Hillis GS, Halliwell R, Li S, Lee VW, Mooney J, Polanczyk CA, Furtado MV, Berwanger O, Suzumura E, Santucci E, Leite K, Santo JA, Jardim CA, Cavalcanti AB, Guimaraes HP, Jacka MJ, Graham M, McAlister F, McMurtry S, Townsend D, Pannu N, Bagshaw S, Bessissow A, Bhandari M, Duceppe E, Eikelboom J, Ganame J, Hankinson J, Hill S, Jolly S, Lamy A, Ling E, Magloire P, Pare G, Reddy D, Szalay D, Tittley J, Weitz J, Whitlock R, Darvish-Kazim S, Debeer J, Kavsak P, Kearon C, Mizera R, O'Donnell M, McQueen M, Pinthus J, Ribas S, Simunovic M, Tandon V, Vanhelder T, Winemaker M, Gerstein H, McDonald S, O'Bryne P, Patel A, Paul J, Punthakee Z, Raymer K, Salehian O, Spencer F, Walter S, Worster A, Adili A, Clase C, Cook D, Crowther M, Douketis J, Gangji A, Jackson P, Lim W, Lovrics P, Mazzadi S, Orovan W, Rudkowski J, Soth M, Tiboni M, Acedillo R, Garg A, Hildebrand A, Lam N, Macneil D, Mrkobrada M, Roshanov PS, Srinathan SK, Ramsey C, John PS, Thorlacius L, Siddiqui FS, Grocott HP, McKay A, Lee TW, Amadeo R, Funk D, McDonald H, Zacharias J, Villar JC, Cortés OL, Chaparro MS, Vásquez S, Castañeda A, Ferreira S, Coriat P, Monneret D, Goarin JP, Esteve CI, Royer C, Daas G, Chan MT, Choi GY, Gin T, Lit LC, Xavier D, Sigamani A, Faruqui A, Dhanpal R, Almeida S, Cherian J, Furruqh S, Abraham V, Afzal L, George P, Mala S, Schünemann H, Muti P, Vizza E, Wang CY, Ong GS, Mansor M, Tan AS, Shariffuddin II, Vasanthan V, Hashim NH, Undok AW, Ki U, Lai HY, Ahmad WA, Razack AH, Malaga G, Valderrama-Victoria V, Loza-Herrera JD, De Los Angeles Lazo M, Rotta-Rotta A, Szczeklik W, Sokolowska B, Musial J, Gorka J, Iwaszczuk P, Kozka M, Chwala M, Raczek M, Mrowiecki T, Kaczmarek B, Biccard B, Cassimjee H, Gopalan D, Kisten T, Mugabi A, Naidoo P, Naidoo R, Rodseth R, Skinner D, Torborg A, Paniagua P, Urrutia G, Maestre ML, Santaló M, Gonzalez R, Font A, Martínez C, Pelaez X, De Antonio M, Villamor JM, García JA, Ferré MJ, Popova E, Alonso-Coello P, Garutti I, Cruz P, Fernández C, Palencia M, Díaz S, Del Castillo T, Varela A, de Miguel A, Muñoz M, Piñeiro P, Cusati G, Del Barrio M, Membrillo MJ, Orozco D, Reyes F, Sapsford RJ, Barth J, Scott J, Hall A, Howell S, Lobley M, Woods J, Howard S, Fletcher J, Dewhirst N, Williams C, Rushton A, Welters I, Leuwer M, Pearse R, Ackland G, Khan A, Niebrzegowska E, Benton S, Wragg A, Archbold A, Smith A, McAlees E, Ramballi C, Macdonald N, Januszewska M, Stephens R, Reyes A, Paredes LG, Sultan P, Cain D, Whittle J, Del Arroyo AG, Sessler DI, Kurz A, Sun Z, Finnegan PS, Egan C, Honar H, Shahinyan A, Panjasawatwong K, Fu AY, Wang S, Reineks E, Nagele P, Blood J, Kalin M, Gibson D, Wildes T; Vascular events In noncardiac Surgery patIents cOhort evaluatioN (VISION) Writing Group, on behalf of The Vascular events In noncardiac Surgery patIents cOhort evaluatioN (VISION) Investigators; Appendix 1. The Vascular events In noncardiac Surgery patIents cOhort evaluatioN (VISION) Study Investigators Writing Group; Appendix 2. The Vascular events In noncardiac Surgery patIents cOhort evaluatioN Operations Committee; Vascular events In noncardiac Surgery patIents cOhort evaluatioN VISION Study Investigators. Myocardial injury after noncardiac surgery: a large, international, prospective cohort study establishing diagnostic criteria, characteristics, predictors, and 30-day outcomes. Anesthesiology 2014; 120: 564–78.
5. Mackey WC, Fleisher LA, Haider S, Sheikh S, Cappelleri JC, Lee WC, Wang Q, Stephens JM.

Perioperative myocardial ischemic injury in high-risk vascular surgery patients: incidence and clinical significance in a prospective clinical trial. J Vasc Surg 2006; 43: 533–8.
6. Bundgaard-Nielsen M, Holte K, Secher NH, Kehlet H. Monitoring of peri-operative fluid administration by individualized goal-directed therapy. Acta Anaesthesiol Scand 2007; 51: 331–40.
7. Atasever B, van der Kuil M, Boer C, Vonk A, Schwarte L, Girbes AR, Ince C, Beishuizen A, Groeneveld AB. Red blood cell transfusion compared with gelatin solution and no infusion after cardiac surgery: effect on microvascular perfusion, vascular density, hemoglobin, and oxygen saturation. Transfusion 2012; 52: 2452–8.
8. Torella F, Haynes SL, McCollum CN. Cerebral and peripheral oxygen saturation during red cell transfusion. J Surg Res 2003; 110: 217–21.
9. Glance LG, Dick AW, Mukamel DB, Fleming FJ, Zollo RA, Wissler R, Salloum R, Meredith UW, Osler TM. Association between intraoperative blood transfusion and mortality and morbidity in patients undergoing noncardiac surgery. Anesthesiology 2011; 114: 283–92.
10. Karkouti K, Stukel TA, Beattie WS, Elsaadany S, Li P, Berger R, Wijeysundera DN. Relationship of erythrocyte transfusion with short- and long-term mortality in a population-based surgical cohort. Anesthesiology 2012; 117: 1175–83.
11. Obi AT, Park YJ, Bove P, Cuff R, Kazmers A, Gurm HS, Grossman PM, Henke PK. The association of perioperative transfusion with 30-day morbidity and mortality in patients undergoing major vascular surgery. J Vasc Surg 2015; 61: 1000–9.e1.
12. Bursi F, Barbieri A, Politi L, Di Girolamo A, Malagoli A, Grimaldi T, Rumolo A, Busani S, Girardis M, Jaffe AS, Modena MG. Perioperative red blood cell transfusion and outcome in stable patients after elective major vascular surgery. Eur J Vasc Endovasc Surg 2009; 37: 311–8.
13. Chatterjee S, Wetterslev J, Sharma A, Lichstein E, Mukherjee D. Association of blood transfusion with increased mortality in myocardial infarction: a meta-analysis and diversity-adjusted study sequential analysis. JAMA Intern Med 2013; 173: 132–9.
14. Carson JL, Brooks MM, Abbott JD, Chaitman B, Kelsey SF, Triulzi DJ, Srinivas V, Menegus MA, Marroquin OC, Rao SV, Noveck H, Passano E, Hardison RM, Smitherman T, Vagaonescu T, Wimmer NJ, Williams DO. Liberal versus restrictive transfusion thresholds for patients with symptomatic coronary artery disease. Am Heart J 2013; 165: 964–71.e1.
15. Murphy GJ, Pike K, Rogers CA, Wordsworth S, Stokes EA, Angelini GD, Reeves BC; TITRe2 Investigators. Liberal or restrictive transfusion after cardiac surgery. N Engl J Med 2015; 372: 997–1008.
16. Jairath V, Kahan BC, Gray A, Dore CJ, Mora A, James MW, Stanley AJ, Everett SM, Bailey AA, Dallal H, Greenaway J, Le Jeune I, Darwent M, Church N, Reckless I, Hodge R, Dyer C, Meredith S, Llewelyn C, Palmer KR, Logan RF, Travis SP, Walsh TS, Murphy MF. Restrictive versus liberal blood transfusion for acute upper gastrointestinal bleeding (TRIGGER): a pragmatic, open-label, cluster randomised feasibility trial. Lancet 2015; 386: 137–44.
17. Carson JL, Terrin ML, Noveck H, Sanders DW, Chaitman BR, Rhoads GG, Nemo G, Dragert K, Beaupre L, Hildebrand K, Macaulay W, Lewis C, Cook DR, Dobbin G, Zakriya KJ, Apple FS, Horney RA, Magaziner J; FOCUS Investigators. Liberal or restrictive transfusion in high-risk patients after hip surgery. N Engl J Med 2011; 365: 2453–62.
18. Docherty AB, O'Donnell R, Brunskill S, Trivella M, Doree C, Holst L, Parker M, Gregersen M, Pinheiro de Almeida J, Walsh TS, Stanworth SJ. Effect of restrictive versus liberal transfusion strategies on outcomes in patients with cardiovascular disease in a non-cardiac surgery setting: systematic review and meta-analysis. BMJ 2016; 352: i1351.
19. Moll FL, Powell JT, Fraedrich G, Verzini F, Haulon S, Waltham M, van Herwaarden JA, Holt PJ, van Keulen JW, Rantner B, Schlösser FJ, Setacci F, Ricco JB; European Society for Vascular Surgery. Management of abdominal aortic aneurysms clinical practice guidelines of the European society for vascular surgery. Eur J Vasc Endovasc Surg 2011; 41(Suppl 1): S1–58.
20. Carson JL, Guyatt G, Heddle NM, Grossman BJ, Cohn CS, Fung MK, Gernsheimer T, Holcomb JB, Kaplan LJ, Katz LM, Peterson N, Ramsey G, Rao SV, Roback JD, Shander A, Tobian AA. Clinical practice guidelines from the AABB: red blood cell transfusion thresholds and storage. JAMA 2016; 316: 2025–35.
21. https://www.sst.dk/da/udgivelser/2014/~/media/847BC14D4714453AA3B4ABE41135E476.ashx (accessed 15 June 2017).
22. Kuper M, Gold SJ, Callow C, Quraishi T, King S, Mulreany A, Bianchi M, Conway DH. Intraoperative fluid management guided by oesophageal Doppler monitoring. BMJ 2011; 342: d3016.

23. Nielsen HB. Systematic review of near-infrared spectroscopy determined cerebral oxygenation during non-cardiac surgery. Front Physiol 2014; 5: 93.
24. http://www.dasaim.dk/wp-content/uploads/2014/12/Rekommandation-for-perioperativ-v%C3%A6ske-v-211214_TIC_JAaKP.pdf (accessed 15 June 2017).
25. PerioPerativ regulering af antitrombotisk. [In Danish] Available at: http://dsth.dk/pdf/PRAB_2016_WEB.pdf (accessed 15 June 2017).
26. Rokamp KZ, Secher NH, Eiberg J, Lonn L, Nielsen HB. O_2 supplementation to secure the near-infrared spectroscopy determined brain and muscle oxygenation in vascular surgical patients: a presentation of 100 cases. Front Physiol 2014; 5: 66.
27. Shehata N, Burns LA, Nathan H, Hebert P, Hare GM, Fergusson D, Mazer CD. A randomized controlled pilot study of adherence to transfusion strategies in cardiac surgery. Transfusion 2012; 52: 91–9.
28. Sorensen H, Nielsen HB, Secher NH. Near-infrared spectroscopy assessed cerebral oxygenation during open abdominal aortic aneurysm repair: relation to end-tidal CO2 tension. J Clin Monit Comput 2016; 30: 409–15.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Data S1. Checklist: Recommended items to address in a clinical trial protocol and related documents*.
Data S2. Definitions and literature on the serious adverse event outcome measures used in the TV trial.
Data S3. SPIRIT schedule of enrolment, assessments and interventions in the TV trial.
Data S4. Detailed statistic analysis plan.
Data S5. The TV trial flow chart.

Paper III

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Regular Article

CLINICAL TRIALS AND OBSERVATIONS

Low vs high hemoglobin trigger for transfusion in vascular surgery: a randomized clinical feasibility trial

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KEY POINTS

- **Perioperative restriction of red cells significantly lowered hemoglobin levels, red cell units transfused, and cerebral tissue oxygenation.**
- **Explorative outcomes indicated harm with the low transfusion trigger and warrants further trials to establish whether such strategy is safe.**

Current guidelines advocate to limit red blood cell (RBC) transfusion during surgery, but the feasibility and safety of such a strategy remain unclear, as the majority of evidence is based on postoperatively stable patients. We assessed the effects of a protocol aiming to restrict RBC transfusion throughout hospitalization for vascular surgery. Fifty-eight patients scheduled for lower limb bypass or open abdominal aortic aneurysm repair were randomly assigned, on hemoglobin drop below 9.7 g/dL, to either a low-trigger (hemoglobin < 8.0 g/dL) or a high-trigger (hemoglobin < 9.7 g/dL) group for RBC transfusion. Near-infrared spectroscopy assessed intraoperative oxygen desaturation in brain and muscle. Explorative outcomes included nationwide registry data on death and major vascular complications. The primary outcome, mean hemoglobin within 15 days of surgery, was significantly lower in the low-trigger group, at 9.46 vs 10.33 g/dL in the high-trigger group (mean difference, −0.87 g/dL; *P* = .022), as were units of RBCs transfused (median [interquartile range (IQR)], 1 [0-2] vs 3 [2-6]; *P* = .0015). Although the duration and magnitude of cerebral oxygen desaturation increased in the low-trigger group (median [IQR], 421 [42-888] vs 127 [11-331] minutes × %; *P* = .0036), muscle oxygenation was unaffected. The low-trigger group associated to a higher rate of death or major vascular complications (19/29 vs 8/29; hazard ratio, 3.20; *P* = .006) and fewer days alive outside the hospital within 90 days (median [IQR], 76 [67-82] vs 82 [76-84] days; *P* = .049). In conclusion, a perioperative protocol restricting RBC transfusion successfully separated hemoglobin levels and RBC units transfused. Exploratory outcomes suggested potential harm with the low-trigger group and warrant further trials before such a strategy is universally adopted. This trial was registered at www.clinicaltrials.gov as #NCT02465125. (*Blood*. 2019;133(25):2639-2650)

Introduction

Fluid resuscitation with red blood cells (RBCs) plays a fundamental role in maintaining tissue oxygenation during blood loss. This is particularly relevant for the vascular surgical patient because of frequent preoperative anemia[1,2] and surgical hemorrhage exceeding 500 mL,[3-5] both of which are associated with improved survival if RBC transfusion is initiated during surgery compared with no transfusion.[6] Furthermore, as these patients often present with cardiac disease,[7-9] they may be particularly vulnerable to a hemoglobin (Hb) level below 10 g/dL.[2,10] However, as allogeneic RBC transfusions are also associated with mortality and cardiovascular morbidity,[11-13] and are an expensive and limited resource, randomized trials are warranted to balance risks and benefits of transfusion strategies in vascular surgery.[14] In other surgical specialties, it has been demonstrated that withholding transfusion until reaching a Hb level of 7.0 to 8.0 g/dL is safe,[15-19] but the majority of the evidence is based on postoperative stable patients. Moreover, previous trials involving blood loss above 500 mL lacked adequate separation of Hb levels and RBC units transfused during surgery,[4,16,18,20] possibly as a result of the recruitment of patients with subsequent minimal blood loss or poor protocol adherence. Thus, the safety and feasibility of restricting RBCs during many types of surgeries remain unsettled.

This led us to design the Transfusion in Vascular surgery (TV) trial, as previously reported.[21] We enrolled patients ahead of surgery. Intraoperative fluid therapy was standardized, using changes in cardiac stroke volume to guide when intravascular volume was adequate. An enrichment strategy secured that only patients with an Hb drop below 9.7 g/dL were randomly assigned to 1 of 2 Hb triggers for RBC transfusion. This ensured that patients with limited blood loss and/or high preoperative Hb were excluded. The objective was to assess whether a perioperative strategy that

aimed to restrict RBC transfusion to an Hb drop below 8 g/dL (5 mmol/L, low-trigger), as compared with an Hb below 9.7 g/dL (6 mmol/L, high-trigger), would separate postoperative Hb levels and units of RBCs transfused, as well as influence intraoperative peripheral tissue oxygenation. We also report the occurrence of nonadherence, protocol suspensions, and as an exploratory outcome, death and major vascular complications.

Patients, material, and methods

Trial design and oversight

The TV trial was an investigator-initiated, single-center, stratified, parallel-group, patient- and partly assessor-blinded clinical trial with central web-based randomization. The protocol complies with the criteria outlined in the Declaration of Helsinki (7th revision, 2013) and was approved by the Scientific Ethical Committee of Region Zealand (Project-ID: SJ-426) and the Danish Data Protection Agency. The trial was registered at www.clinicaltrials.gov (NCT02465125) before enrolment of the first patient. Methods and plan for statistical analyses were published previously in detail in a protocol adhering to *The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT)-guideline*.[21] During trial enrolment, we observed that the number of patients randomly assigned after surgery was higher than expected. To reach the required sample size for the intraoperative outcome measures, we increased the sample size from 50 to 58 patients after the Ethical Committee's approval. The setting was a vascular unit, servicing a population of 820 000, corresponding to approximately 15% of the Danish population.

Patients

Patients older than 40 years, who were referred for elective open infra-renal abdominal aortic aneurism (AAA) repair or lower limb bypass (infra-inguinal arterial bypass surgery or femuro-femoral crossover surgery) were asked to participate at the time of scheduling for their operation. Potential patients were excluded if they refused RBC transfusion, had a previous serious adverse reaction with blood products, had previously participated in the TV trial, or were unable to understand the benefits and risks of participating.

Randomization, blinding, and data collection

After preoperative informed and signed consent, randomization and implementation of the group assignment occurred on Hb drop below 9.7 g/dL at any time between the day before surgery and the 30th postoperative day. Randomization was performed by means of an external, centralized, web-based system at The Copenhagen Trial Unit. The allocation sequence was computer-generated in a 1:1 ratio with fixed block sizes of 6 stratified for type of surgery: open AAA operation vs lower limb bypass. Patients, statisticians (P.W. and J.C.J.) and outcome assessor (D.H.) were blinded to the group assignment. Tissue oxygenation, as assessed by near-infrared spectroscopy (NIRS; INVOS5100C; Somanetics, Troy, MI), was blinded to prevent access to data during anesthesia. NIRS is considered a trend monitor[22] of regional Hb oxygen saturation. With a NIRS probe applied on the right part of the forehead, regional cerebral oxygenation ($rScO_2$) was determined. Another probe was applied over the middle part of the right brachial biceps muscle to assess regional muscle oxygenation ($rSmO_2$). Baseline was established ahead of surgery and was defined as the NIRS reading on reaching normovolemia (defined in "Surgery, anesthesia, and fluid").

Cardiac troponin-I and P-creatinine were assessed the first and second day after randomization. Electrocardiogram was obtained preoperatively, in the case of troponin-I above 45 ng/L, and at follow-up 30 days after surgery. At the same follow-up time, patients had Hb measured and were contacted by telephone to inquire whether they had experienced symptoms related to ischemic heart disease or had been aware of their random assignment. Registry follow-up for the exploratory outcomes was requested half a year after surgery of the last randomized patient.

Surgery, anesthesia, and fluid

Surgery adhered to national guidelines and was performed by, or directly supervised by, a consultant in vascular surgery. All patients received sevoflurane, fentanyl, and rocuronium for general anesthesia. Patients with AAA repair had an epidural catheter inserted for perioperative analgesia. Each milliliter of blood loss was replaced by 2 to 3 mL Ringer's acetate according to national guidelines.[21] If blood loss exceeded 1000 mL, or total crystalloid administration was above 3000 mL, human albumin (5%) was administered until the Hb decreased below the allocated threshold for RBC transfusion. Fluid therapy was guided by optimization of cardiac stroke volume, using arterial waveform analysis (FloTrac ver.4.0; Edwards LifeSciences, Irvine, CA). When a fluid bolus increased stroke volume by less than 10%, the patient was considered normovolemic.[23] Then, adequate perfusion pressure was ensured by keeping mean arterial pressure above 65 mm Hg with continuous infusion of norepinephrine (100 μg/mL). Use of plasma and platelets was guided by rotational thromboelastometry (ROTEM).[24] Further details, including targets for heart rate, ventilation, oxygenation, and coagulation, are provided in the protocol.[21]

Intervention, protocol suspension, and nonadherence

After randomization, patients in the low-trigger group (experimental intervention) awaited RBC transfusion until Hb dropped below 8.0 g/dL (5 mmol/L) and received additional RBC units as needed to maintain an Hb level at or above 8.0 g/dL. Patients in the high-trigger group (control intervention) received 1 RBC unit immediately after randomization and additional units as needed to maintain an Hb level at or above 9.7 g/dL (6 mmol/L). The Hb level was measured every half hour during ongoing surgical bleeding, after every RBC transfusion, at a minimum on day 1 and 2 after surgery and randomization, and when clinically indicated. All RBC transfusions were allogeneic, leucoreduced, and nonradiated.

The assigned transfusion strategy was to be followed until 30 days after surgery, as well as in case of readmission. Physicians could temporarily suspend the protocol in case of uncontrollable bleeding, hypotension (mean arterial pressure $<$65 mm Hg) unresponsiveness to fluid replacement, stroke, or occurrence of ischemia in extremities or intestines. RBC transfusion could also be omitted in the case of suspected decompensated heart failure.

The term nonadherence was based on 2 events: an RBC unit administered at an Hb level above the allocated threshold or on

indications not defined in the protocol suspensions above, and nonadherent failure to transfuse when the Hb level was below the allocated threshold.

Outcomes

The primary outcome was a longitudinal outcome of mean postoperative Hb days 0 to 15, in which day 0 was the Hb measured immediately after surgery on arrival at the post-anesthesia care unit. The secondary outcomes were units of RBCs transfused, randomization rate, proportion of patients with protocol suspensions, adherence to Hb concentrations used for transfusion triggers (definitions in Table 2), intraoperative tissue oxygenation as determined by NIRS, and severe adverse events within 30 days of surgery. The NIRS outcome was the lowest $rScO_2$ and lowest $rSmO_2$ before an RBC transfusion (or at the end of operation if no transfusion was given). The duration and magnitude of cerebral desaturation from baseline, the desaturation load, was defined as the cumulative area (minutes × %) below baseline. Definitions of severe adverse events at day 30 are provided in supplemental Table 4, available on the *Blood* Web site.

Based on The Danish National Patient Registry,[25] a 90-day exploratory outcome measure was added. This included death or major vascular complications, which encompassed severe adverse transfusion reaction (definition provided in the legend of Figure 4), acute myocardial infarction (adhering to the universal definition[26]), stroke, new-onset renal replacement therapy, unscheduled vascular surgery registered as secondary to the index operation, and major or minor amputation of the lower limb, defined as above or below the knee, or as forefoot or digit, respectively. The specific registry coding used to identify the major vascular complications is provided in supplemental Table 7. Days alive outside hospital within 90 days of surgery was also reported as an exploratory outcome to capture a net benefit/harm of the intervention (ie, death or hospital stay from any cause).

Statistical methods

With 50 randomized patients, a maximal type 1 error risk of 5%, and standard deviations as described previously,[21] the trial had 95% power to show a difference in postoperative Hb of 1.6 g/dL (1.0 mmol/L) with 44 patients, 80% power to show a difference of 600 mL of RBC volume transfused with 46 patients, and 80% power to detect a difference in NIRS-determined $rScO_2$ of 6% with 44 patients. Last, we also would be able to produce a 97.5% confidence interval (CI) equal to the adherence proportion plus or minus 8%. The required sample size for the desaturation load could not be estimated because of a lack of background data.[27]

All analyses were based on the intention-to-treat population and adjusted for age and type of surgery.[28] Postoperative Hb (longitudinal outcome) was analyzed with generalized estimated equations (GEE), using STATA (version 15/MP). SAS (version 9.4) was used for all other analyses. Binary outcomes were analyzed using logistic regression, and odds ratios were converted to relative risks (RRs). Continuous outcomes were analyzed using linear regression and were additionally adjusted for the baseline value. We applied van Elteren's test on count or skewed distributed data adjusted for type of surgery. Time to event was analyzed using Cox proportional hazards model and included death or major vascular complications occurring between randomization and right censoring (90 days after the last randomization, March 8, 2017). We tested the proportional hazards assumption for all covariates.[29] Only 2-sided tests were used, and a $P < .05$ was considered statistically significant except for the NIRS hypothesis, where we had adjusted the significance level to $P < .02$ because of multiple comparisons of correlated outcome measures. An exploratory longitudinal analysis of the Hb data was performed, using the mixed-model, as this is unbiased when data are missing at random, as opposed to GEE, which uses the last observation carried forward. R studio (version 1.1.447) was used for generating graphs and tables. Further details are provided in the statistical analysis plan.[21]

Results

Study population

Between July 2015 and December 2016, 112 (78%) of 144 approached patients consented to participate. Eleven patient enrollments were discontinued before surgery (reasons provided in Figure 1). Randomization occurred on Hb decrease below 9.7 g/dL in 58 (57%) of the remaining 101 patients. This yielded a randomization rate of 58/144 (40%; 95% CI, 32%-48%) over the course of 17 months, allocating 29 to each group (Figure 1). This rate was lower than anticipated,[21] which may be explained by an unexpected 20% to 25% reduction in patients referred for AAA repair during the trial period and by the reasons presented in Figure 1. There was no patient drop-out during follow-up. Registry data were provided 9 months after the last randomization. NIRS data were subsequently extracted, which completed the database in October 2017.

Patient characteristics were similar at baseline (Table 1). The stratification distributed surgical procedures equally between the 2 groups. Further surgery specifics are provided in the supplementary material (supplemental Table 1). At day 30 follow-up, 98% of the patients were unaware of or could not recollect their assigned transfusion threshold.

Primary outcome measure

Within 15 days of surgery, mean postoperative Hb was 9.46 g/dL in the low-trigger group vs 10.33 g/dL in the high-trigger group. This difference was statistically significant with GEE ($P = .022$) and when the mixed-model was applied (mean difference, −0.91 g/dL; 95% CI, −1.21 to −0.61; $P < .001$). The mean Hb difference was pronounced on day 0 in the 46 patients randomly assigned before the end of surgery (−1.35 g/dL; $P < .001$) and at follow-up 30 days after surgery (−1.07 g/dL; $P = .004$; Table 2; Figure 2; supplemental Figures 1 and 2). These results were consistent across subgroups and sensitivity analyses (supplemental Table 2).

Secondary outcome measures

The number of RBC units transfused was significantly reduced with the low-trigger strategy, overall (median [interquartile range (IQR)], 1 [0-2] vs 3 [2-6]; $P = .0015$) and during and after surgery (Table 2). The proportion of patients exposed to RBCs was reduced by 34%, and the overall usage of RBC units was more than halved (Table 2). Although nonadherent RBC transfusions seemed more common in the low-trigger group, there was a clear trend toward more cases of nonadherent failure to transfuse in the high-trigger group. Overall, any nonadherence

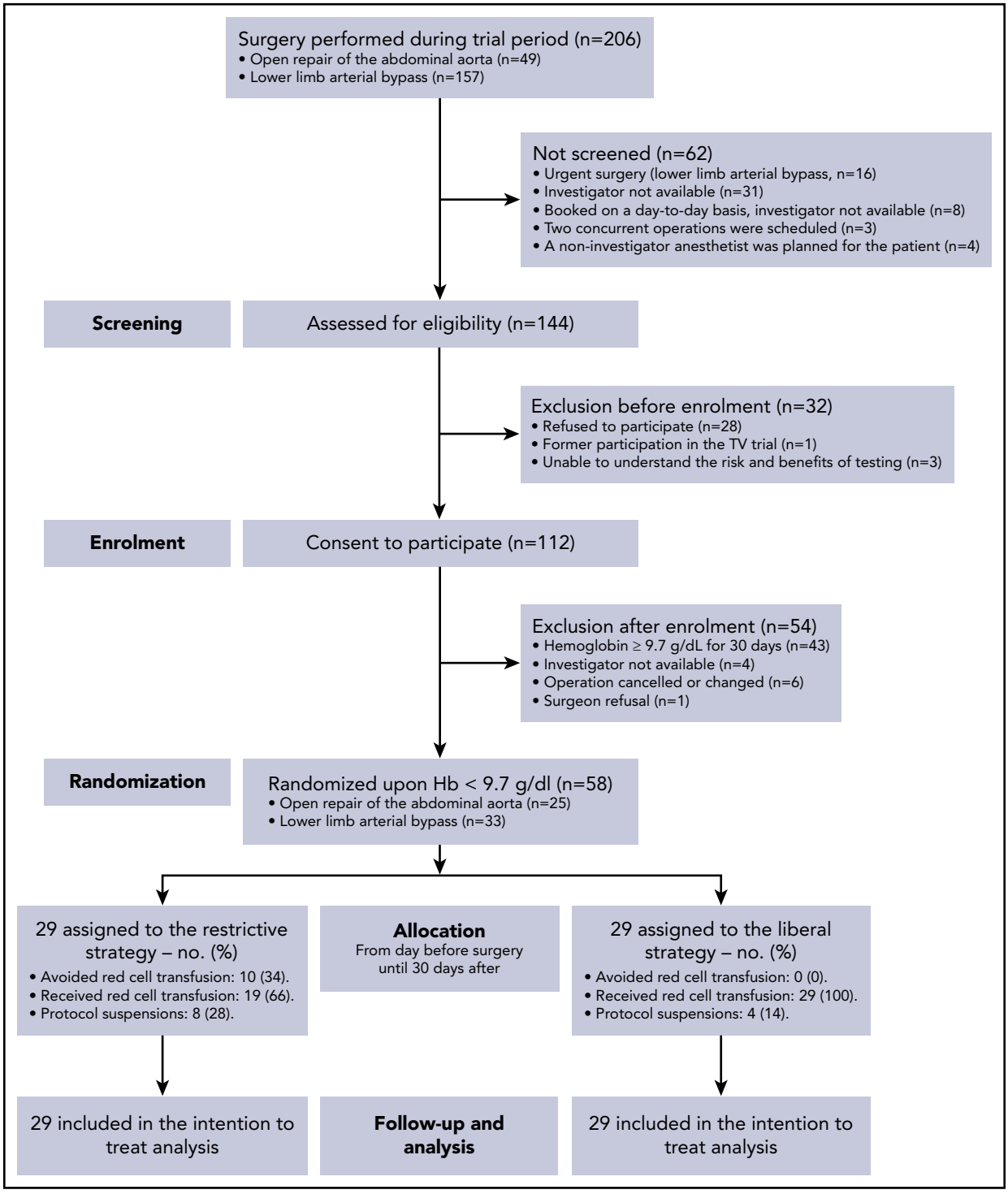

Figure 1. Screening, enrolment, randomization, and follow-up. Randomization occurred in 46 (79%) patients before end of surgery, in 53 patients (91%) within or on the second postoperative day, and in the remaining 5 patients on the third, fourth, sixth, ninth, and fifteenth day.

occurred in 28% and 34% of the patients in the 2 groups (Table 2; supplemental Table 2.6). Apart from the intraoperative infused volume of RBCs, parameters related to intraoperative fluid balance and duration of anesthesia, surgery, and cross clamp were similar in the 2 groups (supplemental Table 1). The overall mean blood loss was 1268 mL, but no intraoperative ROTEM assessments indicated need for plasma or platelet substitution.

The cerebral desaturation load was significantly higher in the low-trigger group (median [IQR], 421 [42-888] minutes × % vs 127 [11-331] minutes × %; $P = .0036$; Table 3; Figure 3). The muscle desaturation load and the lowest $rScO_2$ and $rSmO_2$ values before an RBC transfusion did not differ significantly. Square root transformation of the desaturation load (to approximate a normal distribution for linear regression; supplemental Table 3.1) and the sensitivity analysis (excluding patients who did not receive RBC

Table 1. Patient characteristics

	Trigger < 8 g/dL (n = 29)	Trigger < 9.7 g/dL (n = 29)
Age, mean ± SD, y	71.3 ± 9.4	73.7 ± 7.3
Male sex, n (%)	19 (65.5)	18 (62.1)
BMI, mean ± SD, kg/m^2	25.3 ± 5.8	24.8 ± 3.8
Operation, n (%)		
Lower limb bypass	17 (58.6)	16 (55.2)
Abdominal aortic aneurysm*	12 (41.4)	13 (44.8)
Time of randomization, n (%)		
The day before surgery	2 (6.9)	3 (10.3)
During surgery	20 (69.0)	21 (72.4)
After end of surgery	7 (24.1)	5 (17.2)
Any cardiovascular disease, n (%)	26 (89.7)	23 (79.3)
Lower extremity artery disease	20 (69.0)	17 (58.6)
Claudication	4 (13.8)	1 (3.4)
Pain at rest	4 (13.8)	5 (17.2)
Wound/gangrene	12 (41.4)	11 (37.9)
Angina	3 (10.3)	5 (17.2)
Stroke or TIA	8 (27.6)	5 (17.2)
Previous CABG or PCI	3 (10.3)	5 (17.2)
Congestive heart failure	1 (3.4)	2 (6.9)
Heart valve disease	3 (10.3)	4 (13.8)
Acute myocardial infarction	0 (0.0)	6 (20.7)
Pacemaker	0 (0.0)	0 (0.0)
Any cardiovascular risk factor, n (%)	29 (100.0)	28 (96.6)
Arterial hypertension	19 (65.5)	22 (75.9)
Smoker	12 (41.4)	11 (37.9)
COPD	6 (20.7)	8 (27.6)
Hypercholesterolemia	22 (75.9)	26 (89.7)
Chronic renal failure	3 (10.3)	6 (20.7)
Diabetes mellitus	8 (27.6)	6 (20.7)
ASA score, mean ± SD	3.0 ± 0.27	2.9 ± 0.41
ASA class, n (%)		
ASA1	0 (0.0)	0 (0.0)
ASA2	1 (3.4)	4 (13.8)
ASA3	27 (93.1)	24 (82.8)
ASA4	1 (3.4)	1 (3.4)
Preoperative Hb, mean ± SD, g/dL	12.3 ± 1.8	12.4 ± 2.2
Preoperative creatinine, median (IQR), μmol/L	73 (62 to 98)	87 (66 to 108)
NIRS at baseline†, mean ± SD		
Regional cerebral oxygenation, %	60.8 ± 9.6	59.5 ± 9.1
Regional biceps muscle oxygenation, %	69.8 ± 8.9	68.2 ± 9.8

There was no between-group difference in race, as all patients were white. The only statistically significant baseline difference between groups was previous AMI (P = .023).

ASA, American Society of Anesthesiologists; BMI, body mass index; CABG, coronary artery bypass grafting; COPD, chronic obstructive pulmonary disease; PCI, percutaneous coronary intervention; TIA, transient ischemic attack.

*One patient in the AAA-stratum of the high-trigger (< 9.7g/dL) group underwent aorto-iliac bifurcated prosthesis surgery on the indication aorto-iliac occlusive disease. Otherwise, all patients in the AAA-stratum had aneurysmal disease.

†Baseline was defined as the point at which the patient was normovolemic according to the goal-directed fluid strategy after anesthesia was induced and before starting surgery. The NIRS readings before O_2-supplementation and induction of anesthesia were similar to this baseline (supplemental Table 3.0).

transfusions; supplemental Table 3.2) showed similar point estimates. Within 30 days after the operation, both troponin-I (≥45 ng/L) and P-creatinine (≥26.5 μg/L) had occurred in approximately 33% of patients in both groups, and the composite of any serious adverse events did not differ with statistical significance (RR, 0.74; 95% CI, 0.42-1.30; P = .28; supplemental Table 4).

Table 2. Hb, red cell transfusions and adherence

	Trigger<8 g/dL (n = 29)	Trigger<9.7 g/dL (n = 29)	Treatment effect (95% CI)	P
Hb (day 0 – 15)				
Grand mean, g/dl and GEE coefficient*	9.46	10.33	GEE coefficient: −0.45 (−0.84 to −0.07)	.022
Modeled mean, g/dL†	9.70	10.61	−0.91 (−1.21 to −0.61)	<.001
Hb in recovery room (day 0)‡				
Mean, g/dl (n = 46)†	9.17	10.51	−1.35 (−1.74 to −0.95)	<.001
Hb at follow-up (day 30)				
Mean, g/dL (n = 45)†	10.75	11.83	−1.07 (−1.79 to −0.36)	.004
Hb below lowest trigger, n (%)				
≥1 Hb measurement below 8 g/dL	18 (62)	4 (14)	48% (27% to 70%)	<.001
Red cell transfusions				
Median no. of units (interquartile range)¶				
Overall	1 (0 to 2)	3 (2 to 6)		.002
During surgery§	0 (0 to 1)	1 (1 to 2)		<.001
After surgery	1 (1 to 2)	2 (1 to 3)		.009
≥1 unit of red cells, n (%)‖				
Overall	19 (66)	29 (100)	−34% (−52% to −17%)	.002
Preoperative	0 (0)	3 (10)	−10% (−21% to 1%)	.236
Intraoperative	10 (34)	24 (83)	−48% (−70% to −26%)	<.001
Postoperative	16 (55)	26 (90)	−35% (−56% to −13%)	.008
Total count of red cell units transfused	57	136		
Protocol suspension red cell transfusions**				
≥1 unit of red cells, n (%)‖	8 (28)	4 (14)	14% (−7% to 34%)	.331
Total count¶	23	5		.311
Nonadherence, n (%)‖				
≥1 nonadherent event, overall	8 (28)	10 (34)	−7% (−31% to 17%)	.777
≥1 nonadherent red cell transfusion ††	6 (21)	4 (14)	7% (−13% to 26%)	.728
≥1 nonadherent failure to transfuse‡‡	3 (10)	10 (35)	−24% (−45% to −4%)	.059

*GEE adjusted for age, baseline Hb, and type of operation. The mean Hb values are unadjusted, as this model does not provide mean ± confidence intervals. GEE was applied on a data frame with the Hb unit in mmol/L.

†Mixed model adjusted for age, baseline Hb, and type of operation. Least squared means are showed along with the mean difference. In the case of time point measurement (eg, day 0 or 30), generalized linear model was adjusted for the same covariates.

‡Hb in patients randomized before end of surgery.

¶van Elteren's test adjusted for type of operation.

§No patient received more than 5 units of red blood cell intraoperatively.

‖χ-square with estimated risk difference.

**Reasons for protocol suspension: uncontrollable hemorrhage during surgery 5 (17%) the low-trigger group vs 4 (14%) in the high-trigger group. Protocol suspension resulting from stroke or extremity ischemia exclusively occurred in 3 (10%) patients in the low-trigger group.

††Red cell transfusion at a Hb level above the allocated trigger or not preceded by a Hb measurement. No nonadherence occurred intraoperatively. Adherence proportion (number of red cell units transfused adherently divided by all red cell transfusions) is provided in the supplemental Table 2.6.

‡‡Omission of red cell transfusion despite an Hb level below the trigger. No red cell transfusions were omitted because of volume overload.

Exploratory outcomes

At day 90 after surgery, 2 patients had died in the low-trigger group vs 1 in the high-trigger group (RR, 2.00; $P = .43$). Major vascular complications occurred in significantly more patients allocated to the low-trigger group (18/29 vs 8/29; RR, 2.40; $P = .007$; Figure 4B). Cox regression analysis of time to death or major vascular complication corroborated this finding (hazard ratio, 3.20; $P = .006$; Figure 4A). There was no subgroup heterogeneity between surgery type and transfusion trigger groups (test of interaction, $P = .97$), and the stratified Kaplan-Meier plot also demonstrated similarly increased hazards with the low-trigger strategy regardless of operation type (supplemental Figure 4). Post hoc Cox regressions excluding minor amputations and/or reoperations supported the findings (supplemental Table 9). Last, the low-trigger group had significantly fewer days alive outside of hospital within the 90 days after the operation (median [IQR], 76 [67-82] days vs 82 [76-84] days; $P = .049$; supplemental Table 6).

Discussion

In a carefully designed trial with thorough clinical follow-up and statistical evaluation, we aimed to assess the effects of different Hb trigger levels for RBC transfusion while respecting

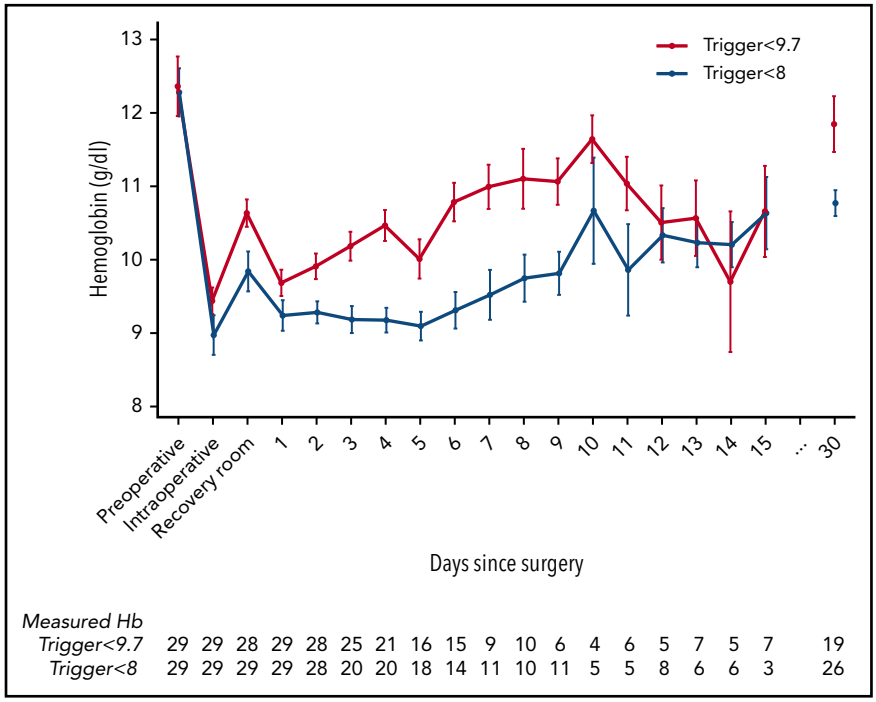

Figure 2. Hb levels in randomized patients at baseline and after vascular surgery. The graph shows the mean daily lowest Hb measurement between the day before surgery (preoperative), during surgery (intraoperative), on arrival in the recovery room (or intensive care unit), and on the first to fifteenth postoperative day followed by the Hb level at follow-up (day 30). Bars indicate ± standard error of the mean. The table below shows the number of patients with available Hb measurements each day. A graph with a separate panel for patients randomized before and after surgery are presented in supplemental Figure 2.

established concepts for intraoperative fluid resuscitation.[30] We observed that for patients undergoing elective AAA or lower limb bypass surgery, a perioperative protocol aiming to restrict RBC transfusion until Hb had reached a level below 8 vs 9.7 g/dL, significantly separated postoperative Hb levels. Importantly, the overall use of RBC units was more than halved. The lowered Hb levels also reflected an increased cerebral desaturation load during surgery compared with the high-trigger strategy. The trial was not powered to detect differences in patient important outcomes, but data suggested a higher rate of death or major vascular complications in patients allocated to the low-trigger group.

Several recent recommendations support RBC transfusion at an Hb of 7 to 8 g/dL in most patients.[31-34] This strategy also seems adopted by the Society of Vascular Surgery,[35] which suggests a threshold of 7 g/dL during or after AAA repair in the absence of rapid ongoing blood loss, while acknowledging the need for trials on RBC, plasma, and platelet transfusion strategies. There are, however, several crucial differences between the available trials that inform RBC transfusion practice and transfusion in vascular surgery. First, most of the evidence is based on postoperatively stable patients with limited surgical hemorrhage, as outlined here. Second, cardiovascular disease (CVD) is particularly prevalent in vascular surgery. In the consecutively screened and unselected TV trial population, 80% to 90% of randomized patients had CVD at baseline compared with 63% in the FOCUS trial, using the same definitions.[15] The FOCUS trial demonstrated that RBC transfusion at Hb below 8 was as safe as 10 g/dL after hip fracture surgery, but inclusion criteria were widened during the trial to also enroll patients with risk factors for CVD, and the inclusion period was extended to 5 years, which raises concern of selection bias. Third, although the recent TRICSIII trial[18,36] provides evidence that a restrictive transfusion strategy is safe, these patients are on cardiopulmonary bypass and have their CVD condition somewhat resolved during surgery, as opposed to the vascular surgery patients, who

Table 3. Near-infrared spectroscopy

	Trigger < 8 g/dL (n = 21)	Trigger < 9.7 g/dL (n = 23)	*P*
Desaturation load*, minutes × %			
Cerebrum, median (IQR)	421 (42 to 888)	127 (11 to 331)	.0036
Biceps muscle, median (IQR)	75 (6 to 234)	112 (16 to 325)	.97
Lowest value before red cell transfusion†, %			
Regional cerebral oxygenation, mean‡	55.9	58.0	.23
Regional biceps muscle oxygenation, mean§	69.6	68.1	.36

All 46 patients (22 vs 24) randomly assigned before the end of surgery were monitored, but because of the loss of NIRS signals, data were missing from 1 patient in each group, yielding the sample of 21 in the low-trigger vs 23 in the high-trigger group.

*The desaturation load was defined as the cumulative area (minutes × %) below baseline and was analyzed by van Elteren's test adjusted for operation type.

†General linear model adjusted for operation type, age, and baseline.

‡Mean difference, −2.11 (95% CI, −5.59 to 1.37).

§Mean difference, 1.50 (95% CI, −1.76 to 4.76).

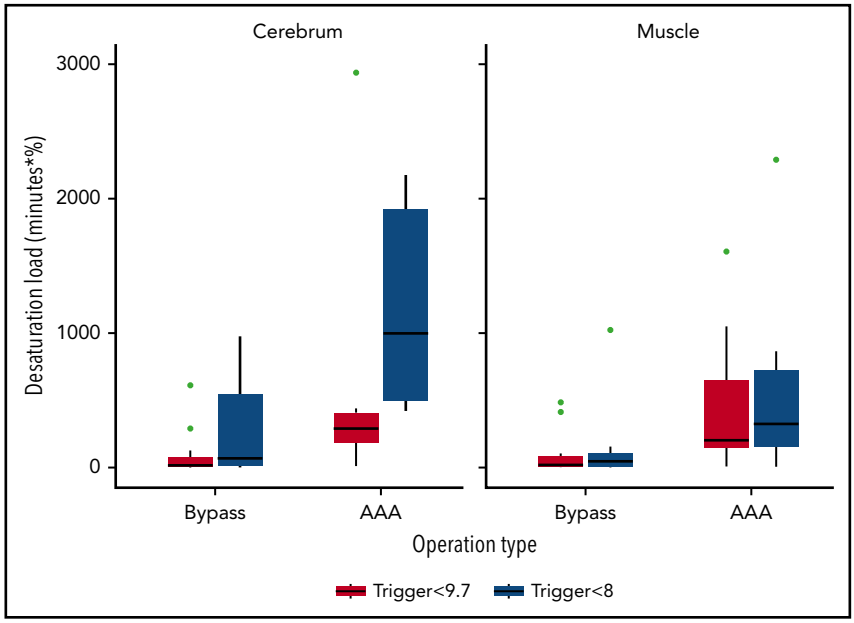

Figure 3. Cerebral and biceps muscle desaturation load stratified by type of surgery. Bypass, lower limb arterial bypass. The desaturation load was defined as the cumulative area (minutes × %) below the baseline tissue oxygenation reading (as determined by near-infrared spectroscopy).

depend on their own ability to increase cardiac output subsequent to both hemorrhage and increased oxygen demand from surgical trauma. In addition, perioperative aggravation of the CVD condition is frequent in vascular surgery, as reported both in the TV trial and in several other cohorts.[37-41]

Recruiting patients for a randomized trial comparing strategies for resuscitating hemorrhage during surgery may be challenged by limited blood loss. In hip fracture, major abdominal, and cardiac surgery, the mean reported blood loss is around 200 to 500 mL.[15,18,42] Preoperative randomization of all comers in such settings may include patients who would never have been considered for RBCs with either strategy as a result of limited Hb drop, which was the case in 22% to 27% of patients in previous cardiac surgery trials of RBC trigger levels.[18,43] When bleeding does occur at higher volumes, patients are often excluded,[44-46] or poor adherence may diminish group separation in terms of Hb levels and units of RBCs transfused.[4,16,20] If randomization is postponed until after surgery, approximately 25% of patients will already have received un-protocolled transfusions.[15,19,44] These details are important because if a clinically significant separation of Hb levels or RBC units transfused is not accomplished, any causal inference on the results becomes spurious. In the TV trial, we used a novel approach in which all enrolled patients received protocolled anesthesia, monitoring, and standardized fluid therapy.[21,30] In addition, blood sampling ensured randomization immediately on an Hb decrease below 9.7 g/dL, whereby 43/112 patients were excluded because of limited blood loss or high preoperative Hb. With this so-called enrichment strategy,[47] all randomized patients encountered a perioperative Hb below 9.7 g/dL. One drawback is a lower randomization rate (40%) than if we had applied preoperative randomization on all 112 consented patients (78%).

Despite the nonadherent events in both groups, and that 12 of 58 were randomly assigned after surgery, data showed significant separation of Hb levels and transfused units of RBCs consistently across all subgroups and sensitivity analyses. We also observed a long-term effect, as significant Hb separation was retained at 30 days follow-up. This was accomplished by using Hb as the main indicator for transfusion, which enables standardized intervention across all phases of hospital admission as a result of the ubiquity of point-of-care devices for Hb assessment. It may be argued that Hb measurement during surgery is imprecise because of the limited time for equilibration of fluid between the vascular beds and the interstitium. We mitigated this phenomenon by exclusively measuring the Hb level during normovolemia and not during uncontrollable bleeding or hypotension. It is our interpretation that the pronounced Hb separation observed in the recovery room demonstrates that this method is feasible and reliable. We acknowledge that other indicators for RBC transfusion may be used[48,49] or are recommended during surgery,[34] but they also need further validation in randomized clinical trials.[31,32] The reduction in RBC units transfused observed with the low-trigger strategy indicates a potentially large cost-saving effect on RBC use, as major surgery, such as AAA repair, is among the most blood product-consuming procedures.[50,51]

The effect of transfusion strategy on tissue oxygenation was evaluated noninvasively by NIRS. Our findings were similar to what has been observed in experimental assessments of brain and muscle oxygen homeostasis during acute hemodilution.[52] With the low-trigger strategy, the cerebral desaturation load increased, whereas the muscle oxygenation was unaffected. This could be explained by the fact that the muscles have a lower oxygen metabolism during anesthesia and surgery, and that muscle oxygenation mostly depends on cardiac output. Cardiac output seems to have been reasonably maintained in both groups, as urinary output (supplemental Table 1) was largely unchanged by different Hb triggers. Apart from cardiac output, $rScO_2$ integrates the influence of CO_2 and blood perfusion pressure on the brain.[53] Cerebral perfusion pressure is vulnerable to carotid stenosis, which is frequent among vascular surgical patients,[54,55] but the prevalence was not assessed in our patients. Also, alfa-adrenergic stimulation by norepinephrine may influence $rScO_2$.[56] The data are taken to reflect that brain was more vulnerable than muscle to the low-trigger strategy, and that oxygen delivery (cardiac output × blood oxygen content)

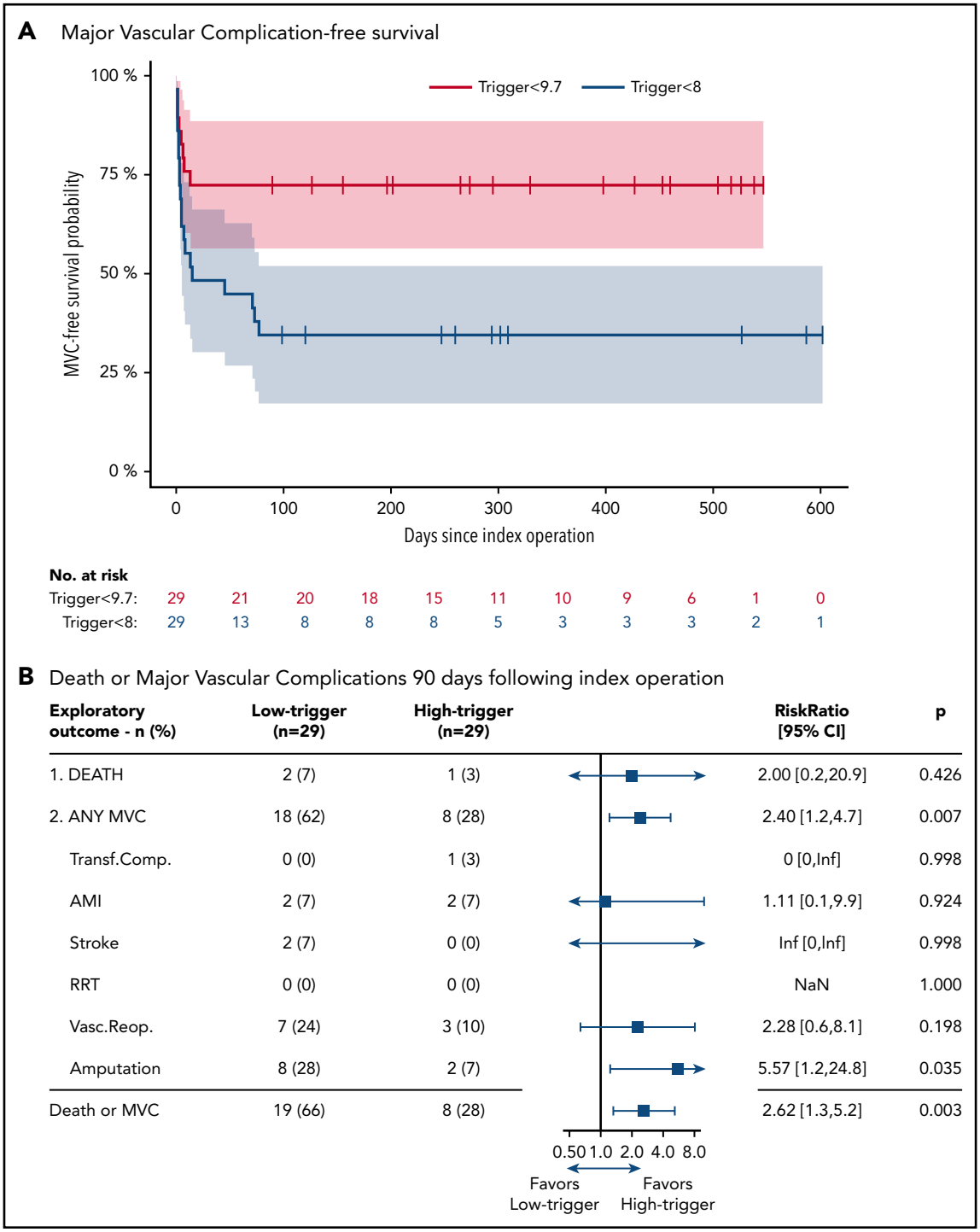

Figure 4. Major vascular complication-free survival and relative risk for death or major vascular complications at 90 days. (*A*) Major vascular complication-free survival probability with 95% CI in the intention-to-treat population. Data were censored 90 days after the last randomized patient. (*B*) shows the relative risks (blue boxes) with 95% CIs (horizontal lines) for the exploratory outcome measures death or major vascular complications at day 90 in the low-trigger group as compared with the high-trigger group. Low-trigger, Hb lower than 8 g/dL; high-trigger, Hb lower than 9.7 g/dL. MVC, major vascular complication. Transf.Compl., severe adverse transfusion reaction (anaphylactic reaction, transfusion-associated circulatory overload, transfusion-related acute lung injury within 6 hours after RBC transfusion or severe acute hemolytic transfusion reaction within 24 hours after RBC transfusion); see trial protocol[21] for further detail. AMI, acute myocardial infarction. RRT, renal replacement therapy. Vasc.Reop., vascular reoperation (specifications provided in supplemental Table 8). Amputation, lower limb amputation from femur to toes. When considering major amputations (femoral or crural) at day 90: 3 (10%) major amputations had occurred in the low-trigger group vs zero (0%) in the high-trigger group; and at right censoring: 7 (24%) vs zero (0%), respectively. *Unadjusted because of lack of model fit with zero events in 1 stratum. All other logistic regressions were adjusted for operation type and age. Odds ratios were converted to RR using the δ-method, where probabilities were derived from the coefficients of the logistic regression using the mean age (73 years) and operation type (0.43); 95% CI was calculated from the standard error.

did not meet the higher metabolic requirements for oxygen in the brain compared with the muscle.

Several reports acknowledge that lowered $rScO_2$ relates to poor clinical outcome,[57,58] but the clinical relevance of our finding is unknown. We did observe an increased risk for death or major vascular complications in patients allocated to the low-trigger strategy, which was notably a result of a higher occurrence of amputations in the patients with lower limb bypass. For patients with AAA allocated to the low-trigger strategy, both the occurrence of death and various major vascular complications was increased (supplemental Table 8). Importantly, the 2 groups were balanced in severity of lower limb artery disease at baseline (Table 1), and the specifics of surgery performed did also not indicate an imbalance (supplemental Table 1). Consistently, patients in the low-trigger group spent almost 1 week less alive outside of the hospital within 90 days. Although the complication rates seem high, it is essential to recall that our trial population was enriched with patients with low preoperative Hb and/or major surgical hemorrhage, and thus reflects a population with relatively high comorbidity burden and more complicated surgery than the general vascular surgery population.

Limitations of this trial are largely lack of blinding as a result of use of open-label design, which is inherent in trials on RBC transfusion. Second, a single-center trial has limited external validity, and it is important that the explorative outcome results are very cautiously interpreted, given the low sample size for detecting or rejecting even large effects, and for now, the explorative outcome results are just hypothesis-generating. Several strengths can be mentioned: the TV trial includes a prepublished protocol and statistical analysis plan, central randomization, assessor blinding, successful patient blinding, and to the best of our knowledge, this is the first transfusion trial using an enrichment strategy, which secures perioperative assessment of transfusion strategies while reducing random noise and heterogeneity through exclusion of patients who are unlikely to be considered for RBC transfusion.

In summary, this feasibility trial successfully separated perioperative Hb levels and units of RBCs transfused, but also demonstrated increased cerebral desaturation with the low-trigger strategy. A large randomized trial, with lowest possible risk for bias, assessing the safety of transfusion strategies in vascular surgery is essential before clinical practice guidelines settle for restrictive RBC transfusion.

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The authors thank the patients who participated in this trial and the staff at the operation theater, vascular surgery, anesthetic, and high-dependency care and intensive care unit and blood bank, making the trial a reality. A special thanks to nurses Helle Witthøft Jørgensen and Izabella Ohm-Hieronymussen for helping with data collection during surgery.

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A. Møller is enrolled as a PhD student at the University of Copenhagen. This work is submitted in partial fulfillment of the requirement for the PhD.

The funders of this study are public organizations and had no role in study design, collection, management, analysis, and interpretation of data, writing of the report, or the decision to submit the report for publication.

Authorship

Contribution: A. Møller is the principal investigator and initiator of the TV Trial and the main author of the protocol, the detailed statistical analysis plan, and the main manuscript, and provided a major contribution to the acquisition, analysis, and interpretation of data; H.B.N. provided a major contribution to the protocol design, interpretation of the data, and drafting of the main manuscript; J.W. provided a major methodological contribution to protocol design, the detailed statistical analysis plan, interpretation of the data, and drafting of the main manuscript; O.B.P. is a coauthor of the protocol and contributed to data acquisition and interpretation of the data; D.H. is a coauthor of the protocol and an investigator with substantial contributions to data acquisition and interpretation of the data, and performed blinded outcome assessment; P.W. is the primary statistician with a major contribution to the analysis and interpretation of data; and K.V.M., B.G.U.R., and A. Mortensen are investigators who made substantial contributions to data acquisition; J.C.J. is a statistician who coauthored the statistical analysis plan, performed analysis of the primary outcome measure by generalized estimated equations, and contributed to the interpretation of data; S.S. is a trial sponsor who contributed to participant recruitment and data acquisition; and all authors provided critical revision of the article and gave approval of the final submitted version.

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Footnotes

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There is a *Blood* Commentary on this article in this issue.

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REFERENCES

1. Bodewes TCF, Pothof AB, Darling JD, et al. Preoperative anemia associated with adverse outcomes after infrainguinal bypass surgery in patients with chronic limb-threatening ischemia. *J Vasc Surg*. 2017;66(6):1775-1785.

2. Kougias P, Sharath S, Barshes NR, Chen M, Mills JL Sr. Effect of postoperative anemia and baseline cardiac risk on serious adverse outcomes after major vascular interventions. *J Vasc Surg*. 2017;66(6):1836-1843.

3. Bisgaard J, Gilsaa T, Rønholm E, Toft P. Optimising stroke volume and oxygen delivery in abdominal aortic surgery: a randomised controlled trial. *Acta Anaesthesiol Scand*. 2013;57(2):178-188.

4. Bush RL, Pevec WC, Holcroft JW. A prospective, randomized trial limiting perioperative red blood cell transfusions in vascular patients. *Am J Surg*. 1997;174(2):143-148.

5. Prinssen M, Verhoeven EL, Buth J, et al; Dutch Randomized Endovascular Aneurysm Management (DREAM)Trial Group. A randomized trial comparing conventional and endovascular repair of abdominal aortic aneurysms. *N Engl J Med*. 2004;351(16):1607-1618.

6. Wu WC, Smith TS, Henderson WG, et al. Operative blood loss, blood transfusion, and 30-day mortality in older patients after major noncardiac surgery. *Ann Surg*. 2010;252(1):11-17.

7. Feringa HH, Karagiannis SE, Vidakovic R, et al. The prevalence and prognosis of unrecognized myocardial infarction and silent myocardial ischemia in patients undergoing major vascular surgery. *Coron Artery Dis*. 2007;18(7):571-576.

8. Ferro CR, de Oliveira DC, Guerra FF, et al. Prevalence and risk factors for combined coronary artery disease and aortic aneurysm. *Arq Bras Cardiol*. 2007;88(1):40-44.

9. Hertzer NR, Beven EG, Young JR, et al. Coronary artery disease in peripheral vascular patients. A classification of 1000 coronary angiograms and results of surgical management. *Ann Surg*. 1984;199(2):223-233.

10. Carson JL, Duff A, Poses RM, et al. Effect of anaemia and cardiovascular disease on surgical mortality and morbidity. *Lancet*. 1996;348(9034):1055-1060.

11. Chatterjee S, Wetterslev J, Sharma A, Lichstein E, Mukherjee D. Association of blood transfusion with increased mortality in myocardial infarction: a meta-analysis and diversity-adjusted study sequential analysis. *JAMA Intern Med*. 2013;173(2):132-139.

12. Goel R, Patel EU, Cushing MM, et al. Association of perioperative red blood cell transfusions with venous thromboembolism in a North American registry. *JAMA Surg*. 2018;153(9):826-833.

13. Obi AT, Park YJ, Bove P, et al. The association of perioperative transfusion with 30-day morbidity and mortality in patients undergoing major vascular surgery. *J Vasc Surg*. 2015;61(4):1000-1009.

14. Curley GF, Shehata N, Mazer CD, Hare GM, Friedrich JO. Transfusion triggers for guiding RBC transfusion for cardiovascular surgery: a systematic review and meta-analysis*. *Crit Care Med*. 2014;42(12):2611-2624.

15. Carson JL, Terrin ML, Noveck H, et al; FOCUS Investigators. Liberal or restrictive transfusion in high-risk patients after hip surgery. *N Engl J Med*. 2011;365(26):2453-2462.

16. Palmieri TL, Holmes JH IV, Arnoldo B, et al. Transfusion Requirement in Burn Care Evaluation (TRIBE): a multicenter randomized prospective trial of blood transfusion in major burn injury. *Ann Surg*. 2017;266(4):595-602.

17. Holst LB, Petersen MW, Haase N, Perner A, Wetterslev J. Restrictive versus liberal transfusion strategy for red blood cell transfusion: systematic review of randomised trials with meta-analysis and trial sequential analysis. *BMJ*. 2015;350(mar24 9):h1354.

18. Mazer CD, Whitlock RP, Fergusson DA, et al; TRICS Investigators and Perioperative Anesthesia Clinical Trials Group. Restrictive or liberal red-cell transfusion for cardiac surgery. *N Engl J Med*. 2017;377(22):2133-2144.

19. Murphy GJ, Pike K, Rogers CA, et al; TITRe2 Investigators. Liberal or restrictive transfusion after cardiac surgery. *N Engl J Med*. 2015;372(11):997-1008.

20. Nielsen K, Dahl B, Johansson PI, Henneberg SW, Rasmussen LS. Intraoperative transfusion threshold and tissue oxygenation: a randomised trial. *Transfus Med*. 2012;22(6):418-425.

21. Møller A, Nielsen HB, Wetterslev J, Pedersen OB, Hellemann D, Shahidi S. Low vs. high haemoglobin trigger for transfusion in vascular surgery: protocol for a randomised trial. *Acta Anaesthesiol Scand*. 2017;61(8):952-961.

22. Madsen PL, Secher NH. Near-infrared oximetry of the brain. *Prog Neurobiol*. 1999;58(6):541-560.

23. Kuper M, Gold SJ, Callow C, et al. Intraoperative fluid management guided by oesophageal Doppler monitoring. *BMJ*. 2011;342(may24 3):d3016.

24. Wikkelsø A, Wetterslev J, Møller AM, Afshari A. Thromboelastography (TEG) or rotational thromboelastometry (ROTEM) to monitor haemostatic treatment in bleeding patients: a systematic review with meta-analysis and trial sequential analysis. *Anaesthesia*. 2017;72(4):519-531.

25. Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sørensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. *Clin Epidemiol*. 2015;7:449-490.

26. Thygesen K, Alpert JS, Jaffe AS, et al; Joint ESC/ACCF/AHA/WHF Task Force for the Universal Definition of Myocardial Infarction. Third universal definition of myocardial infarction. *Circulation*. 2012;126(16):2020-2035.

27. Sørensen H, Nielsen HB, Secher NH. Near-infrared spectroscopy assessed cerebral oxygenation during open abdominal aortic aneurysm repair: relation to end-tidal CO2 tension. *J Clin Monit Comput*. 2016;30(4):409-415.

28. Kahan BC, Morris TP. Improper analysis of trials randomised using stratified blocks or minimisation. *Stat Med*. 2012;31(4):328-340.

29. Lin DYW, Wei LJ, Ying Z. Checking the Cox model with cumulative sums of martingale-based residuals. *Biometrics*. 1993;80(3):557-572.

30. Makaryus R, Miller TE, Gan TJ. Current concepts of fluid management in enhanced recovery pathways. *Br J Anaesth*. 2018;120(2):376-383.

31. Carson JL, Stanworth SJ, Roubinian N, et al. Transfusion thresholds and other strategies for guiding allogeneic red blood cell transfusion. *Cochrane Database Syst Rev*. 2016;10:CD002042.

32. Carson JL, Triulzi DJ, Ness PM. Indications for and adverse effects of red-cell transfusion. *N Engl J Med*. 2017;377(13):1261-1272.

33. Carson JL, Guyatt G, Heddle NM, et al. Clinical practice guidelines from the AABB: red blood cell transfusion thresholds and storage. *JAMA*. 2016;316(19):2025-2035.

34. American Society of Anesthesiologists Task Force on Perioperative Blood Management. Practice guidelines for perioperative blood management: an updated report by the American Society of Anesthesiologists Task Force on Perioperative Blood Management*. *Anesthesiology*. 2015;122(2):241-275.

35. Chaikof EL, Dalman RL, Eskandari MK, et al. The Society for Vascular Surgery practice guidelines on the care of patients with an abdominal aortic aneurysm. *J Vasc Surg*. 2018;67(1):2-77.

36. Mazer CD, Whitlock RP, Fergusson DA, et al; TRICS Investigators and Perioperative Anesthesia Clinical Trials Group. Six-month outcomes after restrictive or liberal transfusion for cardiac surgery. *N Engl J Med*. 2018;379(13):1224-1233.

37. Bursi F, Babuin L, Barbieri A, et al. Vascular surgery patients: perioperative and long-term risk according to the ACC/AHA guidelines, the additive role of post-operative troponin elevation. *Eur Heart J*. 2005;26(22):2448-2456.

38. Landesberg G, Shatz V, Akopnik I, et al. Association of cardiac troponin, CK-MB, and postoperative myocardial ischemia with long-term survival after major vascular surgery. *J Am Coll Cardiol*. 2003;42(9):1547-1554.

39. Marston N, Brenes J, Garcia S, et al. Peak postoperative troponin levels outperform preoperative cardiac risk indices as predictors of long-term mortality after vascular surgery Troponins and postoperative outcomes. *J Crit Care*. 2012;27(1):66-72.

40. Le Manach Y, Perel A, Coriat P, Godet G, Bertrand M, Riou B. Early and delayed myocardial infarction after abdominal aortic surgery. *Anesthesiology*. 2005;102(5):885-891.

41. McFalls EO, Ward HB, Moritz TE, et al. Predictors and outcomes of a perioperative myocardial infarction following elective vascular surgery in patients with documented coronary artery disease: results of the CARP trial. *Eur Heart J*. 2008;29(3):394-401.

42. Myles PS, Bellomo R, Corcoran T, et al; Australian and New Zealand College of Anaesthetists Clinical Trials Network and the Australian and New Zealand Intensive Care Society Clinical Trials Group. Restrictive versus liberal fluid therapy for major abdominal surgery. *N Engl J Med*. 2018;378(24):2263-2274.

43. Hajjar LA, Vincent JL, Galas FR, et al. Transfusion requirements after cardiac surgery: the TRACS randomized controlled trial. *JAMA*. 2010;304(14):1559-1567.

44. de Almeida JP, Vincent JL, Galas FR, et al. Transfusion requirements in surgical oncology patients: a prospective, randomized controlled trial. *Anesthesiology*. 2015;122(1):29-38.

45. Hébert PC, Wells G, Blajchman MA, et al. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. Transfusion Requirements in Critical Care Investigators, Canadian Critical Care Trials Group. *N Engl J Med*. 1999;340(6):409-417.

46. Villanueva C, Colomo A, Bosch A, et al. Transfusion strategies for acute upper gastrointestinal bleeding. *N Engl J Med*. 2013;368(1):11-21.

47. Temple R. Enrichment of clinical study populations. *Clin Pharmacol Ther*. 2010;88(6):774-778.

48. Hahn RG. The transfusion trigger in major surgery. *Acta Anaesthesiol Scand*. 2018;62(2):270.

49. Møller A. Low vs high haemoglobin trigger for transfusion in vascular surgery: protocol for a randomised trial. *Acta Anaesthesiol Scand*. 2018;62(2):271.

50. Dansk Transfusionsdatabase. Årsrapport 2009 (Data fra 2007 og 2008) [in Danish]. http://www.dtdb.dk/download/DTDB_Rapport_2009_43.pdf. Accessed 9 October 2018.

51. Halmin M, Chiesa F, Vasan SK, et al. Epidemiology of massive transfusion: a binational study from Sweden and Denmark. *Crit Care Med*. 2016;44(3):468-477.

52. Kei T, Mistry N, Tsui AKY, et al. Experimental assessment of oxygen homeostasis during acute hemodilution: the integrated role of hemoglobin concentration and blood pressure. *Intensive Care Med Exp*. 2017;5(1):12.

53. Murkin JM. Cerebral oximetry: monitoring the brain as the index organ. *Anesthesiology*. 2011;114(1):12-13.

54. Cahan MA, Killewich LA, Kolodner L, et al. The prevalence of carotid artery stenosis in patients undergoing aortic reconstruction. *Am J Surg*. 1999;178(3):194-196.

55. Marek J, Mills JL, Harvich J, Cui H, Fujitani RM. Utility of routine carotid duplex screening in patients who have claudication. *J Vasc Surg*. 1996;24(4):572-577.

56. Holmgaard F, Vedel AG, Lange T, Nilsson JC, Ravn HB. Impact of 2 distinct levels of mean arterial pressure on near-infrared spectroscopy during cardiac surgery: secondary outcome from a randomized clinical trial [published online ahead of print 9 May 2018]. *Anesth Analg*. doi:10.1213/ANE.0000000000003418.

57. Nielsen HB. Systematic review of near-infrared spectroscopy determined cerebral oxygenation during non-cardiac surgery. *Front Physiol*. 2014;5:93.

58. Zorrilla-Vaca A, Healy R, Grant MC, et al. Intraoperative cerebral oximetry-based management for optimizing perioperative outcomes: a meta-analysis of randomized controlled trials. *Can J Anaesth*. 2018;65(5):529-542.

Supplementary Digital Content
Trial results

This supplementary material has been provided by the authors to give readers additional information about the trial results.

Supplement to: Low vs. high hemoglobin trigger for transfusion in vascular surgery: a randomized clinical feasibility trial.

Supplemental material, Study II, Paper III.

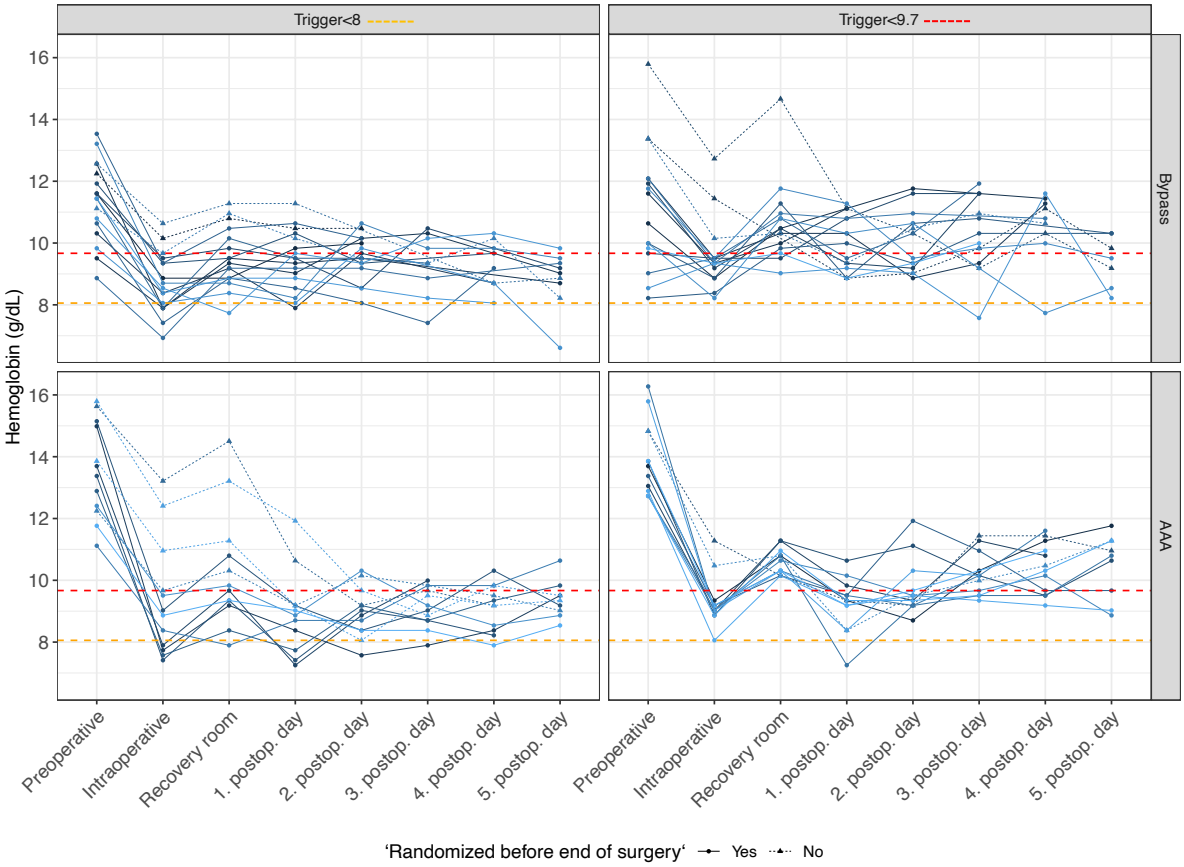
Table of contents

Supplemental material, Study II, Paper III.

Supplemental material, Study II, Paper III.

Supplementary figures

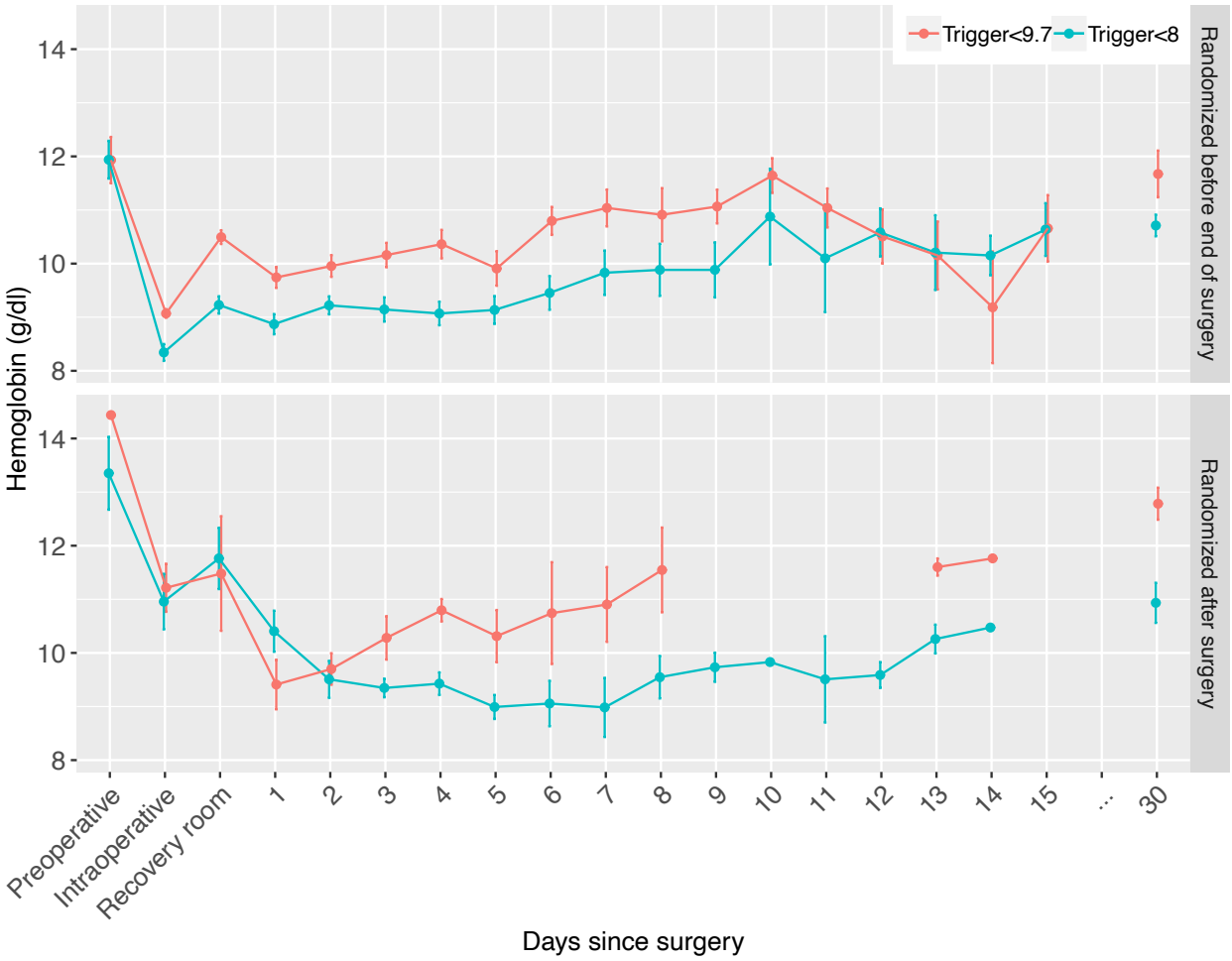
Figure S1. Spaghettiogram of lowest daily hemoglobin level



Legend: Figure S1 shows the development of each patient's lowest hemoglobin (Hb) level between the pre-operative measurement and the 5th postoperative day. Intraoperative, lowest Hb obtained during surgery or between end of surgery and arrival at the recovery room.

The Hb level corresponding to the low trigger threshold is marked with an orange dashed line and the high-trigger threshold is marked with a red dashed line.
Patient randomized after end of surgery are presented with a dashed line and triangular points.

Figure S2. Perioperative hemoglobin levels in patients randomized before (n=46) and after end of surgery (n=12)



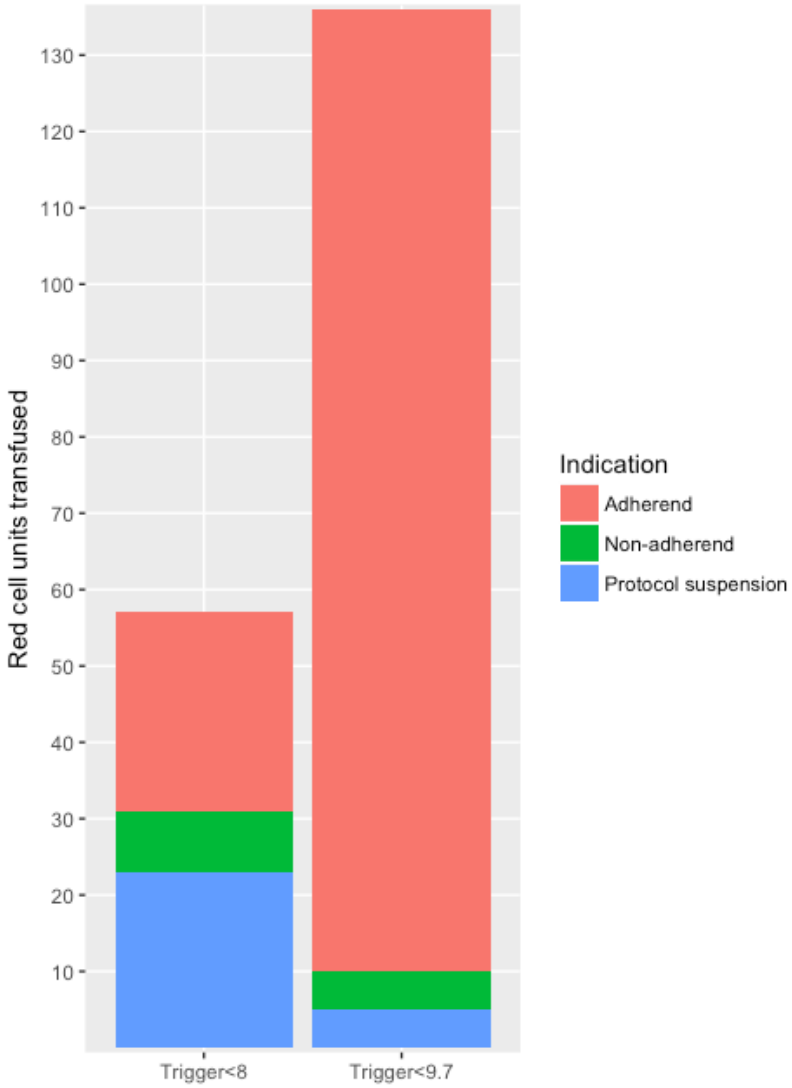
Number of hemoglobin measurements per time point:

Randomized before end of surgery																			
Trigger<9.7	24	24	24	24	23	20	16	12	12	6	7	6	4	6	5	5	4	7	16
Trigger<8	22	22	22	22	22	16	14	13	9	7	6	6	4	3	6	3	5	3	19
Randomized after surgery																			
Trigger<9.7	5	5	4	5	5	5	5	4	3	3	3	0	0	0	0	2	1	0	3
Trigger<8	7	7	7	7	6	4	6	5	5	4	4	5	1	2	2	3	1	0	7

Legend: The graph shows the same as figure 2 in the main paper, but with a separate panel for patients randomized before- (top panel) and after end of surgery (lower panel), with the mean daily lowest hemoglobin measurement between the day before surgery (preoperative), during surgery (intraoperative), upon arrival in the post-anesthesia care unit (recovery room) and on the 1st to 15th postoperative day followed by the hemoglobin level at follow-up (day 30). Bars indicate +/- standard error of the mean. The table below shows the number of patients with available hemoglobin measurements at each time point.

Supplemental material, Study II, Paper III.

Figure S3. Indication for red cell units transfused



Adherent: Red cells transfused according to the assigned Hb trigger.

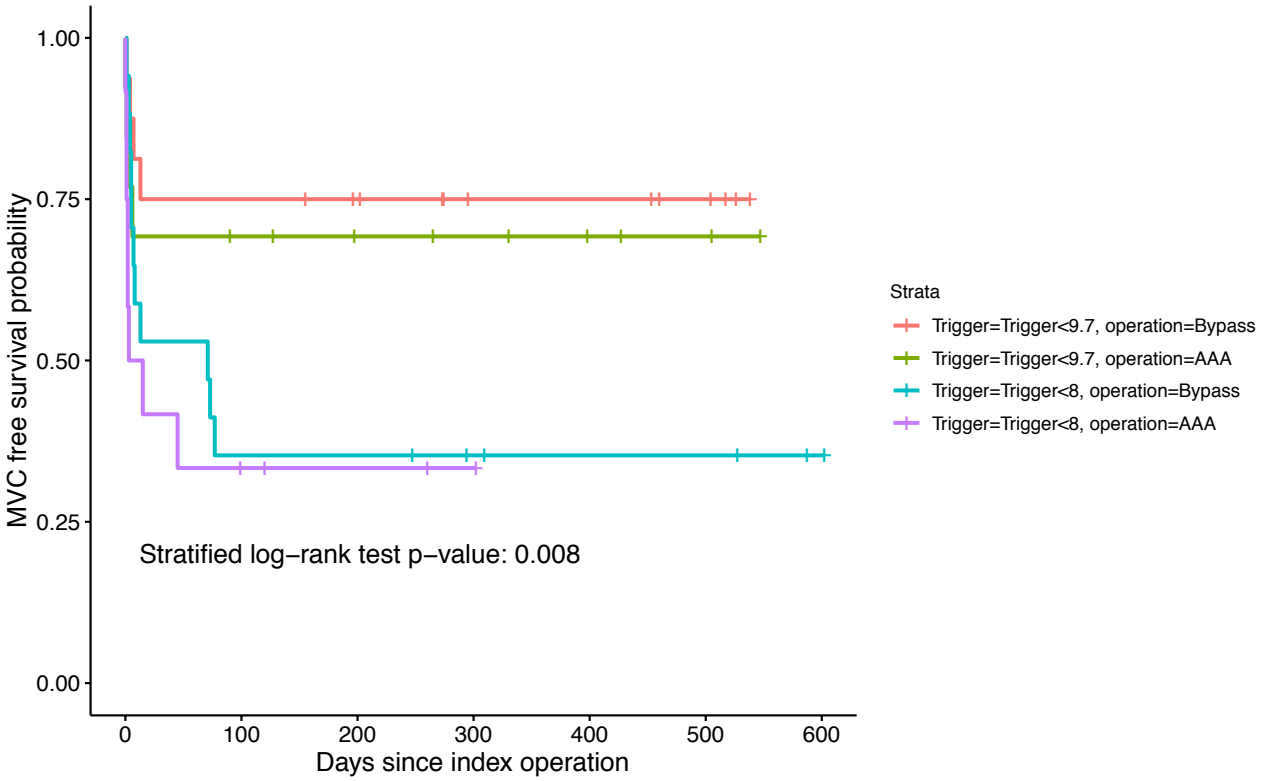
Non-adherent: Red cells were NOT transfused according to the assigned Hb trigger or the indication for red cells did not adhere to the definitions for protocol-suspension.

Protocol suspension: Red cells transfused due to
- uncontrollable hemorrhage (5 patients in low-trigger *vs.* 4 patients units in high-trigger). 3 cases occurred during a reoperation.
- hypotension (mean arterial pressure < 65 mmHg) unresponsive to fluid administration (none registered)
- stroke (1 patient in low-trigger *vs.* none in high-trigger)
- limb ischemia (2 patients in low-trigger *vs.* none in high-trigger)
- bowel ischemia (none registered)

Supplemental material, Study II, Paper III.

Figure S4. Stratified Kaplan-Meier plot

MVC, major vascular complication. Bypass, lower limb bypass. AAA, Abdominal aortic aneurysm. The stratified log-rank test compared the cumulated hazards in the low-trigger groups to the high-trigger group while adjusting for type of operation.



Supplemental material, Study II, Paper III.

Table S1. Intraoperative fluid balance and surgery specifics

	Trigger<8 (n=29)	Trigger<9.7 (n=29)	Total (n=58)	P
FLUID INPUT (ml)				
Crystalloids Mean (sd) Median [iqr]	2675 (1050) 2500 [1800, 3500]	2954 (902) 2850 [2500, 3600]	2815 (980) 2600 [2000, 3575]	0.278
Medicine Mean (sd) Median [iqr]	48 (150) 0 [0, 0]	28.4 (68) 0 [0, 0]	38 (116) 0 [0, 0]	0.972
Human albumin 5 % Mean (sd) Median [iqr]	448 (580) 250 [0, 500]	457 (478) 250 [0, 1000]	453 (527) 250 [0, 750]	0.758
Red blood cells Mean (sd) Median [iqr] ≥1 units (%)	176 (306) 0 [0, 300] 10 (34)	486 (412) 300 [300, 600] 24 (83)	331 (392) 300 [0, 600] 34 (59)	<0.001
Fresh frozen plasma ≥1 units (%)	1 (3)	0 (0)	1 (2)	NA
Platelets ≥1 units (%)	1 (3)	0 (0)	1 (2)	NA
FLUID LOSS (ml)				
Blood loss Mean (sd) Median [iqr]	1129 (1191) 775 [280, 1250]	1407 (1050) 1200 [560, 2100]	1268 (1121) 990 [403, 1894]	0.202
Urine output Mean (sd) Median [iqr]	616 (425) 470 [300, 775]	710 (534) 550 [375, 905]	663 (481) 485 [300, 883]	0.549
FLUID BALANCE (ml)				
Fluid Balance Mean (sd) Median [iqr]	1653 (1042) 1225 [1075, 2130]	1809 (831) 1875 [1525, 2300]	1731 (937) 1660 [1136, 2300]	0.529
RELATIVE BLOOD LOSS				

TBV (ml) Mean (sd) Median [iqr]	4769 (748) 4692 [4380, 5069]	4602 (746) 4596 [4068, 5110]	4685 (746) 4640 [4147, 5102]	0.394
Blood loss/TBV (%) Mean (sd) Median [iqr]	23 (21) 18 [6, 30]	32 (24) 24 [13, 46]	27 (23) 22 [8, 40]	0.140
DURATION (min)				
Anesthesia duration Mean (sd) Median [iqr]	251.0 (85.8) 239 [186, 258]	244.9 (56.4) 251 [206, 276]	247.9 (72.0) 241.5 [196, 273]	0.750
Surgery duration Mean (sd) Median [iqr]	189 (74) 168 [127, 218]	185 (59) 177 [147, 217]	187 (67) 176 [136, 218]	0.796
AAA Cross Clamp Mean (sd) Median [iqr]	91 (50) 82 [48, 108]	82 (24) 75 [64, 103]	86 (38) 77 [63, 103]	0.552
SURGERY SPECIFICS (%)				
AAA surgery Tube prosthesis	5 (17)	9 (31)	14 (21)	
Bifurcated prosthesis	7 (24)	4 (14)	11 (19)	
Bypass surgery Infra-inguinal	9 (31)	14 (48)	23 (40)	
Femuro-femoral cross-over	5 (17)	1 (3)	6 (10)	
Femoral thromb-endarterectomy	2 (7)	1 (3)	3 (5)	
Inoperabel	1 (3)	0 (0)	1 (2)	0.217

NA, not available. TBV, Total blood volume was calculated with Naddler's equation based on weight, height and sex. Normal data are compared with student's t. test, and non-normal data with Wilcoxon test.
AAA, abdominal aortic aneurysm. All AAAs were asymptomatic except for two patients in the Trigger<9.7 g/dl, who group were operated on the claudication indication: one with a thrombosed AAA and one due to aorto-occlusive disease.
See table S5.1 and S5.2 for fluid balance and surgery specifics stratified for type of operation.

Tables S2. Sensitivity analyses of the hemoglobin levels and red-cell units transfused

Table S2.0. Mean baseline hemoglobin values

	Trigger < 8 g/dl	Trigger < 9.7 g/dl	Mean difference (95% CI)	P
Intention to treat population	n=29	n=29		
All patients	12.28	12.36	-0.08 (-1.11 to 0.94)	0.87
	n=17	n=16		
Lower Limb bypass	11.36	11.12	0.25 (-0.90 to 1.39)	0.68
	n=12	n=13		
Abdominal aortic aneurysm	13.58	13.89	-0.32 (-1.40 to 0.76)	0.57
Per-protocol population*	n=18	n=24		
	11.66	11.93	-0.28 (-1.44 to 0.89)	0.65
Patients randomized ≤ 2nd post-operative day	n=26	n=28		
	12.13	12.24	-0.11 (-1.14 to 0.93)	0.84

*Excluded are patients who have less than 50% RBC transfusions adherent to allocated trigger (or were not transfused adherent to the trigger) OR did not fulfill inclusion criteria until after operation.

Table S2.1. Longitudinal analysis of hemoglobin day 0 – 15 by generalized estimated equations

	Trigger < 8 g/dl	Trigger < 9.7 g/dl	GEE coefficient (95% CI)	P
Intention to treat population	n=29	n=29	GEE coefficient:	
Grand mean	9.46	10.33	-0.45 (-0.84 to -0.07)	0.022*
Per-protocol population	n=18	n=24	GEE coefficient:	
Grand mean	9.27	10.30	-0.78 (-1.31 to -0.26)	0.004*
Patients randomized ≤ 2nd post-operative day	n=26	n=28	GEE coefficient:	0.016*
Grand mean	9.41	10.33	-0.46 (-0.84 to -0.09)	

GEE, generalized estimated equations. GEE was applied on a data frame with the Hb unit in mmol/l.

Table S2.2. Mean hemoglobin level at day 0

	Trigger < 8 g/dl	Trigger < 9.7 g/dl	Treatment effect (95% CI)	P
Intention to treat population	n=29	n=29		
	9.76	10.64	-0.89 (-1.45 to -0.32)	0.003
Per-protocol population	n=18	n=24		
	9.13	10.49	- 1.37 (-1.78 to -0.95)	<0.001
Patients randomized ≤ 2nd post-operative day	n=26	n=28		
	9.59	10.50	-0.92 (-1.45 to -0.32)	0.012

General linear model adjusted for the baseline value, operation type and age.

Supplemental material, Study II, Paper III.

Table S2.3. Mean hemoglobin level at day 30

	Trigger < 8 g/dl	Trigger < 9.7 g/dl	Treatment effect (95% CI)	P
Intention to treat population	n=26 10.75	n=19 11.83	-1.07 (-1.79 to -0.36)	0.004
Per-protocol population	n=15 10.89	n=16 11.51	- 0.62 (-1.57 to 0.32)	0.19
Patients randomized ≤ 2nd post-operative day	n=23 10.73	n=19 11.79	-0.92 (-1.81 to -0.30)	0.007

General linear model adjusted for the baseline value, operation type and age.

Post-hoc longitudinal analysis confined to cover Hb measured day 0-8 by mixed model

Mean difference in Hb (n=58): -0.94 g/dl (95% CI -1.25 to -0.64, p<0.001).

Table S2.4. Sensitivity analysis of number of red-cell transfusions

	Trigger < 8 g/dl	Trigger < 9.7 g/dl	P
Intention to treat population median [iqr]	n=29 1.0 [0.0 to 2.0]	n=29 3.0 [2.0 to 6.0]	0.002
Per-protocol population median [iqr]	n=18 1.0 [0.0 to 2.8]	n=24 3.5 [3.0 to 6.0]	0.001
Patients randomized ≤ 2nd post-operative day median [iqr]	n=26 1.5 [0.0 to 2.8]	n=28 3.0 [2.0 to 6.0]	0.004

Supplemental material, Study II, Paper III.

Table S2.5. Distribution of number of red-cell transfusions

	Trigger < 8 g/dl n=29	Trigger < 9.7 g/dl n=29	P
Overall transfusions – no. (%)			
0 units	10 (35)	0 (0.0)	0.002
1 unit	6 (21)	5 (17)	
2 units	6 (21)	4 (14)	
3 units	3 (10)	8 (28)	
4 units	1 (4)	3 (10)	
6-10 units	3 (10)	6 (21)	
11-20 units	0 (0)	9 (31)	
Intraoperative transfusions – no (%)			
0 units	19 (66)	5 (17)	<0.001
1 unit	6 (21)	12 (41)	
2 units	2 (7)	6 (20.7)	
3 units	1 (3)	3 (10.3)	
4 units	1 (3)	1 (3.4)	
5 units	0 (0)	2 (6.9)	
>5 units	0 (0)	0 (0.0)	
Postoperative transfusions – no (%)			
0 units	13 (45)	3 (10)	0.008
1 unit	7 (24)	8 (28)	
2 units	5 (17)	7 (24)	
3 units	1 (3)	6 (21)	
4 units	0 (0)	2 (7)	
5 units	0 (0)	1 (3)	
>5 units	3 (10)	2 (7)	

Chi-square test.

Table S2.6. Adherence

	Trigger<8 g/dl n=29	Trigger<9.7 g/dl n=29	Risk difference (95% CI)	P
Transfusion adherence per patient (%) *				
Median [interquartile range] †	100 [50 to 100]	100 [100 to 100]		0.061
Mean (95 % confidence interval)	71 (56 to 87)	96 (92 to 99)		
Mean (97.5 % confidence interval)	71 (53 to 89)	96 (92 to 100)		
Protocol suspension red-cell transfusions‡				
≥ 1 unit of red cells – no. (%)	8 (28)	4 (14)	14 % (-7 to 34)	
Total count†	23	5		0.311
Non-adherent red-cell transfusions§				
≥ 1 unit of red cells – no. (%)	6 (21)	4 (14)	7 % (-13 to 26)	
Total count†	8	5		0.683
Non-adherent failure to transfuse‖				
≥ 1 red cell transfusion omitted – no. (%)	3 (10)	10 (35)	- 24 % (-45 to -4)	
Total count of Hb levels below the trigger†	3	15		0.090
Overall non-adherence				
≥ 1 non-adherent event – no. (%)	8 (28)	10 (34)	- 7 % (-31 to 17)	
Total count†	11	20		0.495

* Number of adherent red-cell transfusions divided by the number of all red-cell transfusion. Among the ten un-transfused in the low-trigger group, nine patients contribute with 100 % adherence as no deviation from protocol occurred and one patient contribute with 24/25 (96%) adherence to hemoglobin levels.

Comments: The median protocol adherence was 100 % in both groups. Due to skewed distribution, the mean adherence was 71 % in the low-trigger group compared to 96 % in the high-trigger group. This related to protocol suspensions and non-adherence accounting for 31/57 (54%) of all RBC units transfused among the 19 patients (66 %) receiving RBCs in the low-trigger group.

† van Elteren's test adjusted for type of operation.

‡ Reasons for protocol suspension: uncontrollable hemorrhage during surgery 5 (17 %) the low-trigger group vs. 4 (14 %) in the high-trigger group. Protocol suspension due to stroke or extremity ischemia exclusively occurred in 3 (10 %) patients in the low-trigger group.

§ Red-cell transfusion at a hemoglobin level above the allocated trigger or not preceded by a hemoglobin measurement. No non-adherence occurred intra-operatively.

‖ Omission of red-cell transfusion despite a hemoglobin level below the trigger. No red-cell transfusions were omitted due to volume overload.

Near infrared spectroscopy (NIRS)

Table S3.0. NIRS at baseline and before induction of anesthesia and O_2-supplementation

Variable	Level	Trigger<8 g/dl (n=22)	Trigger<9.7 g/dl (n=24)	Total (n=46)	P
Baseline $rScO_2$ (normovolemia)	mean (sd) median [iqr]	60.8 (9.6) 64.0 [56.6, 65.3]	59.5 (9.1) 59.1 [53.7, 65.4]	60.1 (9.3) 60.3 [54.1, 65.4]	0.637
Baseline $rSmO_2$ (normovolemia)	mean (sd) median [iqr]	69.8 (8.9) 71.2 [67.2, 74.8]	68.2 (9.8) 69.5 [63.1, 74.6]	69.0 (9.3) 70.0 [63.8, 74.8]	0.560
Awake rScO2 (no O2 suppl.)	mean (sd) median [iqr] missing	57.4 (10.8) 59.0 [47.9, 61.4] 0	58.9 (8.9) 58.7 [51.3, 63.7] 1	58.2 (9.8) 58.7 [50.3, 62.1] 1	0.596
Awake rSmO2 (no O2 suppl.)	mean (sd) median [iqr]	67.2 (11.9) 71.7 [60.1, 74.8]	66.2 (11.5) 66.8 [60.3, 73.8]	66.6 (11.6) 70.5 [60.0, 74.8]	0.775

rScO2, regional cerebral oxygenation. $rSmO_2$, regional muscle oxygenation.
Baseline was defined as time point where the patient stroke volume was unresponsive to further fluid administration (normovolemia) after inducing anesthesia and before start of surgery.
The awake reading is provided for comparison, as this is a more commonly used baseline in other trials and studies.

Table S3.1. Primary analysis covering both crude and square root transformed data

	Trigger < 8 g/dl	Trigger < 9.7 g/dl	Treatment effect (95% CI)	P
Area under baseline				
Forehead oxygenation ($rScO_2$)	(n=21)	(n=23)		
Crude data, min% [IQR]	421 [42 - 888]	127 [11 - 331]		0.0036*
Transformed data, sqrt(min%)	20.3	13.6	6.77 (0.32 to 13.2)	0.040†
Biceps muscle oxygenation ($rSmO_2$)	(n=21)	(n=23)		
Crude data, min% [IQR]	75 [6 - 234]	112 [16 - 325]		0.97*
Transformed data, sqrt(min%)	13.8	12.8	0.98 (-4.83 to 6.78)	0.74†
Lowest value before red cell transfusion				
Forehead oxygenation ($rScO_2$)	(n=21)	(n=23)		
Mean (%)	55.9	58.0	-2.11 (-5.59 to 1.37)	0.23†
Brachial biceps oxygenation ($rSmO_2$)	(n=22)	(n=23)		
Mean (%)	69.6	68.1	1.07 (0.36 to 1.79)	0.36†

Sqrt, square root.
*van Elteren's test adjusted for operation type. Probabilistic index model adjusted for operation type and baseline p= 0.009758 (age could not be included in this model).

†General linear model adjusted for operation type, age and baseline. There was no statistically significant sub-group heterogeneity between surgery type and transfusion trigger groups (p=0.111).

Table S3.2. Sensitivity analysis, after exclusion of patients who did not receive red cell transfusions during the operation

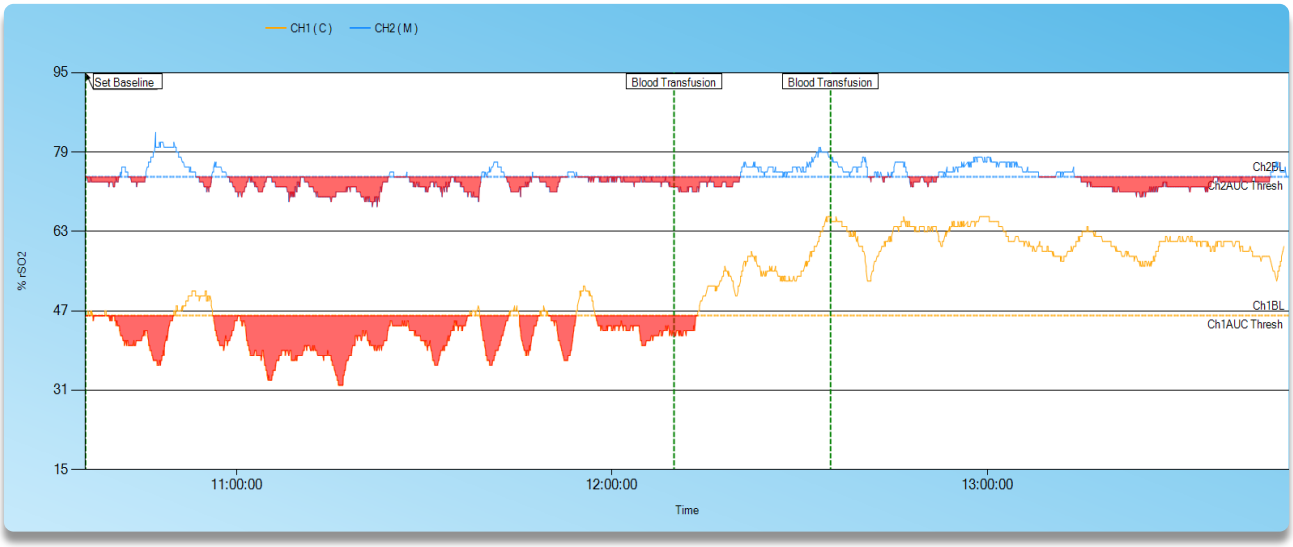
	Trigger < 8 g/dl (n=10)	Trigger < 9.7 g/dl (n=23)	Treatment effect (95% CI)	P
AREA UNDER BASELINE				
Forehead oxygenation				
Crude data, min% [IQR]	284 [24 - 1551]	127 [11 - 232]		0.068*
Transformed data, sqrt(min%)	20.5	12.7	7.73 (-0.280 to 15.7)	0.058†
Biceps muscle oxygenation				
Crude data, min% [IQR]	113 [6 - 234]	112 [16 - 325]		0.78*
Transformed data, sqrt(min%)	12.7	13.3	-0.546 (-8.30 to 7.21)	0.89†
LOWEST VALUE BEFORE RED CELL TRANSFUSION				
Forehead oxygenation ($rScO_2$)				
Mean (%)	55.7	57.4	-1.70 (-6.16 to 2.76)	0.44†
Brachial biceps oxygenation ($rSmO_2$)				
Mean (%)	70.8	68.2	1.07 (0.36 to 1.79)	0.24†

Sqrt=square root
*VanElteren's test adjusted for operation type.
†General linear model adjusted for operation type, age and baseline.

Supplemental material, Study II, Paper III.

Figure describing the area under baseline calculation (desaturation load)

Values were calculated and extracted using the INVOS Analytics Tool Version 1.2 and yielded the area-under-baseline calculation, referred to as desaturation load, which represents the integral between baseline and the rScO2- or SmO2-curve (Time span: from baseline to end of surgery). Area is colored red.



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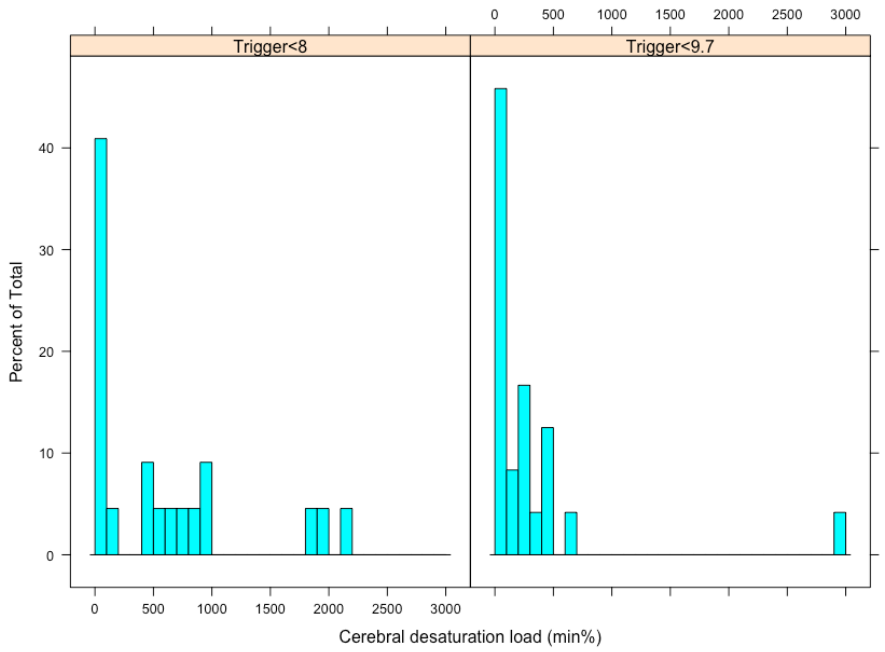
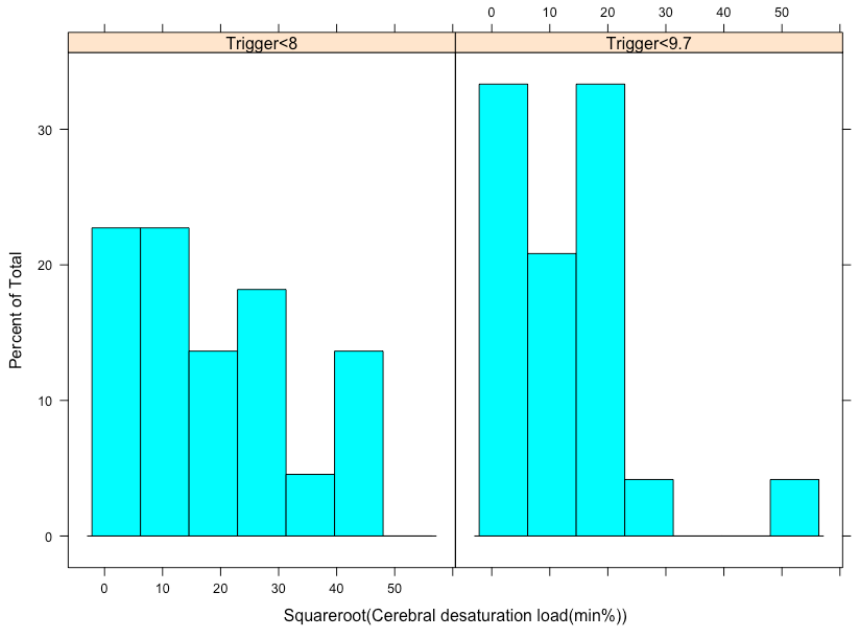
Histograms of the desaturation load data before and after square root transformation

Histogram of the crude cerebral desaturation load (unit: minutes*%)

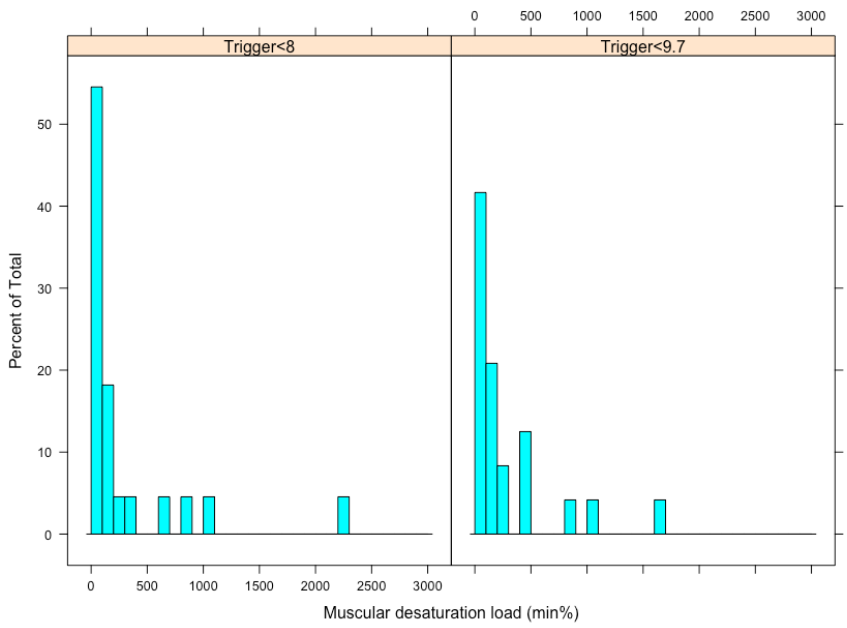


Histogram after square root transformation of the cerebral desaturation load (unit: sqrt(min%)):

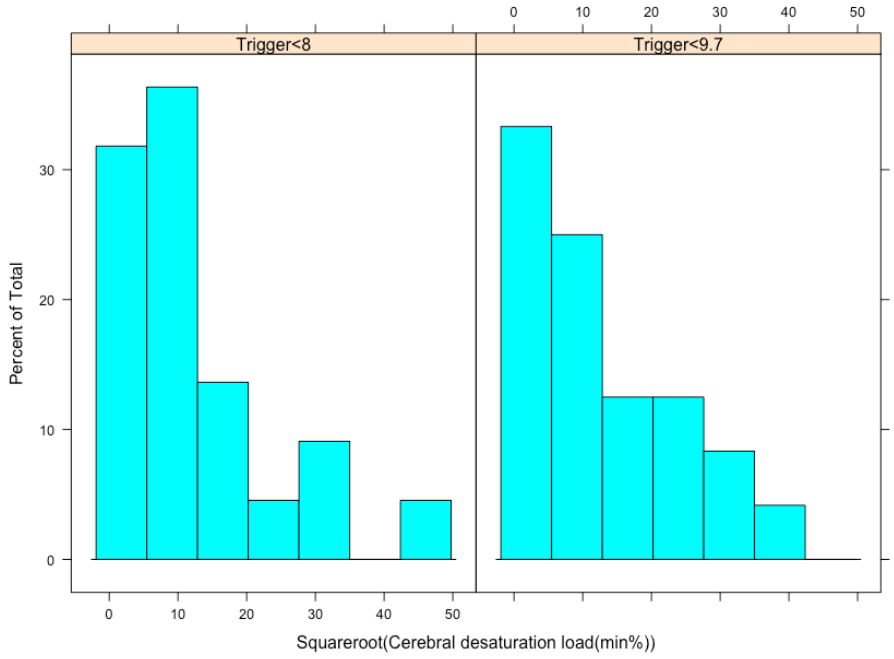


Supplemental material, Study II, Paper III.

Histogram of the crude muscular desaturation load (unit: minutes*%)

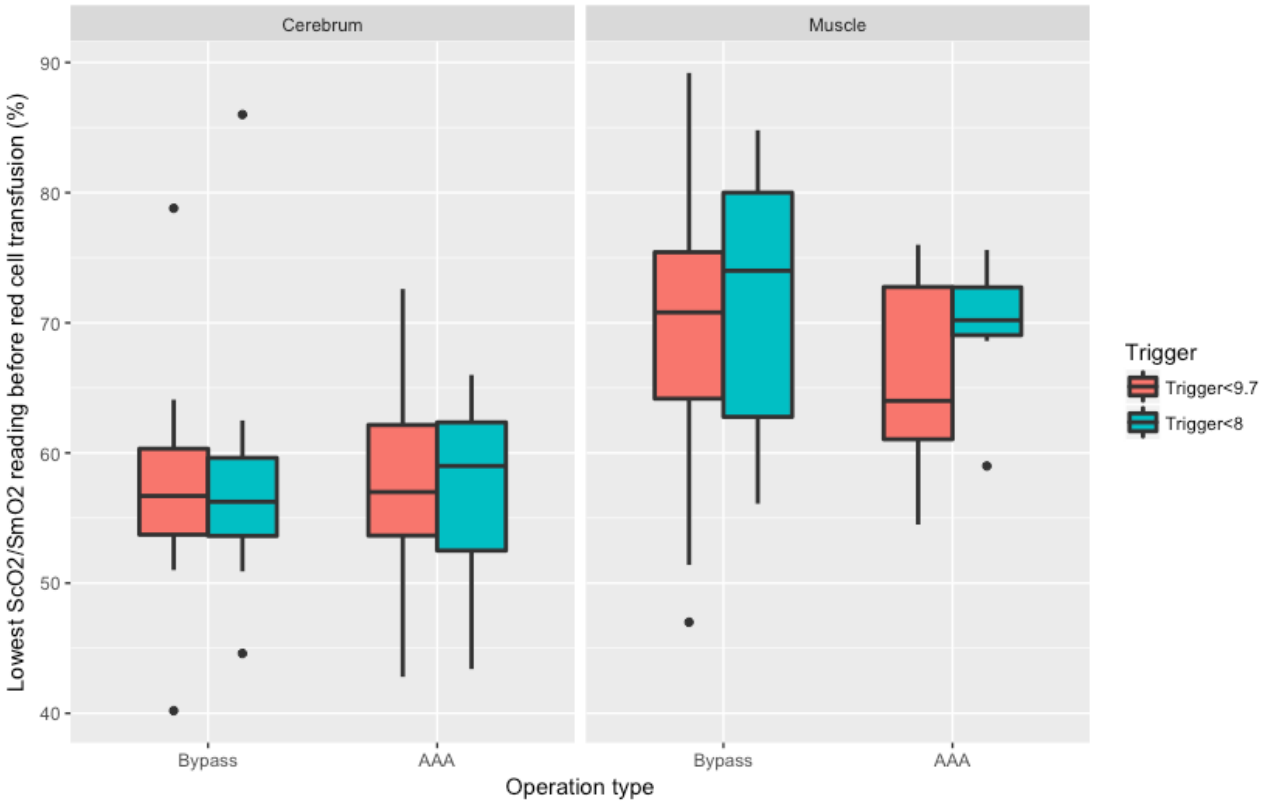


Histogram of square root transformed muscular desaturation load (unit: sqrt(min%))



Supplemental material, Study II, Paper III.

Boxplots of lowest regional cerebral and muscular NIRS reading before an RBC transfusion (%)



Supplemental material, Study II, Paper III.

Table S4. Severe adverse events at day 30

Table S4: Severe adverse events < 30 days	Trigger < 8 mmol/l (n=29)	Trigger < 9.7 mmol/l (n=29)	RR (95% CI)	P
Any event – no./total no. (%)*	13/29 (45)	18/29 (62)	0.74 (0.42 to 1.30)	0.28
Myocardial injury†	10/29 (34)	9/29 (31)		
Troponin-I ≥ 45 ng/l	9	8		
ECG suggestive of ischemia at day 30	2	2		
Acute kidney injury‡	8/29 (28)	11/29 (38)		
Stage 1	4	9§		
Stage 2	4	1		
Stage 3	0	1		
Ischemic stroke	1/29 (3)	0/29 (0)		
Death	1/29 (3)	1/29 (3)		
Severe adverse transfusion reaction‖	0/29 (0)	1/29 (3)		

*Logistic regression adjusted for age and type of operation. This was converted to relative risk (RR) using the delta-method, where probabilities were derived from the coefficients of the logistic regression using the mean age (73) and operation type (0.43); 95 % confidence interval was calculated from the standard error.

†Cardiac troponin-I ≥ 45 ng/L measured 1st and 2nd day after randomization or new onset of adverse electro cardiographic recordings on day 30 (bundle-branch block, Q-wave, inverted or flattened T-wave, ST-elevation or depression of 1 mm or more in two or more contiguous leads). Electrocardiography on day 30 is missing from 6 patients in each group. One patient in each group reported chest pain < 30 days following surgery, but none of these events could be related to ischemic heart disease.

‡ Kidney disease improving global outcome "KDIGO"-criteria. No acute kidney injury occurred due to urine output decline.
§ 3/9 patients in the high-trigger group did not have sustained creatinine increase > 24 hours. A post-hoc sensitivity excluding these 3 patients yields a relative risk of 0.95 (95 % CI: 0.49 to 1.82; p=0.87).

Staging of acute kidney injury:

Stage 1: S-creatinine increase x 1.5 of baseline within 7 days or ≥ 26.5 µmol/L within 48 hours **OR**
Urine production < 0.5 ml/kg/hour for 6 hours

Stage 2: S-creatinine increase x 2.0 of baseline **OR**
Urine production < 0.5 ml/kg/hour for 12 hours

Stage 3: S-creatinine increase x 3.0 of baseline or S-creatinine ≥ 354 µmol/L **OR**
Urine production < 0.3 ml/kg/hour for 24 hours, anuria for 12 hours **OR**
Patients requiring renal replacement therapy

‖ Anaphylactic / allergic reaction, transfusions-associated circulatory overload, transfusion-related acute lung injury within 6 hours after RBC transfusion or severe acute hemolytic transfusion reaction within 24 hours after RBC transfusion. The definitions are defined in our protocol and correspond to those use in The TRISS trial by Holst et al. 2014, NEJM.

Table S5. Anesthesia and fluids stratified according to surgery

Table S5.1. Open repair of abdominal aortic aneurysm

Abdominal aortic aneurysm surgery	Trigger < 8 g/dl (n=12)	Trigger < 9.7 g/dl (n=13)	Total (n=25)	P
FLUID INPUT (ml)				
Crystalloids Mean (sd) Median [iqr]	3642 (742) 3600 [3225, 4000]	3611 (683) 3600 [3150, 4200]	3626 (697) 3600 [3150, 4100]	0.916
Medicine Mean (sd) Median [iqr]	24 (60) 0 [0, 0]	6 (21) 0 [0, 0]	15 (44) 0 [0, 0]	0.441
Human albumin 5 % Mean (sd) Median [iqr]	813 (700) 500 [500, 1063]	865 (333) 1000 [750, 1000]	840 (530) 750 [500, 1000]	0.436
Red blood cells Mean (sd) Median [iqr] ≥1 units (%)	250 (380) 0 [0, 375] 5 (42)	508 (333) 600 [300, 600] 11 (85)	384 (373) 300 [0, 600] 16 (64)	0.043
Fresh frozen plasma ≥1 units (%)	1 (8)	0 (0)	1 (4)	NA
Platelets ≥1 units (%)	1 (8)	0 (0)	1 (4)	NA
FLUID LOSS (ml)				
Blood loss Mean (sd) Median [iqr]	1921 (1435) 1225 [1143, 2115]	2140 (855) 2100 [1875, 2400]	2035 (1150) 1900 [1200, 2400]	0.165
Urine output Mean (sd) Median [iqr]	461 (245) 415 [296, 478]	692 (488) 600 [300, 960]	581 (400) 470 [300, 705]	0.314
FLUID BALANCE				
Fluid Balance Mean (sd) Median [iqr]	2471 (1155) 2615 [1835, 3229]	2159 (577) 2300 [1875, 2450]	2309 (897) 2300 [1875, 2850]	0.387

RELATIVE BLOOD LOSS				
TBV (ml)* Mean (sd) Median [iqr]	 5126 (670) 4936 [4668, 5357]	 4786 (737) 5050 [4596, 5213]	 4949 (712) 4982 [4596, 5213]	0.229
Blood loss/TBV (%) Mean (sd) Median [iqr]	 37 (23) 26 [22, 47]	 47 (20) 44 [37, 58]	 42 (22) 38 [24, 58]	0.266
DURATION (min)				
Anesthesia duration Mean (sd) Median [iqr]	 282 (102) 242 [216, 341]	 253 (56) 251 [221, 278]	 267 (81) 247 [221, 316]	0.369
Surgery duration Mean (sd) Median [iqr]	 210 (86) 178 [151, 276]	 181 (55) 176 [158, 197]	 195 (72) 176 [158, 237]	0.307
AAA Cross Clamp Mean (sd) Median [iqr]	 91 (50) 82 [48, 108]	 82 (24) 75 [64, 103]	 86 (38) 77 [63, 103]	0.552

NA, not available. TBV, Total blood volume was calculated with Naddler's equation based on weight, height and sex. Normal data are compared with student's t. test, and non-normal data with Wilcoxon test.

Table S5.2. Lower limb bypass surgery

Lower limb bypass surgery	Trigger < 8 g/dl (n=17)	Trigger < 9.7 g/dl (n=16)	Total (n=33)	P
FLUID INPUT (ml)				
Crystalloids Mean (sd) Median [iqr]	 1993 (596) 1850 [1750, 2000]	 2420 (682) 2500 [1888, 2813]	 2200 (666) 2000 [1800, 2600]	0.055
Medicine Mean (sd) Median [iqr]	 64 (191) 0 [0, 0]	 47 (87) 0 [0, 37.5]	 56 (147) 0 [0, 0]	0.650
Human albumin 5 % Mean (sd) Median [iqr]	 191 (287) 0 [0, 250]	 125 (274) 0 [0, 63]	 159 (278) 0 [0, 250]	0.343
Red blood cells Mean (sd)	 124 (239)	 469 (477)	 291 (407)	0.003

Median [iqr] ≥1 units (%)	0 [0, 300] 5 (29)	300 [300, 450] 13 (81)	300 [0, 300] 18 (55)	
Fresh frozen plasma ≥1 units (%)	0 (0)	0 (0)	0 (0)	NA
Platelets ≥1 units (%)	0 (0)	0 (0)	0 (0)	NA
FLUID LOSS (ml)				
Blood loss Mean (sd) Median [iqr]	571 (514) 400 [245, 775]	811 (793) 580 [229, 920]	687 (665) 450 [235, 900]	0.552
Urine output Mean (sd) Median [iqr]	725 (495) 575 [300, 1000]	725 (584) 513 [394, 819]	725 (531) 550 [375, 905]	0.914
FLUID BALANCE				
Fluid Balance Mean (sd) Median [iqr]	1076 (353) 1135 [1065, 1225]	1525 (912) 1723 [1499, 2094]	1294 (708) 1420 [1075, 1660]	0.059
RELATIVE BLOOD LOSS				
TBV (ml)* Mean (sd) Median [iqr]	4517 (713) 4441 [4176, 4701]	4452 (743) 4273 [3944, 4695]	4486 (717) 4409 [3994, 4701]	0.798
Blood loss/TBV (%) Mean (sd) Median [iqr]	13 (13) 6 [5, 18]	20 (21) 14 [6, 23]	17 (17) 12 [6, 22]	0.270
DURATION (min)				
Anesthesia duration Mean (sd) Median [iqr]	229 (67) 227 [183, 244]	239 (58) 244 [200, 268]	234 (62) 235 [183, 257]	0.663
Surgery duration Mean (sd) Median [iqr]	175 (64) 167 [122, 198]	188 (64) 198 [143, 219]	181 (63) 168 [132, 217]	0.548

NA, not available. TBV, Total blood volume was calculated with Naddler's equation based on weight, height and sex. Normal data are compared with student's t. test, and non-normal data with Wilcoxon test.

Supplemental material, Study II, Paper III.

Days alive outside hospital (DAOH) within 90 days

Data extraction from the Danish National Patient Registry files:

Every hospital visit has a unique record number (RECNR). We selected 133 RECNR of interest using the method described in the flow chart below

Visits lasting zero days were not accounted for if transfer to another unit (yielding a new RECNR) occurred the same day. This was done to avoid that the day of admission (and transfer) would be counted twice.

DAOH was calculated as follows

DAOH = 90 - "days dead within 90 days" - "cumulated length of hospital stay within 90 days"

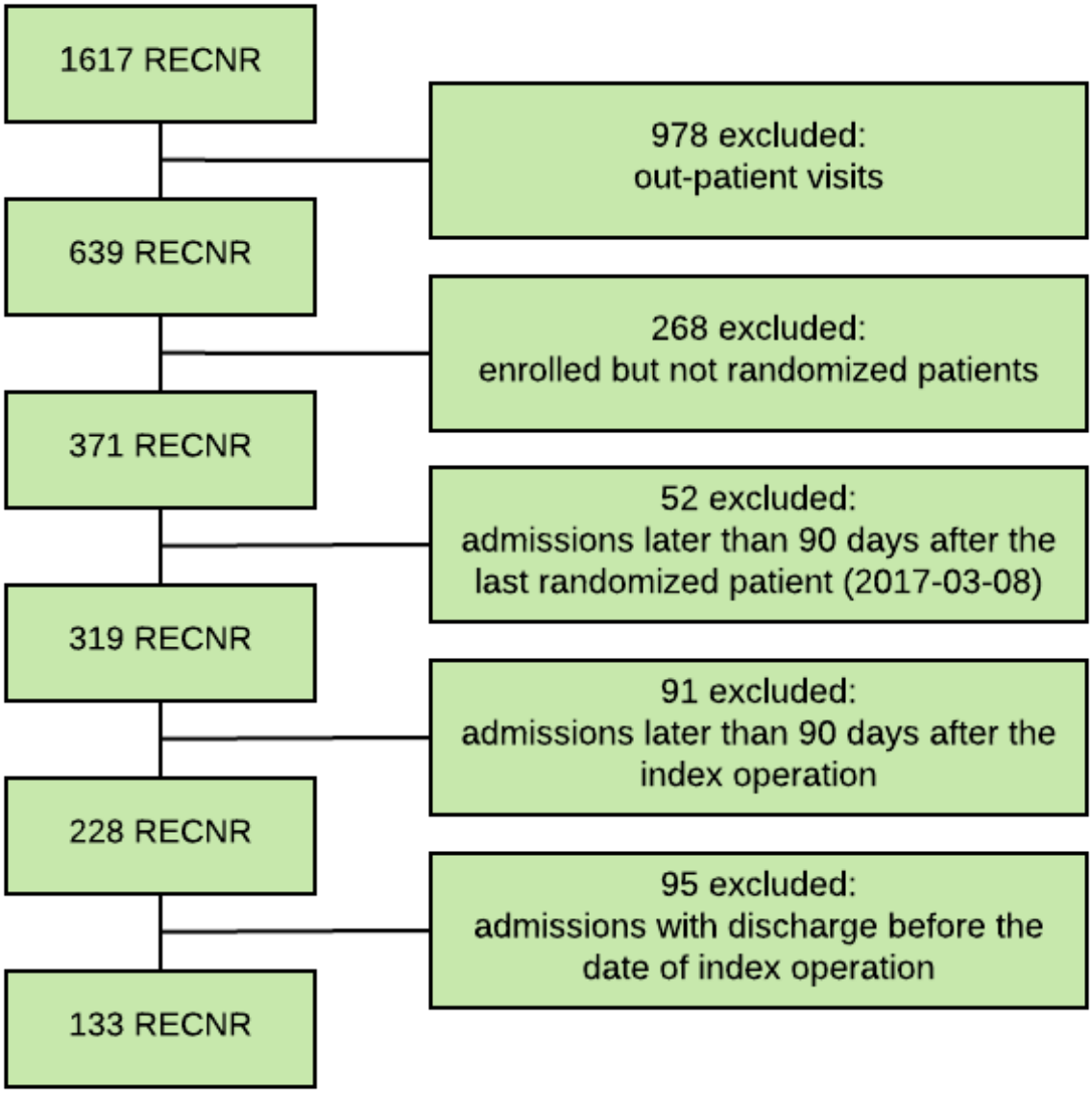


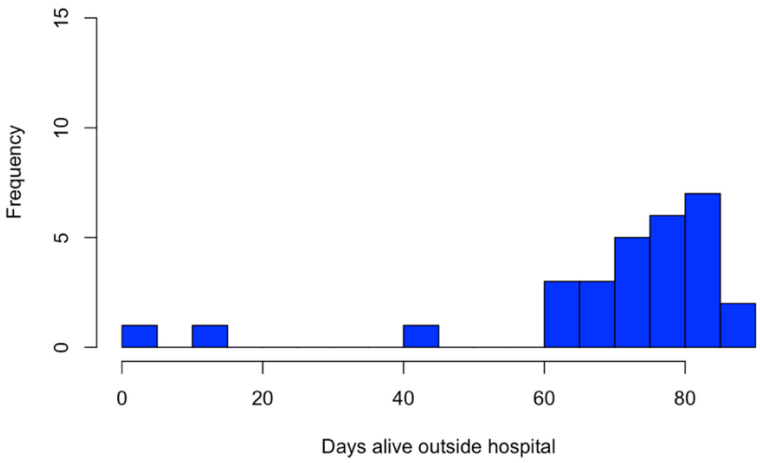
Table S6. Days alive outside of hospital within 90 days following the operation (DAOH)

	Trigger<8.0 (n=29)	Trigger<9.7 (n=29)	P*	P†	P‡
DAOH, median [iqr]	76.0 [67.0 - 82.0]	82.0 [76.0, 84.0]	0.049	0.020	0.033

*van Elteren's test adjusted for type of operation.
†Probabilistic index model adjusted for type of operation and age.
‡Cox regression model adjusted for type of operation and age. Status of all subjects set to 1 and time-to-status is the DAOH count.

Comment: the Probabilistic index model is a semi-parametric regression model, which extends the Wilcoxon–Mann–Whitney test in a similar fashion as that the linear model extends the two-sample t-test (Thas 2012).

Histogram of DAOH < 90 days for the low-trigger group (Hb < 8 g/dl).



Histogram of DAOH < 90 days for the low-trigger group (Hb < 9.7 g/dl).

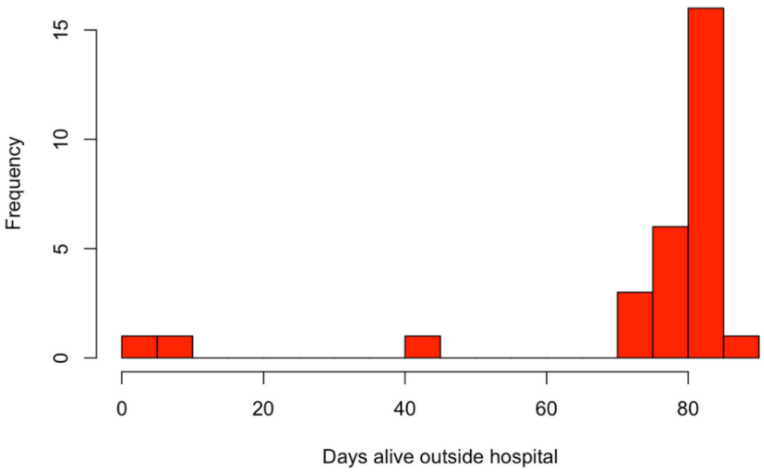


Table S7. Danish National Patient Registry data extraction method

The table shows the exploratory outcomes (death or major vascular complications), their coding and how they were extracted and included for analysis. We included events between randomization and up until right censoring (March 8th 2017).

As it came to our knowledge that three major vascular complications registered in the case report forms were not included in the registry data, we transferred these three to the exploratory outcome, as described below. These three events were: one type-1 acute myocardial infarction in each group and one severe adverse transfusion reaction in the high-trigger group. All three events were adjudicated through blinded ECG or chest X-ray assessment.

OUTCOME		DATA SOURCE OF EVENT		EVENTS
	DNPR coding	DNPR (hits)	Supplemental events from the case report form*	Included in the analysis of the exploratory outcome
1. Severe adverse transfusion complications	DT80†	0	1	1
2. Acute myocardial infarction	DI21.0-23.9	2	2	4
3. Stroke‡	DI60-64.9	2	(1)	2
4. Renal Replacement therapy	BJFD BJFZ DZ992	0	0	0
5. Vascular reoperation§	KPW	10	NR	10
6. Amputation‖	KNEQ, KNFQ, KNGQ, KNHQ	13	NR	13
7. Death	CPR	3	(2)#	3

DNPR, Danish National Patient Registry. NR, not registered in CRF as these outcomes (5 and 6) were exclusively reported at day 90 according to the protocol. CPR is the name of the Danish civil registry which contains vitality data on all inhabitants unless they have moved abroad (which was not the case in any of the patients).

* Numbers in parenthesis indicate that the events were registered both in the CRF and in the DNPR.

† We also searched for ARDS with no results/hits.

‡ All Strokes were ischemic.

§ Further three patients had vascular reoperations between the index operation and randomization but were not included for analysis, as it was the very reoperation that led to further bleeding and subsequent randomization (one wound revision, one hematoma evacuation, and one graft occlusion).

‖ 12/13 amputations occurred after lower limb bypass surgery. The amputation registered in the AAA-group, was in a patient referred for left lower extremity artery disease where preoperative arteriography (CT angiography) revealed a 6.5 cm AAA, and the patient was scheduled for AAA surgery. Within the following month, the patient had bilateral femur amputation due to loss of perfusion.

An additional patient in the low-trigger group experienced non-fatal cardiac arrest secondary to a torsade de Pointes ventricular tachycardia immediately following administration of macrolide infusion, which is a well-known side effect to this antibiotic. The patient, however, did not experience sequelae and is thus event-free with using our pre-planned definitions.

Coding specifications can be obtained from: www.medinfo.dk/sks/. For the medical conditions (no. 1-4) we used A-coding (primary diagnosis of admission). These were supplemented with B-coding, but only if a patient file review could confirm new-onset of the condition. Otherwise, unselected B-coding would also have included historical diagnoses.

Table S8. Death or major vascular complications 90 days following index operation (stratified for type of operation)

	Low Trigger Bypass (n=17)	Low Trigger AAA (n=12)	High Trigger Bypass (n=16)	High Trigger AAA (n=13)
DEATH, no. (%)	0 (0)	2 (17)	1 (6)	0 (0)
ANY MVC, no. (%)	11 (65)	7 (58)	4 (25)	4 (31)
Transf.Compl.	0 (0)	0 (0)	0 (0)	1 (8)
AMI	0 (0)	2 (17)	0 (0)	2 (15)
Stroke	0 (0)	2 (17)	0 (0)	0 (0)
RRT	0 (0)	0 (0)	0 (0)	0 (0)
Vascular reoperation	5 (29)	2 (17)	2 (13)	1 (8)
Wound revision			*1**	*1†*
Deep wound infection + tissue necrosis	*2*			
Deep bleeding			*1*	
Fascia rupture		*1*		
Thrombectomy of bypass	*2*			
Thrombectomy of groin		*1*		
Crural fasciotomy	*1*			
Amputation	7 (41)	1 (8)	2 (13)	0 (0)
Major	*3*	*1*	*0*	*0*
Minor	*4*	*0*	*2*	*0*
DEATH OR MVC, no. (%)	11 (65)	8 (67)	4 (25)	4 (31)

*lymphocele

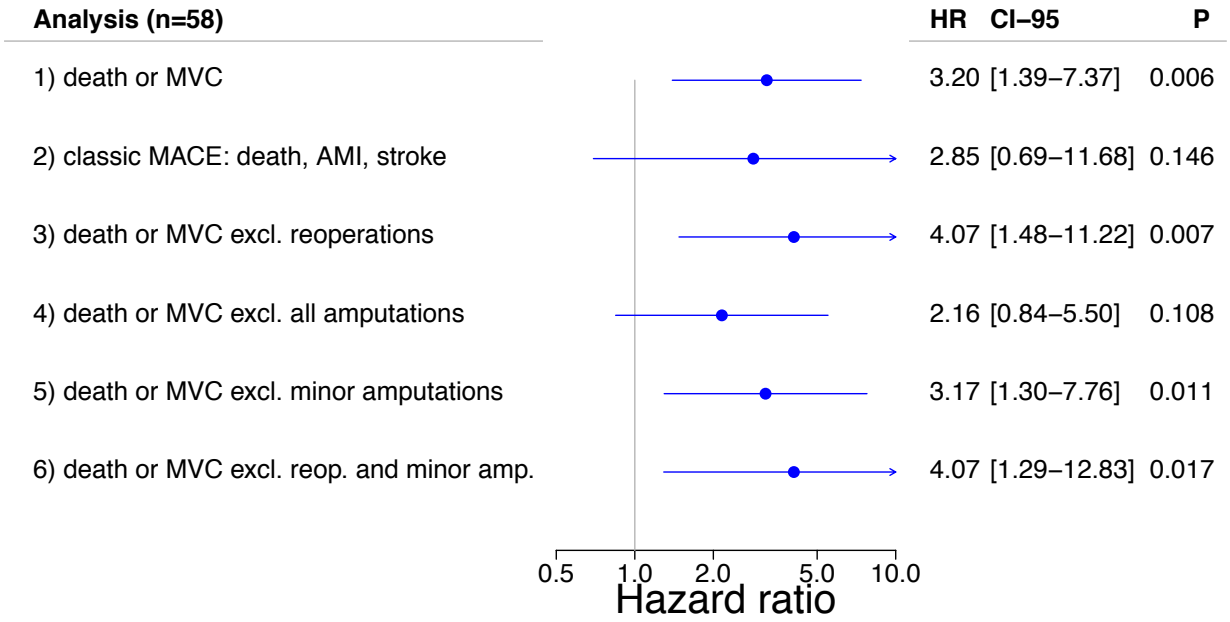
†suspected fascia rupture

Supplemental material, Study II, Paper III.

Abbreviations: Low-trigger, hemoglobin < 8 g/dl. High-trigger, hemoglobin < 9.7 g/dl. Bypass, lower limb bypass. AAA, abdominal aortic aneurysm. MVC, major vascular complications. Transf.Compl., Severe adverse transfusion reaction (Anaphylactic reaction, transfusions-associated circulatory overload, transfusion-related acute lung injury within 6 hours after RBC transfusion or severe acute hemolytic transfusion reaction within 24 hours after RBC transfusion), see[21] for further detail. AMI, acute myocardial infarction. RRT, renal replacement therapy. Vasc.Reop., vascular reoperation. Amputation, lower limb amputation from femur to toes. When considering major amputations (femoral or crural) at day 90: three major amputations had occurred in the low-trigger group vs. zero (0 %) in the high-trigger group; and at right censoring: seven (24%) vs. zero (0 %), respectively.

Table S9. Post-hoc sensitivity analyses of the intention to treat population excluding elements of the exploratory outcome: death or major vascular complications

The first analysis is the same as presented in the main manuscript.



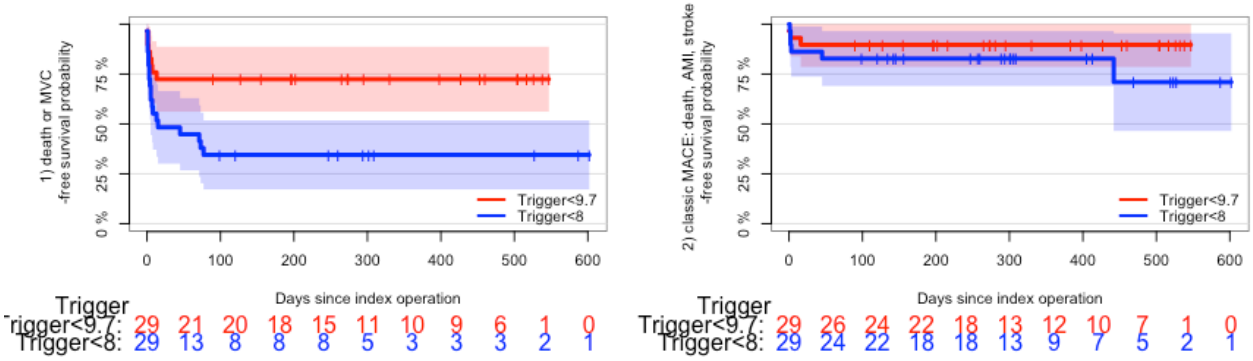
Supplemental material, Study II, Paper III.

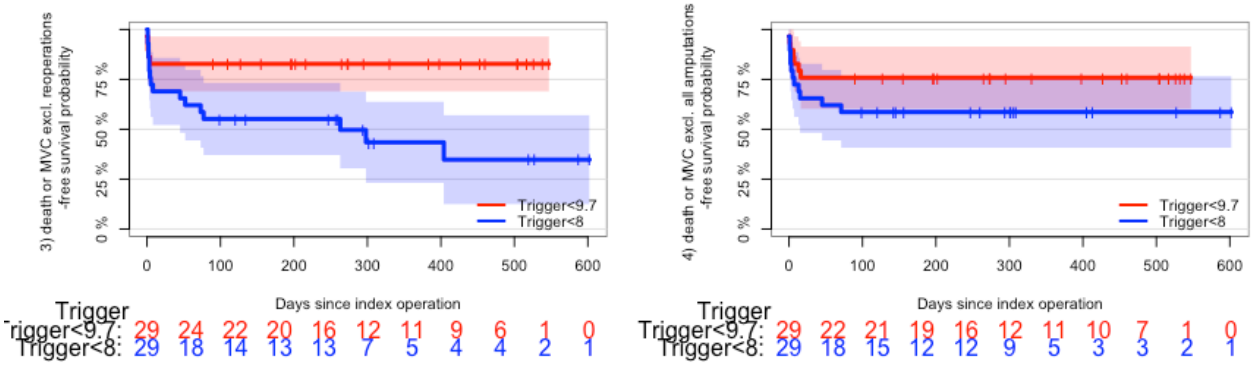
MVC, major vascular complications. AMI, acute myocardial infarction. Reop, reoperations. Amp, amputations. HR, hazard ratio. CI-95, 95 % confidence interval.

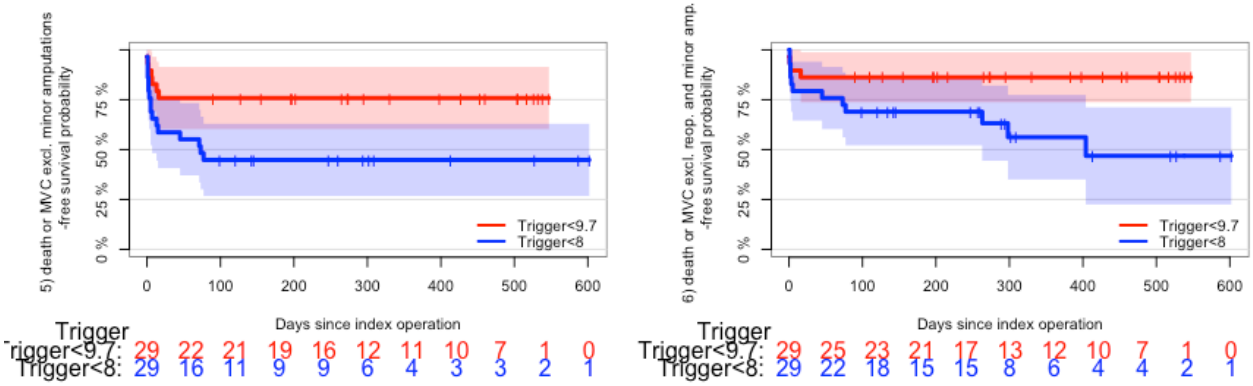
All models were adjusted for operation type and age. There was no statistically significant interaction between trigger and type of operation, or between trigger and age in any of the models. Proportional hazards assumption for the “type of operation”-covariate was not fulfilled in the sensitivity analysis 3 and 6. We adjusted for this by performing a cox regression stratified for type of operation and adjusted for age. The previous cox regression model (including operation type as a non-stratified covariate) yielded similar estimates (model 3: hazard ratio, 4.46; P=0.004, model 6: hazard ratio 4.75, P=0.009).

The corresponding Kaplan-Meier plots of complication free survival probability:

Supplemental material, Study II, Paper III.







Supplemental material, Study II, Paper III.

Paper IV

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CLINICAL INVESTIGATION

Effect of low vs high haemoglobin transfusion trigger on cardiac output in patients undergoing elective vascular surgery: Post-hoc analysis of a randomized trial

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Abstract

Background: During vascular surgery, restricted red-cell transfusion reduces frontal lobe oxygen (ScO_2) saturation as determined by near-infrared spectroscopy. We evaluated whether inadequate increase in cardiac output (CO) following haemodilution explains reduction in ScO_2.

Methods: This is a post-hoc analysis of data from the Transfusion in Vascular surgery (TV) Trial where patients were randomized on haemoglobin drop below 9.7 g/dL to red-cell transfusion at haemoglobin below 8.0 (low-trigger) vs 9.7 g/dL (high-trigger). Fluid administration was guided by optimizing stroke volume. We compared mean intraoperative levels of CO, haemoglobin, oxygen delivery, and CO at nadir ScO_2 with linear regression adjusted for age, operation type and baseline. Data for 46 patients randomized before end of surgery were included for analysis.

Results: The low-trigger resulted in a 7.1% lower mean intraoperative haemoglobin level (mean difference, −0.74 g/dL; $P < .001$) and reduced volume of red-cell transfused (median [inter-quartile range], 0 [0-300] vs 450 mL [300-675]; $P < .001$) compared with the high-trigger group. Mean CO during surgery was numerically 7.3% higher in the low-trigger compared with the high-trigger group (mean difference, 0.36 L/min; 95% confidence interval (CI.95), −0.05 to 0.78; $P = .092$; n = 42). At the nadir ScO_2-level, CO was 11.9% higher in the low-trigger group (mean difference, 0.58 L/min; CI.95, 0.10-1.07; $P = .024$). No difference in oxygen delivery was detected between trial groups (MD, 1.39 dL_{O2}/min; CI.95, −6.16 to 8.93; $P = .721$).

Conclusion: Vascular surgical patients exposed to restrictive RBC transfusion elicit the expected increase in CO making it unlikely that their potentially limited cardiac capacity explains the associated ScO_2 decrease.

An account of the results has been provided in an abstract for the Scandinavian Society of Anaesthesiology and Intensive Care Medicine (SSAI) congress (2019-08-28, Copenhagen, Denmark).

1 | INTRODUCTION

Red blood cell (RBC) transfusion is often considered during vascular surgery to compensate for lowered blood O_2 transport capacity when haemoglobin (Hb) is reduced by blood loss or haemodilution.[1] Vascular surgical patients may present left ventricular dysfunction[2] and coronary[3] as well as carotid[1,4] atherosclerotic lesions which could compromise both global- and regional blood flow with risk of post-operative ischaemic events.[5,6] Thus, especially in the vascular surgical patient, fluid therapy needs to balance concern for both cardiac output (CO) and blood O_2 carrying capacity. Yet, both anaesthesia- and vascular surgical societies recommend restricted use of RBCs[7-10] as non-randomized studies demonstrate RBC transfusion associates to a poor outcome in patients with cardiovascular disease.[11] Also, restricted RBC transfusion is reported to be safe in the post-operative period.[12,13] However, the safety of restricting RBC transfusion during surgery lacks confirmation in randomized trials.[1,10]

The randomized transfusion in vascular surgery (TV) feasibility trial compared a low-trigger for RBC transfusion (Hb < 8 g/dL) with a high-trigger (Hb < 9.7 g/dL) in patients undergoing elective open abdominal aortic aneurism (AAA) repair or lower-limb bypass.[1,14] During surgery, patients allocated to the low-trigger strategy demonstrated more pronounced duration and magnitude of frontal lobe tissue oxygen (ScO_2) desaturation compared with patients allocated to the high-trigger transfusion strategy, 421 vs 127 min·%.[1] The outcome was defined as the cumulative area below the ScO_2 baseline and was referred to as the ScO_2 desaturation load. The data fuelled the hypothesis that the CO response to haemodilution was lower or inadequate to preserve oxygen delivery (DO_2) and explained the ScO_2 in the low-trigger group. To test that hypothesis, we carried out a post-hoc analysis of the CO data that were continuously recorded during surgery. Other potential explanatory variables including arterial oxygenation (paO_2), ventilation ($paCO_2$), mean arterial pressure (MAP) and noradrenaline dosage were included in the computation. We present a novel description of the intraoperative time-trend and dynamics of the Hb and near-infrared spectroscopy (NIRS) data to juxtapose the intervention effect on Hb, CO, DO_2, and tissue oxygenation.

Editorial Comment

In this post-hoc analysis from a randomized trial comparing two haemoglobin concentration triggers (8.0 vs 9.7 g/dL) for erythrocyte transfusion during vascular surgery, measurements of cardiac output and oxygen delivery was compared between groups. While oxygen delivery was comparable between the groups, cardiac output was increased in the group with the more restrictive transfusion strategy. This was interpreted as an adequate physiological reserve to compensate for lower oxygen carrying capacity.

2 | MATERIALS AND METHODS

2.1 | Trial design and setting

The TV trial is an investigator-initiated, single-centre, stratified, parallel-group, patient- and partly assessor-blinded clinical feasibility trial with external web-based randomization evaluating the effects of a restrictive vs a liberal RBC transfusion strategy throughout hospitalization for vascular surgery. This post-hoc analysis is confined to patients who were randomized before end of surgery. Patients above 40 years of age scheduled for AAA repair or lower-limb bypass (infra-inguinal bypass or femoro-femoral cross-over surgery) were randomized immediately following a drop in Hb to below 9.7 g/dL to receive RBC transfusion triggered by a Hb < 8 g/dL (5 mmol/L, intervention) vs Hb < 9.7 g/dL (6 mmol/L, control). Randomization was stratified by type of surgery (AAA vs lower-limb bypass). Exclusion criteria are provided in Figure 1. All patients gave written consent ahead of surgery. The protocol was approved by the Scientific Ethical Committee of Region Zealand (Project-ID: SJ-426) and the Danish Data Protection Agency. This trial was registered at www.clinicaltrials.gov as NCT02465125.

2.2 | Red blood cell transfusion and protocol suspension

Patients assigned to the low-trigger group awaited RBC transfusion until Hb dropped to below 8.0 g/dL and received transfusions to maintain Hb at or above 8.0 g/dL, while the high-trigger group received one RBC unit immediately after randomization and additional units to maintain Hb at or above 9.7 g/dL. The Hb was determined every half hour during on-going bleeding, after every RBC transfusion, when considered clinically indicated, and on arrival at the recovery room. RBC transfusions were allogeneic and leuco-reduced. Physicians could suspend the protocol in case of uncontrollable bleeding or if there was suspicion of extremity, intestine or brain ischaemia. Hb triggered RBC transfusion was resumed when haemodynamic stability was restored.

2.3 | Anaesthesia and fluid therapy

General anaesthesia was by inhalation of sevoflurane and fentanyl with rocuronium used for muscle relaxation. Patients undergoing open AAA repair had an epidural catheter inserted for peri-operative analgesia. Fluid therapy was guided by cardiac stroke volume (SV) using invasive arterial waveform analysis (FloTrac ver.4.0 and EV1000 platform; Edwards LifeSciences, Irvine, CA). If a 250 mL fluid bolus increased SV by less than 10%, the patient was considered normovolaemic and no further boluses were administered

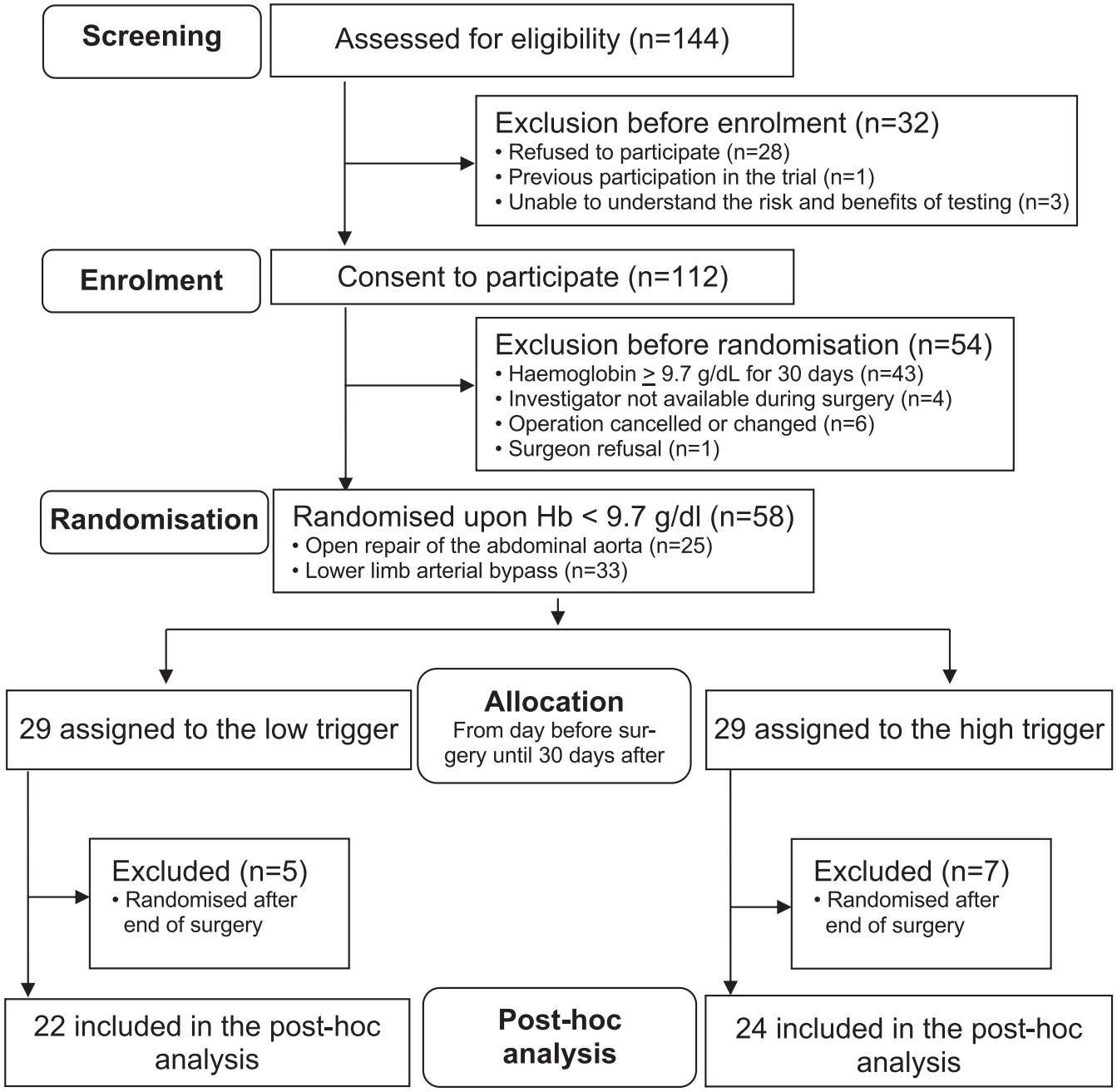


FIGURE 1 Patient flow. Patients randomized before end of surgery were included in the post-hoc analysis. Potential participants were excluded if they refused RBC transfusion, had experienced serious adverse reaction to blood products, had previously participated in the TV trial, or were unable to understand the benefits and risks of the trial

unless hypovolaemia developed, or the aortic cross-clamp was about to be released. The primary iv fluid was Ringer's acetate solution, but if blood loss exceeded 1 L or the total crystalloid administration was above 3 L, human albumin (5%) was administered as guided by SV until the Hb decreased to below the allocated transfusion threshold. When patients were normovolaemic, continuous infusion of norepinephrine kept MAP above 65 mmHg. The fraction of inspiratory O_2 was 0.5 and the ventilation targeted a p_aCO_2 of 4.5-6 kPa. Plasma and platelet transfusion was guided by rotational thromboelastometry. Intraoperative blood salvage, tranexamic acid and diuretics were not used.

2.4 | Data collection

FloTrac-data (CO, cardiac index (CI), heart rate (HR), MAP, and SV) were recorded every 20th second on the EV1000 platform. NIRS probes were placed on the right side of the forehead and on the right biceps muscle parallel to the fibres to assess Hb oxygen saturation of the frontal lobe (ScO_2) and muscle (SmO_2). The NIRS reading was blinded during anaesthesia. Baseline CO was established ahead of surgery and was defined as the mean reading over 1 minute before reaching normovolaemia as defined. The baseline Hb was the value from the day before surgery. Blood samples were analysed by ABL90

flex (Radiometer®, Copenhagen, Denmark). Baseline blood gas variables were analysed after placing an arterial line before start of surgery and used to calculate arterial oxygen content (CaO_2) which multiplied by CO yielded DO_2. Noradrenaline dosing and pulse oximetry saturation were recorded real time by Metavision (iMDsoft®, Tel Aviv, Israel).

2.5 | Outcomes

The primary outcome was mean CO during surgery from baseline to skin closure. Also, we report the CO at nadir ScO_2, the mean Hb, DO_2, ScO_2 and SmO_2 during surgery besides mean paO_2, $paCO_2$, MAP and noradrenaline dosing. Nadir ScO_2 was the lowest ScO_2-reading before a RBC transfusion or at skin closure if the transfusion threshold was not met in the low-trigger group.[17] The nadir Hb and DO_2 were the lowest values measured during surgery.

2.6 | Statistical analysis

Analyses adhere to the detailed statistical analysis plan[14] and were based on the intention-to-treat population. Continuous outcomes were compared between trial groups with linear regression adjusted for age, type of operation, and the baseline value of the outcome. Results are presented as mean (standard deviation). Non-normal data are presented as median [inter-quartile range] and are compared with Van Elteren's test adjusted for the stratum. We tested variables for normality with quantile-quantile plots and used area under the curve to compute a mean intraoperative level of repeated measurements between baseline and skin closure. To describe relative differences of Hb and CO, we divided the modelled mean of the low-trigger with the modelled mean of the high-trigger. The evaluation of DO_2 was based on the CaO_2, but for five patients computed from the saturation obtained by pulse oximetry.

To indicate the detectable change in CO, we performed a post-hoc power calculation based on a sample size of 42, alfa of 5%, beta of 20%, and the standard deviation of all participants mean CO during surgery, 1.05, which yielded a delta of 0.93 L/min. Since we adjust the linear model for baseline values, operation type and age, we expect reduced variance and higher actual power.

Most covariates were available (except from one patient's baseline $paCO_2$) and, therefore multiple imputation for missing data was not needed.[14] Two-sided tests were used and *P*-values below .05 were considered statistically significant.

3 | RESULTS

Written consent was obtained from 112 of 144 screened patients and 58 patients were subsequently randomized during the trial and the 46 patients randomized before end of surgery were included (Figure 1). Baseline patient characteristics and risk factors were balanced among the included patients (Table 1). Five patients were randomized pre-operatively as Hb was below 9.7 g/dL on hospital admission and, accordingly, three patients allocated to the high-trigger received RBC transfusion pre-operatively and two allocated to

TABLE 1 Baseline characteristics

	Trigger < 8 g/dL (n = 22)	Trigger < 9.7 g/dL (n = 24)
Age (years), mean (SD)	71.5 (9.5)	74.8 (7.2)
BMI (kg/m^2), mean (SD)	24.8 (4.67)	24.8 (3.96)
Male sex, n (%)	13 (59.1)	13 (54.2)
Pre-operative Hb (g/dL), mean (SD)	11.94 (1.65)	11.93 (2.12)
Operation, n (%)		
Abdominal aortic aneurysm	8 (36.4)	11 (45.8)
Lower limb bypass	14 (63.6)	13 (54.2)
Cardiovascular disease, n (%)	20 (90.9)	18 (75.0)
Lower extremity artery disease	16 (72.7)	14 (58.3)
Claudication	2 (9.1)	1 (4.2)
Pain at rest	3 (13.6)	3 (12.5)
Wound/gangrene	11 (50.0)	10 (41.7)
Angina	3 (13.6)	5 (20.8)
Stroke or TIA	5 (22.7)	3 (12.5)
Previous CABG or PCI	3 (13.6)	4 (16.7)
Congestive heart failure	1 (4.5)	2 (8.3)
Heart valve disease	3 (13.6)	4 (16.7)
Acute myocardial infarction	0 (0.0)	5 (20.8)
Pacemaker	0 (0.0)	0 (0.0)
Cardiovascular risk factor, n (%)	22 (100.0)	23 (95.8)
Arterial hypertension	14 (63.6)	18 (75.0)
Smoker	8 (36.4)	7 (29.2)
COPD	5 (22.7)	5 (20.8)
Hypercholesterolaemia	18 (81.8)	21 (87.5)
Chronic renal failure	1 (4.5)	6 (25.0)
Diabetes mellitus	8 (36.4)	6 (25.0)
Pre-operative ECG, n (%)		
Sinus rythm	21 (95.5)	22 (91.7)
Atrial fibrillation	1 (4.5)	2 (8.3)
ASA physical status, n (%)		
1	0 (0.0)	0 (0.0)
2	1 (4.5)	4 (16.7)
3	20 (90.9)	19 (79.2)
4	1 (4.5)	1 (4.2)

Note: There was no statistically significant baseline difference between groups in any of the variables, nor in race as all patients were white.

Abbreviations: ASA, American Society of Anaesthesiologists; BMI, body mass index; CABG, coronary artery bypass grafting; COPD, chronic obstructive pulmonary disease; ECG, electrocardiography; PCI, percutaneous coronary intervention; TIA, transient ischaemic attack.

the low-trigger did not. Reasons for missing data are provided in the table legends.

Peri-operative characteristics and fluid balance were similar in the two trial groups except for the infused RBC volume which, as intended was lower in the low-trigger compared with the high-trigger group (0 mL [0-300] vs 450 [300-675]; $P < .001$; Table 2 and Table S2). The blood loss was 898 mL [403-1695] in the low-trigger vs 1650 [590-2160] in the high-trigger group ($P = .429$) and larger for AAA: 2085 mL [1775-2612] than for lower-limb bypass surgery: 560 mL [240-1000].

3.1 | Haemoglobin

The mean pre-operative Hb was 11.9 g/dL in both trial groups. The mean Hb during surgery was 9.6 g/dL in the low-trigger group vs 10.4 g/dL in the high-trigger group (mean difference, −0.74 g/dL; $P < .001$; Table 3) and corresponded to a 7.1% lower Hb. On arrival in the recovery room, the mean difference in Hb was −1.35 g/dL ($P < .001$).[1]

3.2 | Haemodynamic variables

At baseline, CO was 4.80 L/min in the low-trigger and 4.54 L/min in the high-trigger group ($P = .494$). The mean CO during surgery was 7.3% higher in the low-trigger group compared with the high-trigger (mean difference, 0.36 L/min; 95% confidence interval (CI.95), −0.05 to 0.78; $P = .092$, Table 3, Figures 2 and 3). At the nadir ScO_2-reading, CO was 11.9% higher with the low-trigger strategy (mean difference, 0.58 L/min; 95.CI, 0.07 to 1.10; $P = .024$). A sensitivity analysis excluding patients randomized

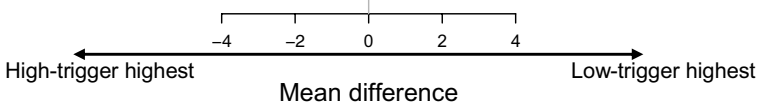
TABLE 2 Fluid balance, noradrenaline dose and duration of anaesthesia, surgery and cross clamp

	Trigger < 8 g/dL (n = 22)	Trigger < 9.7 g/dL (n = 24)	P
Fluid input (mL)			
Crystalloids	2300 [1800-3500]	2900 [2075-3704]	0.235
Human albumin 50 g/L	500 [0-875]	250 [0-1000]	0.186
Red blood cells	250 [0-1000]	450 [300-675]	<0.001
Total	3000 [2100-4378]	4025 [2700-5238]	0.083
Fluid loss (mL)			
Blood loss	898 [403-1695]	1650 [590-2160]	0.429
Urine	468 [288-869]	648 [394-964]	0.190
Total	1528 [936-2436]	2260 [1321-3108]	0.368
Fluid balance (mL)			
Total	1190 [1081-2098]	1855 [1499-2300]	0.312
Ratio (mL/mL)			
Total fluid input/blood loss	3.5 [2.6-5.4]	2.9 [2.3-4.6]	0.584
Duration (minutes)			
Anaesthesia	238 [189-282]	252 [207-277]	0.808
Surgery	174 [153-232]	181 [152-213]	0.843
AAA Cross Clamp (n = 8 *vs* n = 11)	89 [64-140]	75 [64-90]	0.225
Noradrenaline (mg)			
Cumulated dose (n = 21 *vs* n = 22)	0.39 [0.05-0.91]	0.44 [0.00-0.74]	0.840

Note: Results presented as median [inter-quartile range]. Test statistic is Van Elteren's test adjusted for operation type. In the low-trigger group 12/22 (55%) avoided RBC transfusion whereas all patients in the high-trigger group received RBCs. One patient in the low-trigger group received fresh frozen plasma transfusion due to massive bleeding caused by iliac vein laceration during insertion of aorto-bifurcated prosthesis. No patients required platelet transfusion. No cases of non-adherence to transfusion strategies were registered during surgery, but protocol suspension was used in five patients in the low-trigger and in four patients in the high-trigger group due to rapid haemorrhage.[1] An additional patient in the low-trigger group received RBCs under protocol suspension due to extremity ischaemia. See Supplementary Material for further data. Three patients had missing data on cumulated noradrenaline dose because the change in infusion rate was not recorded in the electronic anaesthesia files.

TABLE 3 Haemodynamic profile

Haemodynamic variables (n 22 vs 24)	Trigger<8 mean (sd)	Trigger<9.7 mean (sd)	MD	95 % CI	P
Haemoglobin (g/dL)					
Nadir	8.4 (0.78)	9.12 (0.34)	−0.75	[−1.10 to −0.39]	<0.001
Mean	9.54 (0.85)	10.36 (0.67)	−0.76	[−1.07 to −0.45]	<0.001
Recovery room	9.23 (0.76)	10.49 (0.63)	−1.35	[−1.73 to −0.96]	<0.001
Cardiac output (L/min), (n 20 vs 22)					
CO at nadir ScO2	5.6 (1.1)	4.8 (0.92)	0.58	[0.10 to 1.07]	0.024
Mean	5.49 (1.15)	4.85 (0.88)	0.36	[−0.05 to 0.78]	0.092
Skin Closure	5.84 (1.28)	5.16 (1.31)	0.42	[−0.17 to 1.01]	0.172
DO2 (dL_O2/min), (n 20 vs 22)					
Nadir	63.4 (16.2)	58 (11.1)	2.64	[−4.48 to 9.76]	0.472
Mean	71.3 (14.9)	67.3 (13.5)	1.39	[−6.16 to 8.93]	0.721
Last Measurement	70.8 (18.2)	68.2 (17.3)	−0.86	[−10.20 to 8.49]	0.858
ScO2 (%), (n 22 vs 23)					
Nadir	57.45 (8.74)	57.66 (8.5)	−2.11	[−5.48 to 1.27]	0.228
Mean	59.77 (7.76)	61.25 (7.01)	−2.86	[−5.34 to −0.38]	0.029
Skin Closure	59.77 (7.76)	61.22 (7.03)	−2.84	[−5.31 to −0.36]	0.030
SmO2 (%), (n 22 vs 23)					
Nadir	71.21 (8.4)	67.32 (9.65)	1.50	[−1.66 to 4.67]	0.358
Mean	71.34 (8.3)	69.34 (9.05)	−0.26	[−2.73 to 2.21]	0.836
Skin Closure	71.33 (8.3)	69.34 (9.04)	−0.27	[−2.74 to 2.20]	0.833
MAP (mmHg), (n 20 vs 22)					
MAP at nadir ScO2	69.34 (12.34)	65.95 (7.85)	4.23	[−2.38 to 10.83]	0.218
Mean	69.24 (6.08)	69.04 (4.51)	0.92	[−2.25 to 4.10]	0.572
Skin Closure	70.97 (10.31)	66.75 (11.63)	4.71	[−2.52 to 11.94]	0.209
Respiration (kPa) (n 20 vs 21)					
Mean paO2	19.7 (5.2)	19.5 (6.2)	0.22	[−3.50 to 3.93]	0.910
Mean paCO2	5.4 (0.4)	5.3 (0.5)	0.12	[−0.16 to 0.40]	0.411



Abbreviations: Cardiac output, CO. DO_2, oxygen delivery. MAP, mean arterial pressure. ScO_2, frontal lobe tissue oxygenation. SmO_2, biceps muscle oxygenation.

The trial group means are presented as grand means (standard deviation, sd). MD, modelled mean difference. CI, confidence interval. The vertical line indicates zero MD. CO data were missing from two patients in each group (the first three patients were randomized before we had the option to download the CO-data from the EV1000-platform and one patient had missing CO-data because the EV1000-power supply short-circuited as iv-fluid entered by accident and the data was lost). DO_2 was calculated based on Hb bound O_2 alone. Results from DO_2 based on Hb-bound and plasma dissolved O2 is in the results section. DO_2 at skin closure could not be calculated because an arterial blood gas was not consequently obtained at skin closure. Conversely, recovery room DO_2 could not be calculated because CO was not recorded in the recovery room. Thus, last DO_2 obtained during surgery is presented. Recovery room Hb, and nadir ScO_2 and SmO_2 have been reported previously1 but are also presented in this table for comparison.

pre-operatively did not change the mean difference in CO during surgery (0.40 L/min; CI.95, −0.06 to 0.86; P = .094, n = 37). Median number of blood gases analysed after baseline was 4 [2.75-4.25] vs 4 [3-5.75]. The mean Hb bound DO_2 was similar in the two trial groups at baseline (71.0 (18.3) vs 69.1 (20.8) dL_{O2}/min) as during surgery (MD, 1.39 dL_{O2}/min; 95.CI, −6.16 to 8.93, P = .721, n = 42). When adding physiologic dissolved O_2, fewer measurements were available (3 [2.25-4] vs 4 [3-5.5]) because central venous blood gases were occasionally drawn for Hb measurement, but demonstrated similar DO_2 (MD −3.19 dL_{O2}/

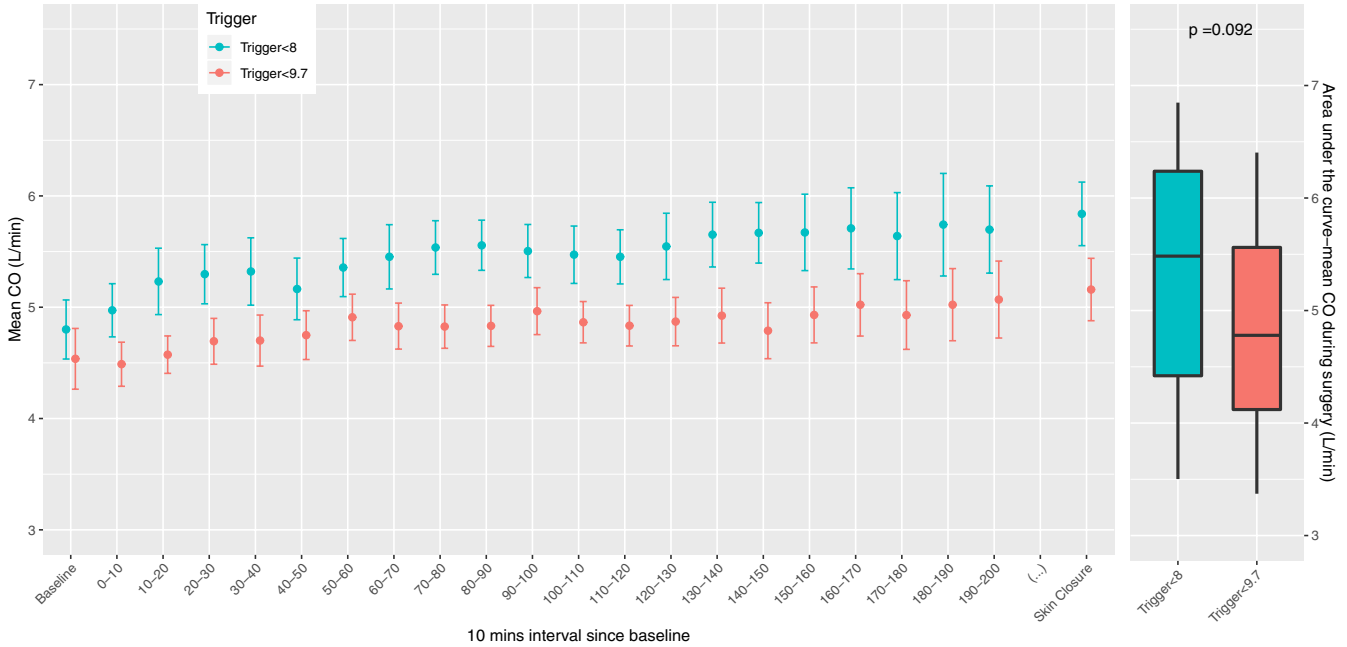


FIGURE 2 Cardiac output

Number of patients observed in each 10 minutes interval.

	Baseline	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	100-110	110-120	120-130	130-140	140-150	150-160	160-170	170-180	180-190	190-200	(...)	Skin closure
Trigger < 8	20	20	20	20	20	20	20	20	20	20	20	20	19	17	16	16	15	15	14	14	10		20
Trigger < 9.7	22	22	22	22	21	22	22	22	22	22	22	22	22	20	19	18	18	17	15	13	12		22

Baseline was defined as the time point when the patient's stroke volume was unresponsive to further fluid administration, that is, was considered normovolaemic ahead of surgery.

min; CI.95, −10.59 to 4.22; $P = .405$; n = 37). The mean MAP was 69 mmHg in both trial groups ($P = .572$). For analyses of CI see Table S3. The median urine output during anaesthesia was above 2 mL/kg/h in both trial groups (2.1 [1.2-2.7] vs 2.4 [1.6-4.1] mL/kg/h; $P = .159$).

The mean ScO_2 during surgery was lower with the low-trigger strategy (mean difference, −2.9%-point; CI.95, −5.3 to −0.3; $P = .029$) and ScO_2 separation was retained at skin closure, Table 3. The SmO_2 did not differ with statistical significance in any of the models.

3.3 | Oxygenation, ventilation and noradrenaline

We observed no difference in median SaO_2 (1.00 [0.99-1.00] vs 0.99 [0.97-1.00]; $P = .331$), mean paO_2 or $paCO_2$ (Table 3), or median cumulated noradrenaline dose (Table 2).

4 | DISCUSSION

This post-hoc analysis presents novel data on central and peripheral circulatory variables from the TV trial for vascular surgical patients who underwent an intraoperative protocol restricting RBC transfusion until Hb was below 8 vs 9.7 g/dL. In patients allocated to the low-trigger group, CO was higher at the nadir ScO_2-reading and numerically higher during surgery. This post-hoc analysis complements the finding from the main trial,[1] that the low-trigger increased the ScO_2 desaturation load despite a higher CO. Importantly, the ScO_2 desaturation load was not explained by differences in paO_2, DO_2, $paCO_2$, MAP or noradrenaline infusion. It can be estimated that the expected CO increase following normovolaemic haemodilution from a Hb of 10.4-9.6 g/dL is about 0.5 L/min[15] and the mean difference in CO was similar (0.36 L/min). The net effect of lowered Hb and higher CO in the low-trigger group was a DO_2 comparable to that of the high-trigger group, as reflected by a similar SmO_2. The results indicate, that an inadequate increase in CO following haemodilution does not explain the lower ScO_2 in the low-trigger group. We suggest that regional differences in the adaptive response to haemodilution explain the heterogeneous effect on ScO_2 and SmO_2.

Balancing the circulation during surgical haemorrhage is a challenge. A goal-directed fluid strategy appears to reduce post-operative complications,[16] but the target Hb level remains little explored. Restrictive RBC transfusion seems safe in the intensive care unit patient[17] and for hip-fracture surgery,[12] and a restrictive RBC transfusion strategy is advocated during surgery.[7-9] However, systematic reviews demonstrate increased risk of ischaemic complications with restrictive RBC transfusion in the peri-operative setting.[5,18]

A blood loss exceeding 500 mL has been associated with increased odds of mortality if RBC transfusion is withheld during

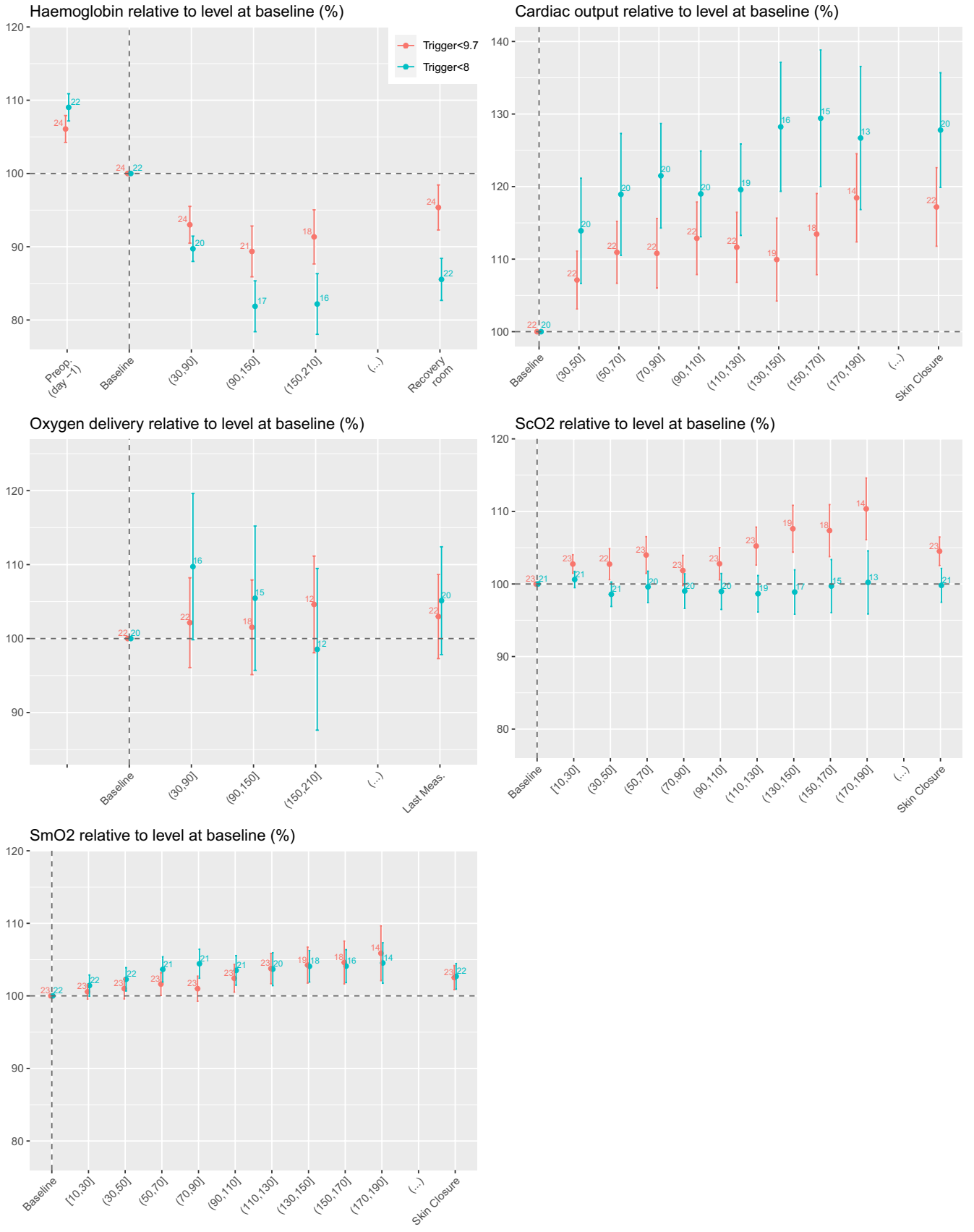


FIGURE 3 Haemoglobin, cardiac output, oxygen delivery and tissue oxygenation during surgery. *X*-axis: time elapsed since baseline (minutes). *Y*-axis: mean ± SE (%). Numerals indicate the number of patients observed. The *x*-axis is truncated at 180 minutes, the mean duration of surgery. Baseline was defined as the time point when the patient's stroke volume was unresponsive to further fluid administration. Oxygen delivery calculation was based on Hb bound O_2

surgery compared to intraoperative RBC transfusion.[19] However, randomized trials comparing Hb transfusion triggers during surgery involving blood loss of more than 500 mL report no or limited separation of Hb levels and infused volume of RBCs during surgery[20,21] which precludes firm conclusions on the safety of restrictive RBC transfusion. Mazer et al[13] compared a Hb trigger of 9.5 vs 7.5 g/dL for RBC transfusion during cardiac surgery. The difference in nadir Hb during surgery was 0.2 g/dL and the CI at chest closure was almost identical (2.47 vs 2.43 L/min/m^2). As patients were randomized before surgery and the mean blood loss was limited to 0.5 L, only about half of the patients were exposed to Hb levels below 9.5 g/dL intra-operatively. In the TV trial the mean difference in nadir Hb and CI was 0.75 g/dL and 0.28 L/min/m^2, respectively (Table 3 and Table S3), that is, a three- to fivefold larger separation. Most likely, this separation was due to a large bleeding volume (1175 mL) and because the Hb level was used as an inclusion criterion thereby excluding patients with minimal blood loss and enriching the population with patients at risk of ischaemic complications secondary to anaemia.

The low-trigger group received a numerically lower total fluid volume than the high-trigger. However, due to a larger blood loss in the high-trigger group, the ratio of total fluid administration to blood loss indicated the opposite or no difference. More RBC units transfused in the high-trigger group may have provoked a larger blood loss, although the difference was not statically significant. A median urine output above 2 mL/kg/h in both trial groups indicates that central blood volume was maintained in both groups.

The explanation for a lower ScO_2 despite preserved global DO_2 in the low-trigger groups may be found in the cerebral flow regulation. A review on human and animal studies reports that cerebral DO_2 is impaired when CaO_2 is reduced by haemodilution. Mechanisms controlling cerebral blood flow following a decrease in CaO_2 are multifactorial, but increased cerebral perfusion seems to be provoked by deoxygenation of haemoglobin, and haemodilution may reduce the signal transduction originating from the RBCs.[22] Also, anaesthetized rats exposed to acute normotensive haemodilution to a Hb < 7 g/dL demonstrate a decrease in cerebral oxygenation while muscle oxygenation seems unaffected as determined by invasive tissue oximetry.[23] That animal model corroborates the present findings and suggests that haemodiluting Hb below 7-8 g/dL is too large to maintain cerebral oxygenation although increased arterio-venous O_2-extraction may secure cerebral oxygen consumption. Carotid atherosclerosis is seen in 20%-30% of vascular surgical patients[1,4] and may reduce brain perfusion pressure, the capacity to increase oxygen extraction, and impair cerebral autoregulation.[24] Cerebral perfusion is sensitive to changes in MAP[25] but MAP was identical in the two groups. A depressive effect of anaesthesia on the autonomic nervous system could influence CO or regulation of cerebral perfusion. However, reduced cerebral perfusion during propofol anaesthesia is offset by reduced cerebral oxygen metabolism.[26]

The present re-analysis of the NIRS data shows that the difference in cerebral desaturation load in the two groups (421 vs 127 min·%; $P = .0036$)[1] translates into a mean ScO_2 difference of 3%. The clinical relevance of ScO_2 desaturation remains to be established. However, the cerebral desaturation load in the low-trigger group was approximately fourfold larger than that observed in a trial on patients undergoing CABG surgery.[27] Such desaturation is superimposed on brain extracting more than 40% of the arterial oxygen content as reflected by the pre-anaesthesia ScO_2 of 58.2% (Table S1.1) vs approximately 66% in the CABG patient.[28] Only a limited section of brain and skull is, however, monitored and more evidence demonstrating whether ScO_2 targeted algorithms improve outcome is in need. Nonetheless, the potential to reduce cerebral ischaemic damage in surgery was disclosed by the NeuroVISION study,[29] which established a 7% incidence of covert stroke following non-cardiac surgery. The exploratory outcomes in the TV trial were indicative of increased hazard of complications and death with the low-trigger strategy (hazard ratio, 3.20; $P = .006$)[1] compared with the high-trigger. Although it is tempting to interpret that as a result of impaired tissue oxygenation, the exploratory outcome finding is not confirmative, and the risk of a chance finding is high given the small sample size. Trials on RBC transfusion triggers could include post-operative cognitive impairment, or covert stroke[29] to test whether mitigating ScO_2 desaturation with liberal RBC transfusion translates into clinical benefit.

The limitations of this study include the sample size and concerns for precision of CO-monitoring. Un-calibrated pulse contour analysis precision for CO measurements is sensitive to changes in systemic vascular resistance[30] and the arteries are clamped during vascular surgery. Lack of precision may increase the risk of both type-I and type-II errors. Strictly, we cannot reject the null hypothesis, or even that CO was lower in the low-trigger group. However, taken together the consistent CO separation throughout surgery, the similar DO_2, and the physiologic rationale for an increase in CO following haemodilution, our overall conclusion remains, that the lower ScO_2 saturation in the low-trigger group was mainly an effect of lowered Hb.

In summary, this post-hoc analysis established that for patients allocated to the low-trigger group CO reached higher values at the nadir ScO_2-reading although the mean CO reached only a numerically higher value. Thus, in the low-trigger group ScO_2 desaturation occurred despite that global DO_2 was preserved at levels obtained with the high-trigger, which suggests a regional attenuation of brain DO_2 secondary to haemodilution. We believe the next step is further randomized clinical trials in vascular surgical patients with power to detect differences in patient important outcomes. Recommendations for RBC transfusion during vascular surgery should await such trials and until then, the decision to transfuse be based on individual considerations; preferably in collaboration between the anaesthetist and the surgeon responsible for the patient.

CONFLICTS OF INTEREST

All authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

A. Møller is the principal investigator and initiator of the TV Trial, the main author of the protocol, the detailed statistical analysis plan and this manuscript. Major contribution to the acquisition, analysis and interpretation of data. HBN provided major contribution to the protocol design, interpretation of the data and drafting of the main manuscript. JW Major methodological contribution to protocol design, the detailed statistical analysis plan, interpretation of the data, drafting of the main manuscript. OBP: Co-author of the protocol and contributed to data acquisition and interpretation of the data. DH: Co-author of the protocol. Investigator with substantial contributions to data acquisition and interpretation of the data. BGUR, A. Mortensen and KVM: Investigators with substantial contributions to data acquisition. SS: Trial sponsor. Contributed with participant recruitment and data acquisition. NHS proposed the hypothesis for this post-hoc analysis and contributed with interpretation of the data. Drs. AM, DH and SS are guarantors of the trial conduct. All authors provided critical revision of the article and gave approval of the final submitted version.

The trial group means are presented as grand means (standard deviation, sd). MD, modelled mean difference. CI, confidence interval. The vertical line indicates zero MD. CO data were missing from two patients in each group (the first three patients were randomized before we had the option to download the CO-data from the EV1000 platform and one patient had missing CO-data because the EV1000 power supply short-circuited as iv-fluid entered by accident and the data were lost).

DO_2 was calculated based on Hb bound O_2 alone. Results from DO_2 based on Hb bound and plasma dissolved O_2 is in the results section. DO_2 at skin closure could not be calculated because an arterial blood gas was not consequently obtained at skin closure. Conversely, recovery room DO_2 could not be calculated because CO was not recorded in the recovery room. Thus, last DO_2 obtained during surgery is presented. Recovery room Hb, and nadir ScO_2 and SmO_2 have been reported previously[1] but are also presented in this table for comparison.

Abbreviations: CaO_2, Arterial oxygen content; CO, cardiac output; DO_2, oxygen delivery; MAP, mean arterial pressure; ScO_2, frontal lobe tissue oxygenation; SmO_2, biceps muscle oxygenation.

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REFERENCES

1. Moller A, Nielsen HB, Wetterslev J, et al. Low vs high hemoglobin trigger for transfusion in vascular surgery: a randomized clinical feasibility trial. *Blood*. 2019;133:2639-2650.
2. Feringa HH, Karagiannis SE, Vidakovic R, et al. The prevalence and prognosis of unrecognized myocardial infarction and silent myocardial ischemia in patients undergoing major vascular surgery. *Coron Artery Dis*. 2007;18:571-576.
3. Hertzer NR, Beven EG, Young JR, et al. Coronary artery disease in peripheral vascular patients. A classification of 1000 coronary angiograms and results of surgical management. *Ann Surg*. 1984;199:223-233.
4. Cahan MA, Killewich LA, Kolodner L, et al. The prevalence of carotid artery stenosis in patients undergoing aortic reconstruction. *Am J Surg*. 1999;178:194-196.
5. Hovaguimian F, Myles PS. Restrictive versus liberal transfusion strategy in the perioperative and acute care settings: a context-specific systematic review and meta-analysis of randomized controlled trials. *Anesthesiology*. 2016;125:46-61.
6. Kougias P, Sharath S, Barshes NR, Chen M, Mills JL Sr. Effect of postoperative anemia and baseline cardiac risk on serious adverse outcomes after major vascular interventions. *J Vasc Surg*. 2017;66:1836-1843.
7. American Society of Anesthesiologists Task Force on Perioperative Blood Management. Practice guidelines for perioperative blood management: an updated report by the American Society of Anesthesiologists Task Force on Perioperative Blood Management*. *Anesthesiology*. 2015;122:241-275.
8. Kozek-Langenecker SA, Afshari A, Albaladejo P, et al. Management of severe perioperative bleeding: guidelines from the European Society of Anaesthesiology. *Eur J Anaesthesiol*. 2013;30:270-382.
9. Chaikof EL, Dalman RL, Eskandari MK, et al. The Society for Vascular Surgery practice guidelines on the care of patients with an abdominal aortic aneurysm. *J Vasc Surg*. 2018;67:2-77.e2.
10. Shah A, Stanworth SJ, Docherty AB. Restrictive blood transfusion - is less really more? *Anaesthesia*. 2020;75:433-437.
11. Chatterjee S, Wetterslev J, Sharma A, Lichstein E, Mukherjee D. Association of blood transfusion with increased mortality in myocardial infarction: a meta-analysis and diversity-adjusted study sequential analysis. *JAMA Intern Med*. 2013;173:132-139.
12. Carson JL, Terrin ML, Noveck H, et al. Liberal or restrictive transfusion in high-risk patients after hip surgery. *N Engl J Med*. 2011;365:2453-2462.
13. Mazer CD, Whitlock RP, Fergusson DA, et al.; TRICS Investigators and Perioperative Anesthesia Clinical Trials Group. Restrictive or liberal red-cell transfusion for cardiac surgery. *N Engl J Med*. 2017;377:2133-2144.
14. Moller A, Nielsen HB, Wetterslev J, Pedersen OB, Hellemann D, Shahidi S. Low vs. high haemoglobin trigger for transfusion in vascular surgery: protocol for a randomised trial. *Acta Anaesthesiol Scand*. 2017;61:952-961.
15. Gonzalez-Alonso J, Mortensen SP, Dawson EA, Secher NH, Damsgaard R. Erythrocytes and the regulation of human skeletal muscle blood flow and oxygen delivery: role of erythrocyte count and oxygenation state of haemoglobin. *J Physiol*. 2006;572:295-305.
16. Miller TE, Myles PS. Perioperative fluid therapy for major surgery. *Anesthesiology*. 2019;130:825-832.
17. Holst LB, Haase N, Wetterslev J, et al.; TRISS Trial Group; Scandinavian Critical Care Trials Group. Lower versus higher hemoglobin threshold for transfusion in septic shock. *N Engl J Med*. 2014;371:1381-1391.
18. Docherty AB, O'Donnell R, Brunskill S, et al. Effect of restrictive versus liberal transfusion strategies on outcomes in patients with cardiovascular disease in a non-cardiac surgery setting: systematic review and meta-analysis. *BMJ*. 2016;352:i1351.
19. Wu WC, Smith TS, Henderson WG, et al. Operative blood loss, blood transfusion, and 30-day mortality in older patients after major noncardiac surgery. *Ann Surg*. 2010;252:11-17.
20. Bush RL, Pevec WC, Holcroft JW. A prospective, randomized trial limiting perioperative red blood cell transfusions in vascular patients. *Am J Surg*. 1997;174:143-148.
21. Palmieri TL, Holmes JHT, Arnoldo B, et al. Transfusion requirement in burn care evaluation (TRIBE): a multicenter randomized prospective trial of blood transfusion in major burn injury. *Ann Surg*. 2017;266:595-602.

22. Hoiland RL, Bain AR, Rieger MG, Bailey DM, Ainslie PN. Hypoxemia, oxygen content, and the regulation of cerebral blood flow. *Am J Physiol Regul Integr Comp Physiol*. 2016;310:R398-R413.
23. Kei T, Mistry N, Tsui AKY, et al. Experimental assessment of oxygen homeostasis during acute hemodilution: the integrated role of hemoglobin concentration and blood pressure. *Intensive Care Med Exp*. 2017;5:12.
24. Vlisides PE, Kunkler B, Thompson A, et al. Cerebrovascular disease and perioperative neurologic vulnerability: a prospective cohort study. *Front Neurol*. 2019;10:560.
25. Olesen ND, Frederiksen HJ, Storkholm JH, et al. Internal carotid artery blood flow is enhanced by elevating blood pressure during combined propofol-remifentanil and thoracic epidural anaesthesia: a randomised cross-over trial. *Eur J Anaesthesiol*. 2020;37:482-490.
26. Oshima T, Karasawa F, Satoh T. Effects of propofol on cerebral blood flow and the metabolic rate of oxygen in humans. *Acta Anaesthesiol Scand*. 2002;46:831-835.
27. Holmgaard F, Vedel AG, Lange T, Nilsson JC, Ravn HB. Impact of 2 distinct levels of mean arterial pressure on near-infrared spectroscopy during cardiac surgery: secondary outcome from a randomized clinical trial. *Anesth Analg*. 2019;128:1081-1088.
28. Chan MJ, Chung T, Glassford NJ, Bellomo R. Near-infrared spectroscopy in adult cardiac surgery patients: a systematic review and meta-analysis. *J Cardiothorac Vasc Anesth*. 2017;31:1155-1165.
29. Neuro VI. Perioperative covert stroke in patients undergoing non-cardiac surgery (NeuroVISION): a prospective cohort study. *Lancet*. 2019;394:1022-1029.
30. Grensemann J. Cardiac output monitoring by pulse contour analysis, the technical basics of less-invasive techniques. *Front Med (Lausanne)*. 2018;5:64.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Supplementary Digital Content
Trial results

This supplementary material has been provided by the authors to give readers additional information about the results of the post-hoc analysis.

Supplement to:
Effect of low vs. high haemoglobin transfusion trigger on cardiac output in patients undergoing elective vascular surgery: post-hoc analysis of a randomised trial.

Table of content

Supplemental tables

Table S1. Baseline readings

Table S1.1. Baseline readings of circulatory variables from the EV1000 platform

Variable	Trigger<8 (n=22)	Trigger<9.7 (n=24)	Total	P
EV1000 data available, n (%)	20 (91)	22 (92)	42 (91.30)	1.000
Baseline cardiac output (L/min) mean (sd)	4.80 (1.19)	4.54 (1.28)	4.66 (1.23)	0.490
Baseline cardiac index (L/min /m^2), mean (sd)	2.61 (0.66)	2.54 (0.76)	2.57 (0.71)	0.760
Baseline heart rate (min^{-1}), mean (sd)	67.22 (11.67)	59.30 (11.07)	63.07 (11.92)	0.024
Baseline mean arterial pressure (mmHg), mean (sd)	65.35 (14.07)	61.31 (10.41)	63.23 (12.31)	0.287

Table S1.2. NIRS at baseline and before O_2-supplementation ahead of anaesthesia induction

Variable	Level	Trigger<8 g/dl (n=22)	Trigger<9.7 g/dl (n=24)	Total (n=46)	P
Baseline ScO_2 (normovolaemia)	mean (sd) median [iqr]	60.8 (9.6) 64.0 [56.6, 65.3]	59.5 (9.1) 59.1 [53.7, 65.4]	60.1 (9.3) 60.3 [54.1, 65.4]	0.637
Baseline SmO_2 (normovolaemia)	mean (sd) median [iqr]	69.8 (8.9) 71.2 [67.2, 74.8]	68.2 (9.8) 69.5 [63.1, 74.6]	69.0 (9.3) 70.0 [63.8, 74.8]	0.560
Awake ScO_2 (no O_2 suppl.)	mean (sd) median [iqr] missing	57.4 (10.8) 59.0 [47.9, 61.4] 1	58.9 (8.9) 58.7 [51.3, 63.7] 1	58.2 (9.8) 58.7 [50.3, 62.1] 1	0.596
Awake SmO_2 (no O_2 suppl.)	mean (sd) median [iqr]	67.2 (11.9) 71.7 [60.1, 74.8]	66.2 (11.5) 66.8 [60.3, 73.8]	66.6 (11.6) 70.5 [60.0, 74.8]	0.775

Baseline was defined as time point where the patient stroke volume was unresponsive to further fluid administration (normovolaemia) after inducing anaesthesia and before start of surgery.
The awake reading is provided for comparison, as this is a more commonly used baseline in other trials and studies.

Table S2. Detailed table of fluid balance and surgery specifics

(her er det VanElterens test hvis qq-plot indiceret ikke-normalfordelte data. I tabel 2 i main doc er alle data præsenteret som median [IQR] og sammenlignet med VanElteren's test)

Variable	Level	Trigger<8 (n=22)	Trigger<9.7 (n=24)	Total (n=46)	p-value
FLUID INPUT (ml)					
Crystalloids	mean (sd) median [iqr]	2617 (1046) 2300 [1800, 3500]	2965 (974) 2900 [2075, 3704]	2798 (1013) 2600 [1863, 3575]	0.220
Medicines	mean (sd) median [iqr]	63 (170) 0 [0, 0]	34 (74) 0 [0, 0]	48 (129) 0 [0, 0]	0.890
Human albumin 5 %	mean (sd) median [iqr]	546 (625) 500 [0, 875]	458 (482) 250 [0, 1000]	500 (550) 375 [0, 1000]	0.186
Red blood cells	mean (sd) median [iqr]	232 (33) 0 [0, 300]	588 (380) 450 [300, 675]	417 (397) 300 [75, 600]	<0.001
Fresh frozen plasma ≥ 1 units, n (%)	≥1 units (%)	1 (4)	0 (0)	1 (2)	NA
Platelets ≥ 1 units, n (%)	≥1 units (%)	0 (0)	0 (0)	0 (0)	NA
TOTAL	mean (sd) median [iqr]	3475 (1903) 3000 [2100, 4378]	4010 (1396) 4025 [2700, 5238]	3754 (1661) 3715 [2250, 5054]	0.300
FLUID LOSS (ml)					
Blood loss	mean (sd) median [iqr]	1267 (1321) 898 [403, 1695]	1531 (1064) 1650 [590, 2160]	1405 (1188) 1175 [475, 2039]	0.714
Urine output	mean (sd) median [iqr]	648 (470) 468 [288, 869]	793 (548) 648 [394, 964]	724 (511) 555 [363, 946]	0.193
TOTAL	mean (sd) median [iqr]	1915 (1337) 1528 [936, 2436]	2324 (1249) 2260 [1321, 3108]	2128 (1294) 2050 [1002, 2892]	0.366
FLUID BALANCE (ml)					
FLUID BALANCE	mean (sd) median [iqr]	1597 (932) 1190 [1081, 2098]	1721 (873) 1855 [1499, 2300]	1662 (894) 1593 [1131, 2298]	0.822
RELATIVE BLOOD LOSS (ml)					
Total blood volume*	mean (sd) median [iqr]	4685 (766) 4552 [4270, 5039]	4437 (684) 4416 [3944, 5057]	4556 (727) 4482 [4072, 5072]	0.251
Blood loss/Total blood volume (%)	mean (sd) median [iqr]	26 (23) 20 [7, 38]	35 (25) 34 [15, 51]	31 (24) 24 [12, 44]	0.328
DURATIONS (minutes)					

Anaesthesia	mean (sd) median [iqr]	255 (88) 238 [189, 282]	247 (58) 252 [207, 277]	250 (73) 244 [200, 278]	0.678
Surgery	mean (sd) median [iqr]	195 (78) 174 [153, 232]	186 (61) 181 [152, 213]	190 (69) 177 [153, 218]	0.663
AAA Cross Clamp (n=8 vs n=11)	mean (sd) median [iqr]	102 (56) 89 [64, 140]	81 (23) 75 [64, 90]	90 (40) 77 [64, 110]	0.225
Start of surgery to randomisation	Mean (sd) Median [iqr]	62 (58) 63 [11, 107]	51 (61) 64 [0, 97]	56 (59) 64 [0, 99]	0.506
Baseline to randomisation	Mean (sd) Median [iqr]	75 (59) 77 [20, 124]	62 (56) 71 [0, 101]	68 (57) 71 [4, 116]	0.472
Surgery specifics					
AAA tube prosthesis	No (%)	2 (9)	8 (33)	10 (22)	
AAA bifurcated prosthesis	No (%)	6 (27)	3 (13)	9 (20)	
Infra-inguinal bypass	No (%)	7 (32)	11 (46)	18 (39)	
Femofemoral cross-over	No (%)	4 (18)	1 (4)	5 (11)	
Inguinal thromb-endarterectomy	No (%)	2 (9)	1 (4)	3 (7)	
Inoperabel	No (%)	1 (5)	0 (0)	1 (2)	0.129

* TBV, Total blood volume was calculated with Naddler's equation based on weight, height and sex. AAA, abdominal aortic aneurysm.

Table S3. Data from the EV1000 platform: CO, CI, SV, MAP and HR

See next page.

T0, baseline. T2, skin closure. Nadir is the reading at nadir frontal lobe oxygenation (ScO_2)

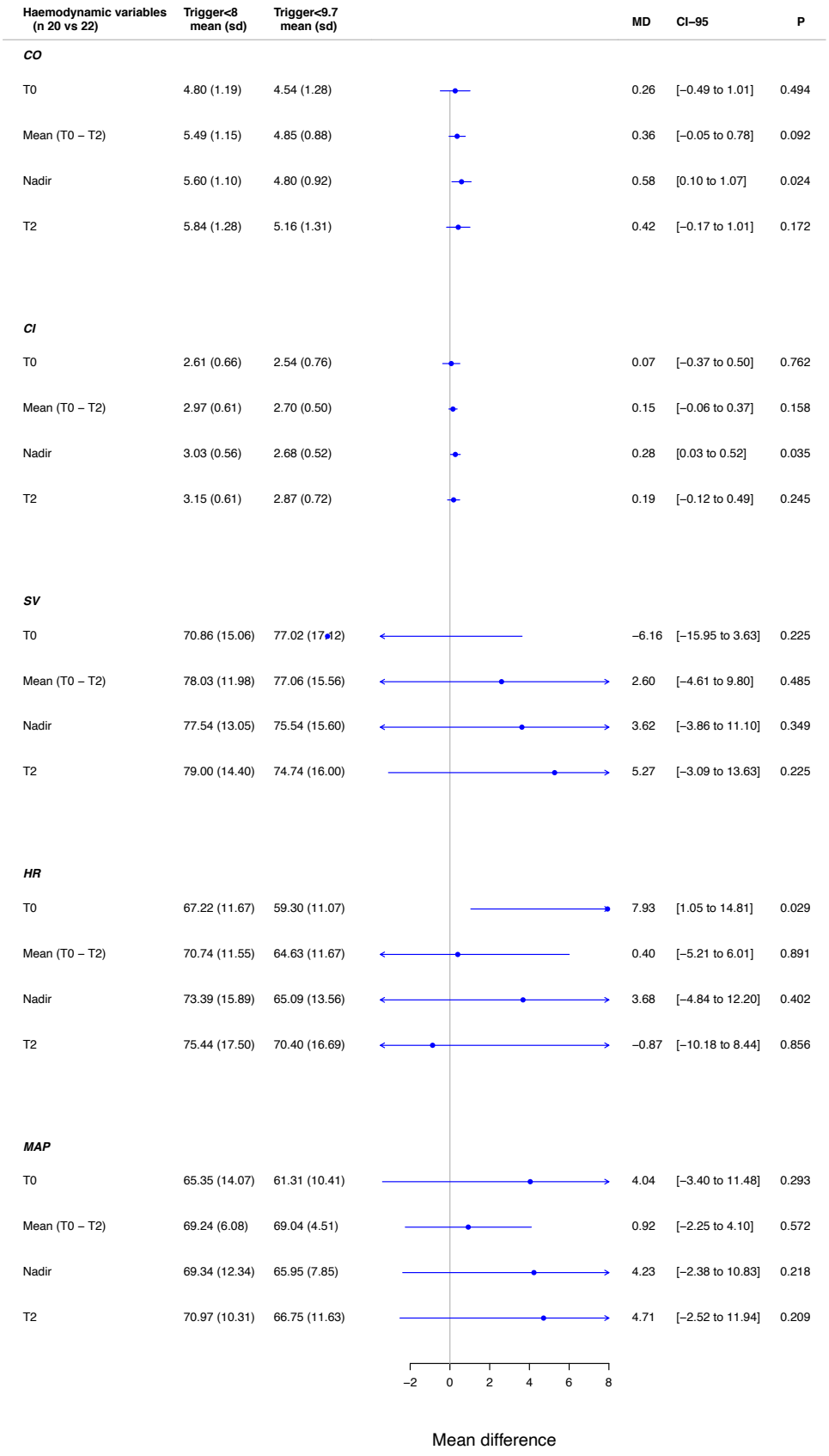

Haemodynamic variables (n 20 vs 22)
Trigger<8 mean (sd)
Trigger<9.7 mean (sd)
MD
CI–95
P
CO
T0 4.80 (1.19) 4.54 (1.28) 0.26 [–0.49 to 1.01] 0.494
Mean (T0 – T2) 5.49 (1.15) 4.85 (0.88) 0.36 [–0.05 to 0.78] 0.092
Nadir 5.60 (1.10) 4.80 (0.92) 0.58 [0.10 to 1.07] 0.024
T2 5.84 (1.28) 5.16 (1.31) 0.42 [–0.17 to 1.01] 0.172
CI
T0 2.61 (0.66) 2.54 (0.76) 0.07 [–0.37 to 0.50] 0.762
Mean (T0 – T2) 2.97 (0.61) 2.70 (0.50) 0.15 [–0.06 to 0.37] 0.158
Nadir 3.03 (0.56) 2.68 (0.52) 0.28 [0.03 to 0.52] 0.035
T2 3.15 (0.61) 2.87 (0.72) 0.19 [–0.12 to 0.49] 0.245
SV
T0 70.86 (15.06) 77.02 (17.12) –6.16 [–15.95 to 3.63] 0.225
Mean (T0 – T2) 78.03 (11.98) 77.06 (15.56) 2.60 [–4.61 to 9.80] 0.485
Nadir 77.54 (13.05) 75.54 (15.60) 3.62 [–3.86 to 11.10] 0.349
T2 79.00 (14.40) 74.74 (16.00) 5.27 [–3.09 to 13.63] 0.225
HR
T0 67.22 (11.67) 59.30 (11.07) 7.93 [1.05 to 14.81] 0.029
Mean (T0 – T2) 70.74 (11.55) 64.63 (11.67) 0.40 [–5.21 to 6.01] 0.891
Nadir 73.39 (15.89) 65.09 (13.56) 3.68 [–4.84 to 12.20] 0.402
T2 75.44 (17.50) 70.40 (16.69) –0.87 [–10.18 to 8.44] 0.856
MAP
T0 65.35 (14.07) 61.31 (10.41) 4.04 [–3.40 to 11.48] 0.293
Mean (T0 – T2) 69.24 (6.08) 69.04 (4.51) 0.92 [–2.25 to 4.10] 0.572
Nadir 69.34 (12.34) 65.95 (7.85) 4.23 [–2.38 to 10.83] 0.218
T2 70.97 (10.31) 66.75 (11.63) 4.71 [–2.52 to 11.94] 0.209
–2
0
2
4
6
8
Mean difference

Table S4. Noradrenaline doses

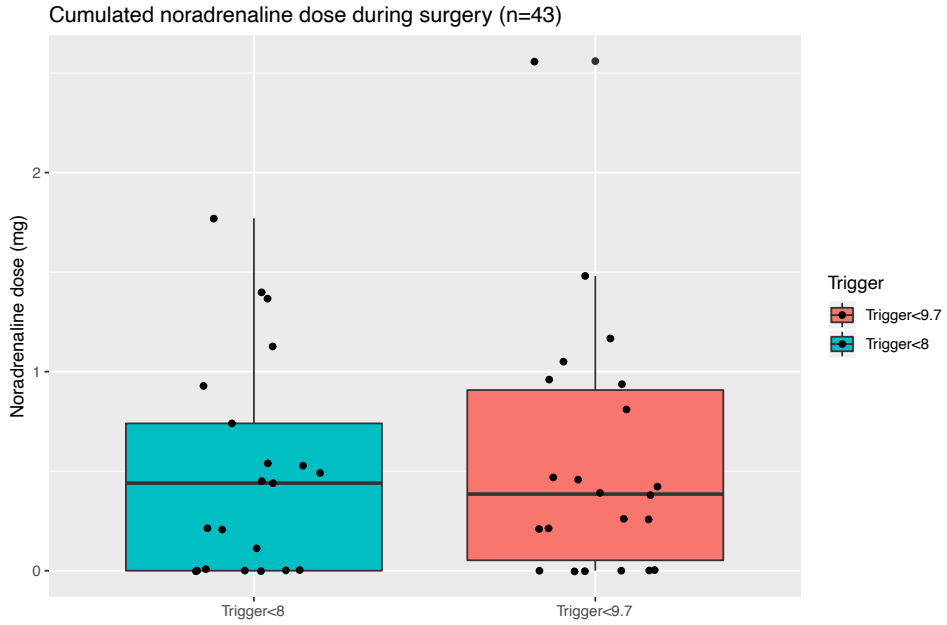
Variable	Level	Trigger<8 (n=22)	Trigger<9.7 (n=24)	Total (n=46)	p-value
Cumulated noradrenaline dose during surgery	median [iqr]	0.44 [0.00, 0.74]	0.39 [0.05, 0.91]	0.39 [0.00, 0.87]	0.840
	missing	1	2	3	
Duration of noradrenaline infusion above 0.1 ug/kg/min (minutes)	median [iqr]	0 [0, 3]	0 [0, 10]	0 [0, 6]	0.602
	missing	1	2	3	
Intraoperative noradrenaline	Yes	16 (73)	19 (79)	35 (76)	0.869
Postoperative noradrenaline, n (%)	Yes	4 (18)	3 (13)	7 (15)	0.901

Both noradrenaline dose and the duration of infusion above 0.1 were analysed with van Elteren's test.

One patient in the low-trigger group deviated from the protocol and received phenylephrine infusion instead of noradrenaline. Thus, 17 patients in the low-trigger and 19 patients in the high-trigger received some form of vasopressor. Conversely, six patients in both trial groups did not receive any vasopressor.

Reason for missing data: in three patients only the administration of noradrenaline, but not the infusion rate, was registered in the electronic anaesthesia file and the cumulated dose could therefore not be calculated.

Plot of cumulated noradrenaline dose during surgery



Plot of duration of noradrenaline-infusion rate above 0.1 µg/kg/min during surgery

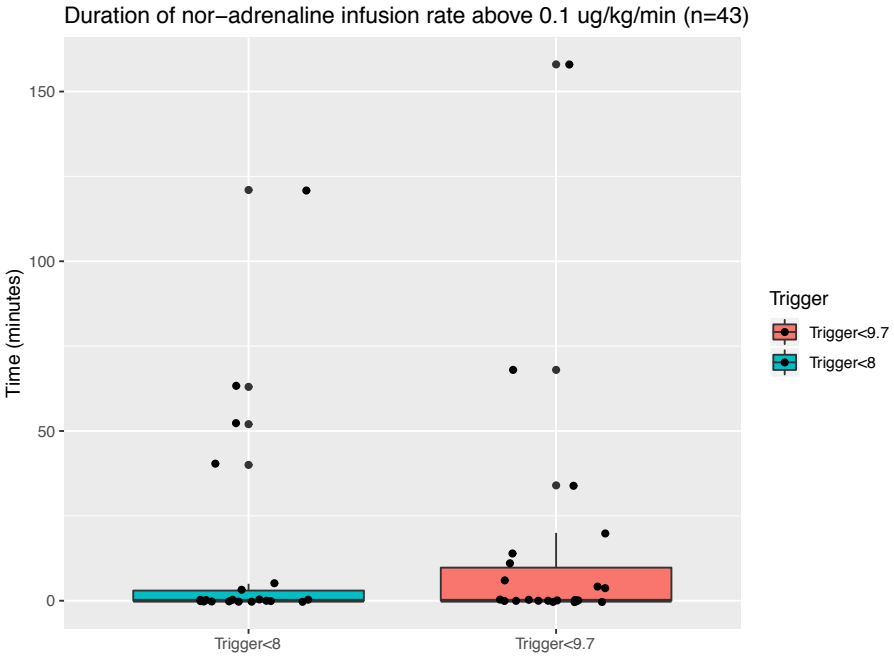


Table S5. Anaesthesia doses during surgery

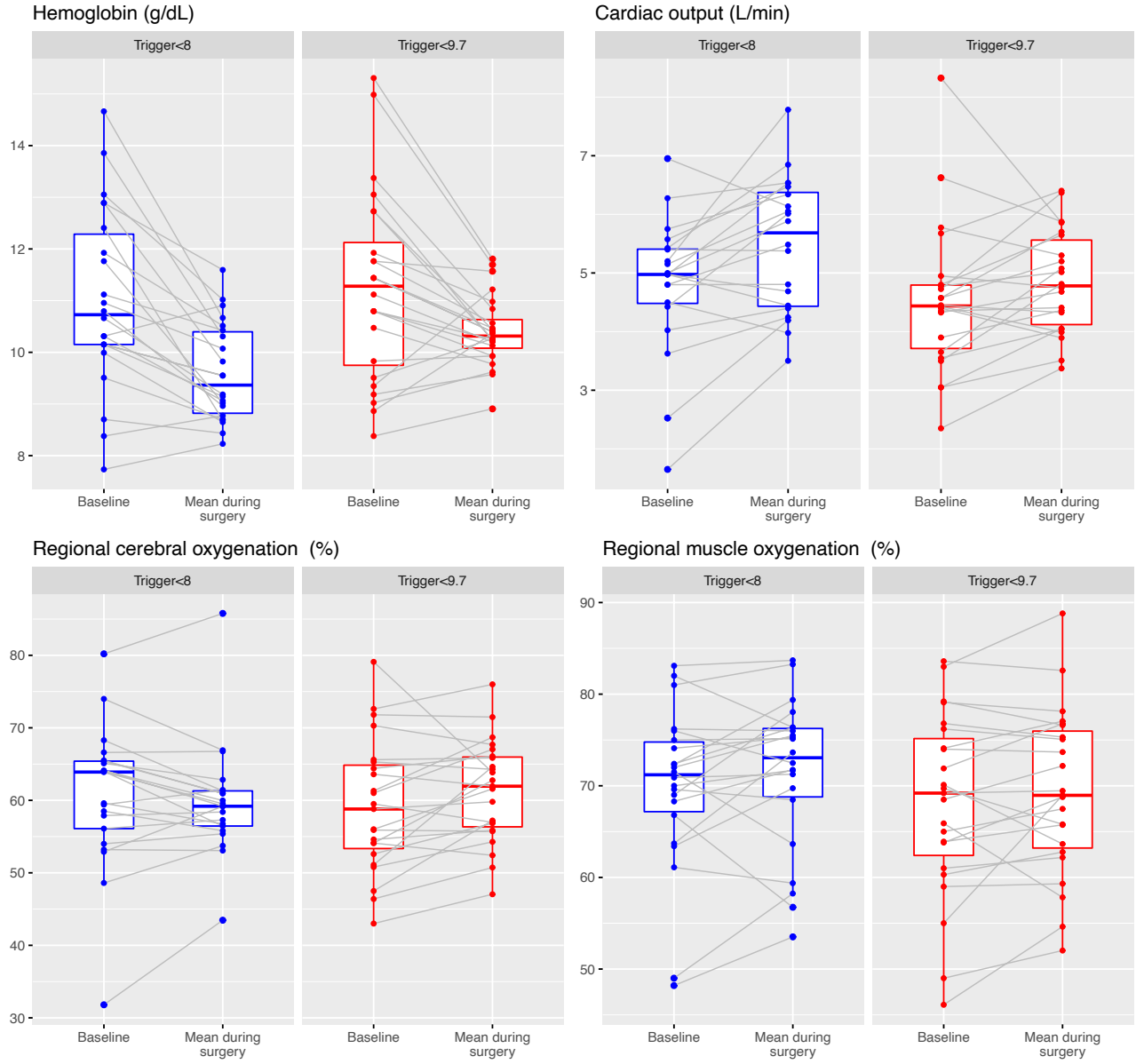
	Trigger<8 (n=22)	Trigger<9.7 (n=24)	Total (n=46)	p unadj.
Total epidural bupivacaine bolus dose (mg)	14.0 (21.5)	9.5 (15.4)	11.7 (18.5)	0.417
Bupivacaine 5mg/ml (ml)	2.3 (4.1)	1.3 (2.7)	1.8 (3.5)	0.336
Bupivacaine 2.5mg/ml (ml)	1.0 (2.5)	1.1 (2.8)	1.1 (2.6)	0.806
Epidural infusion during surgery, n (%)	9 (41)	9 (38)	18 (39.1)	1.000
Calcium chloride (mg)	2.5 (4.0)	2.3 (2.5)	2.4 (3.3)	0.832
Fentanyl dose (mg)	0.49 (0.18)	0.51 (0.34)	0.50 (0.27)	0.812

Data are presented as mean (standard deviation) and number (%).

Supplemental Figures

Individual plots "Spaghettiograms" of baseline vs. mean reading during anaesthesia with superimposed boxplot.

Merged in one figure: Hb, CO, ScO_2 and SmO_2



See fig.S1.5 for DO_2 plot.

Figure S.1.1. Haemoglobin (g/dL) at baseline and mean level during surgery

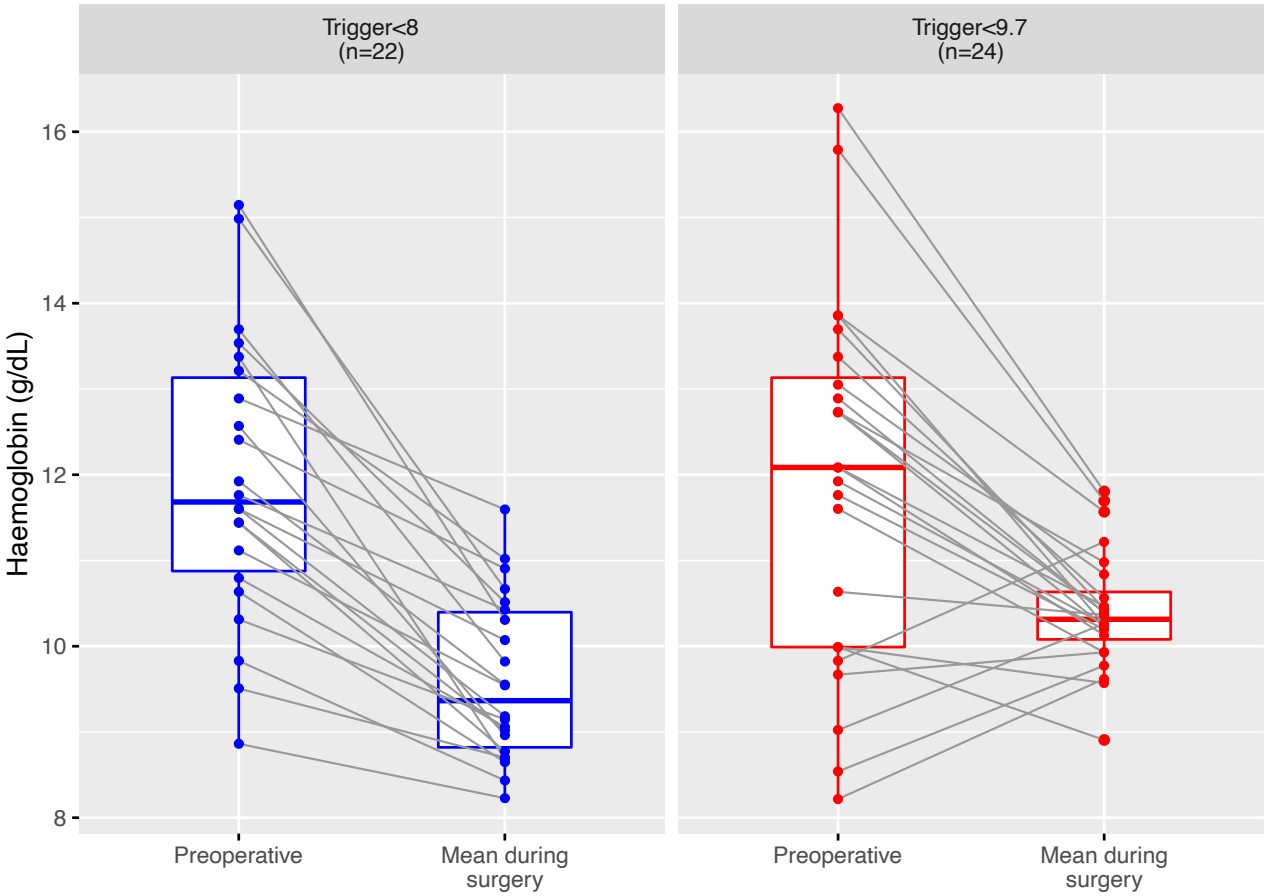


Figure S.1.2. Cardiac output (L/min) at baseline and mean level during surgery

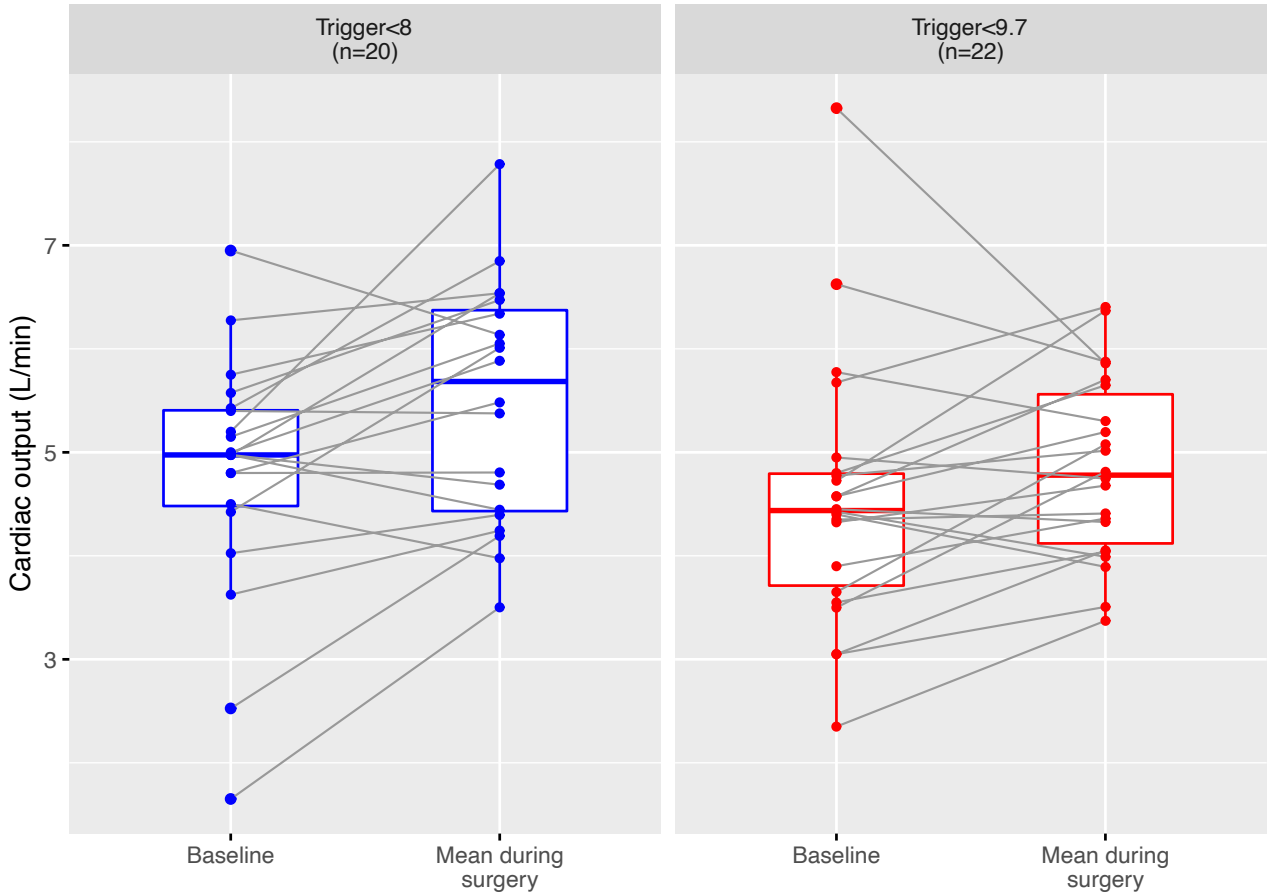


Figure S.1.3. Regional cerebral oxygen saturation (ScO_2) at baseline and mean level during surgery

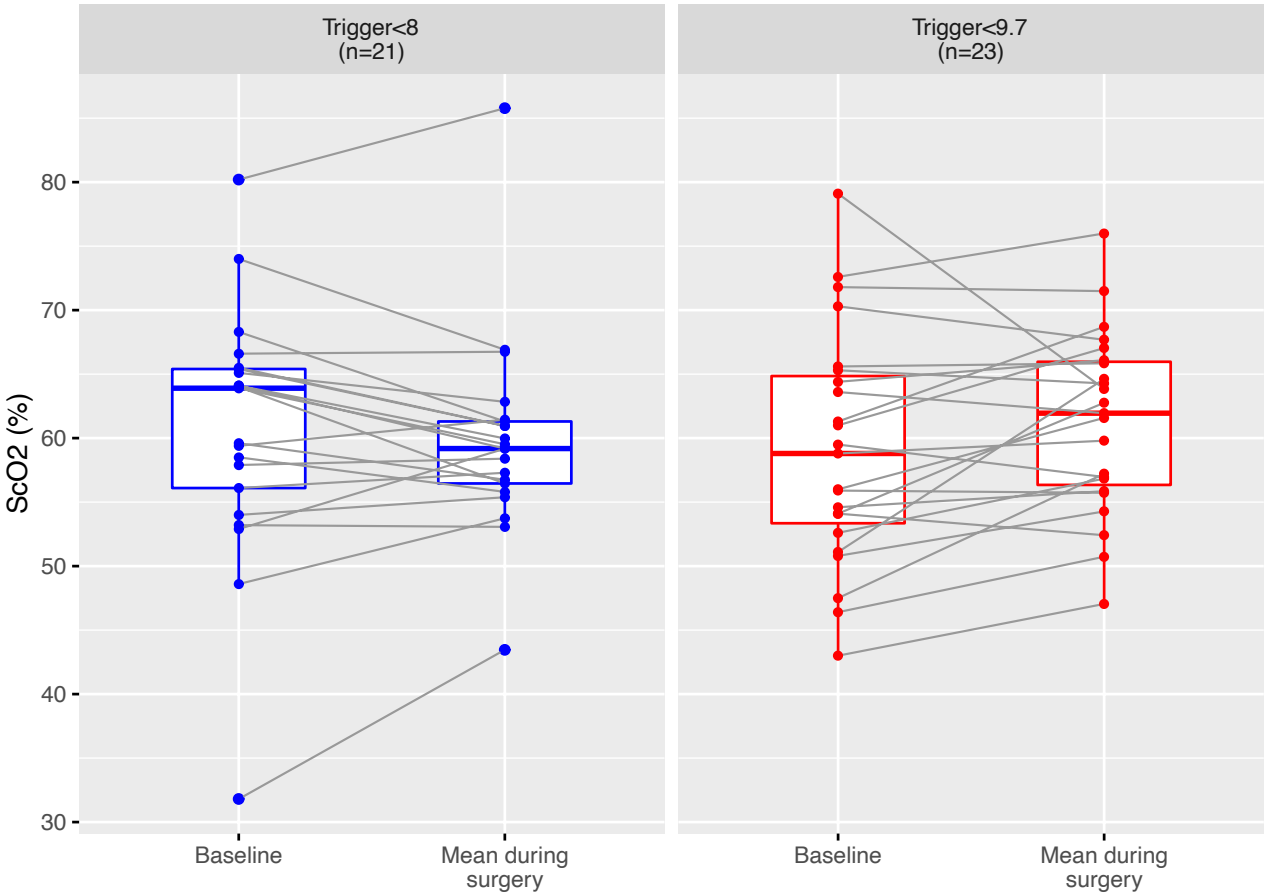


Figure S.1.4. Regional biceps muscle oxygen saturation (SmO_2) at baseline and mean level during surgery

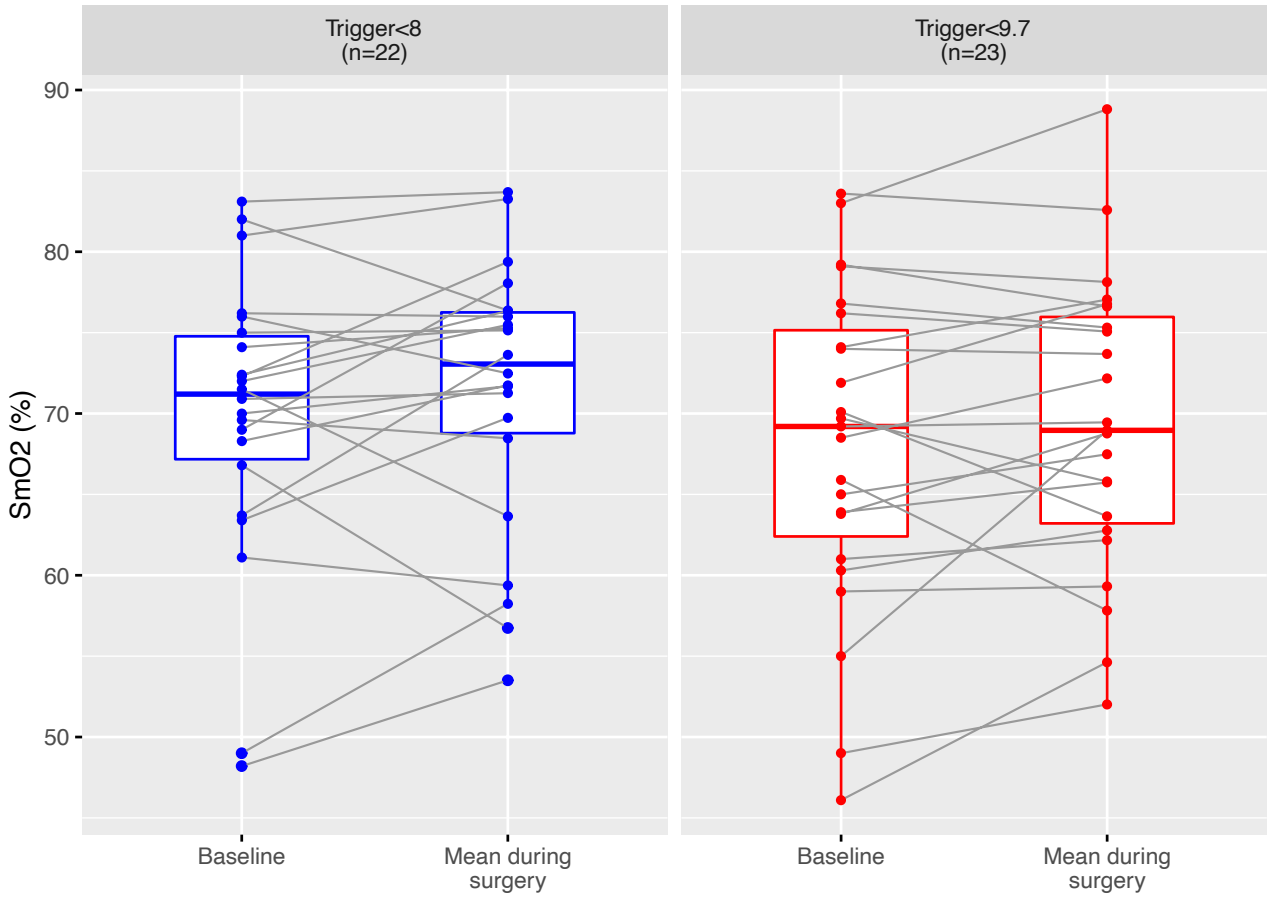


Figure S.1.5. Oxygen delivery (Hb bound O_2) at baseline and at the mean level during surgery, stratified by type of surgery

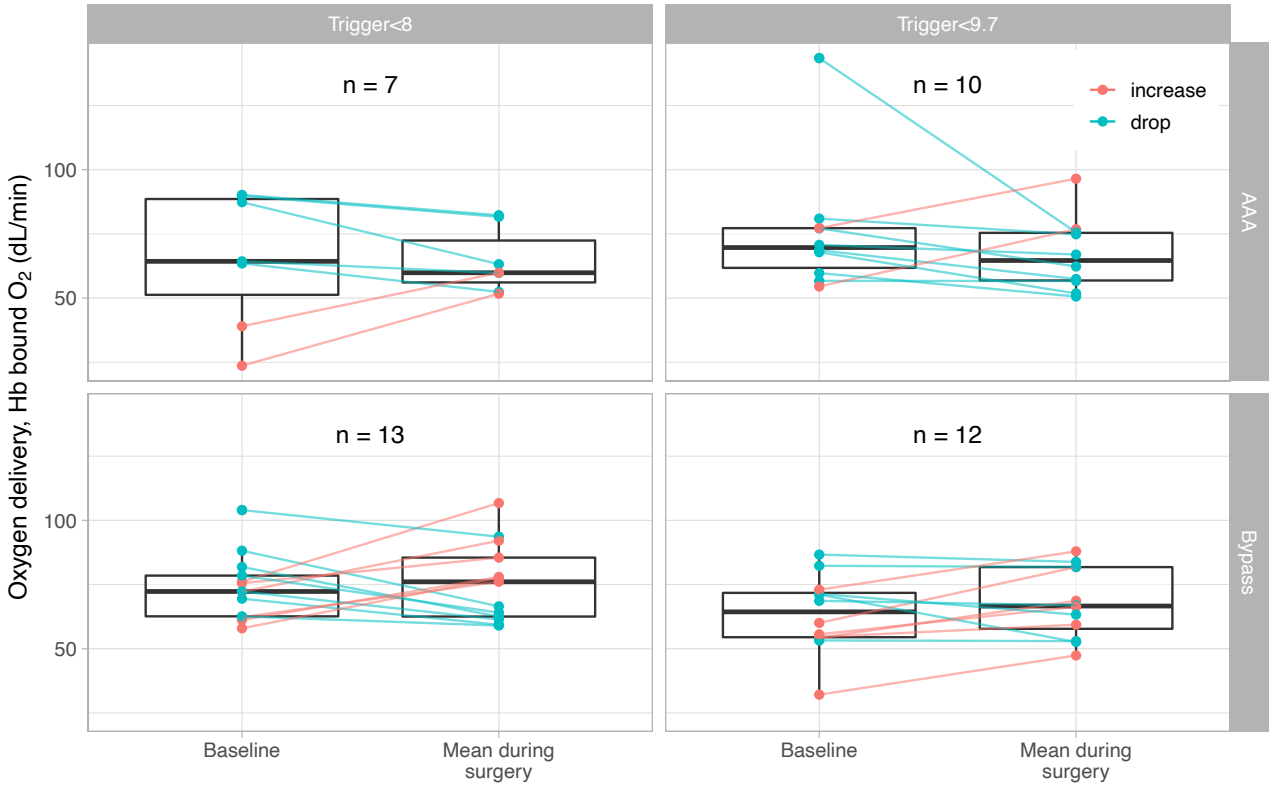


Figure S.1.6. Longitudinal individual haemoglobin plot stratified for type of surgery

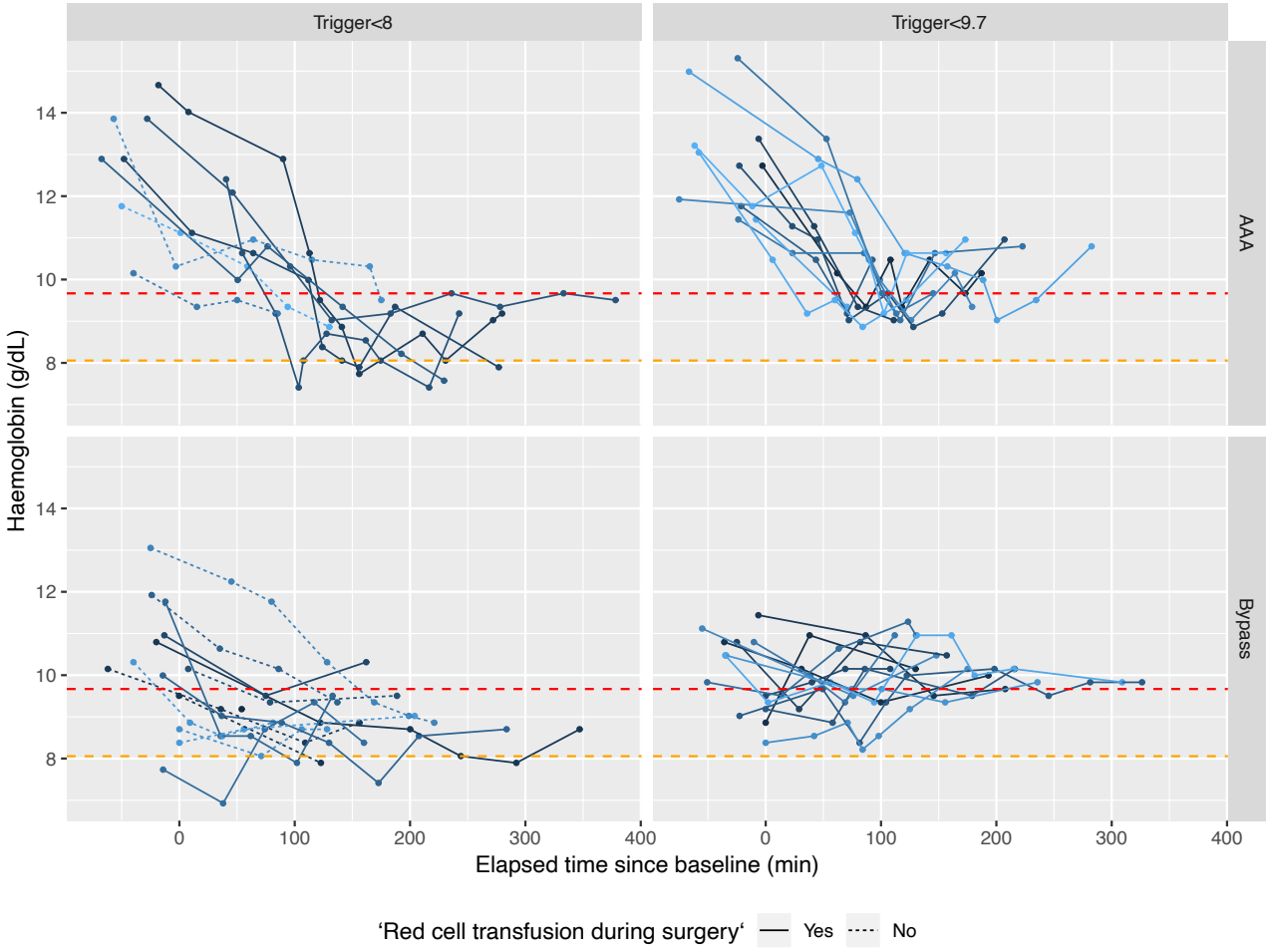
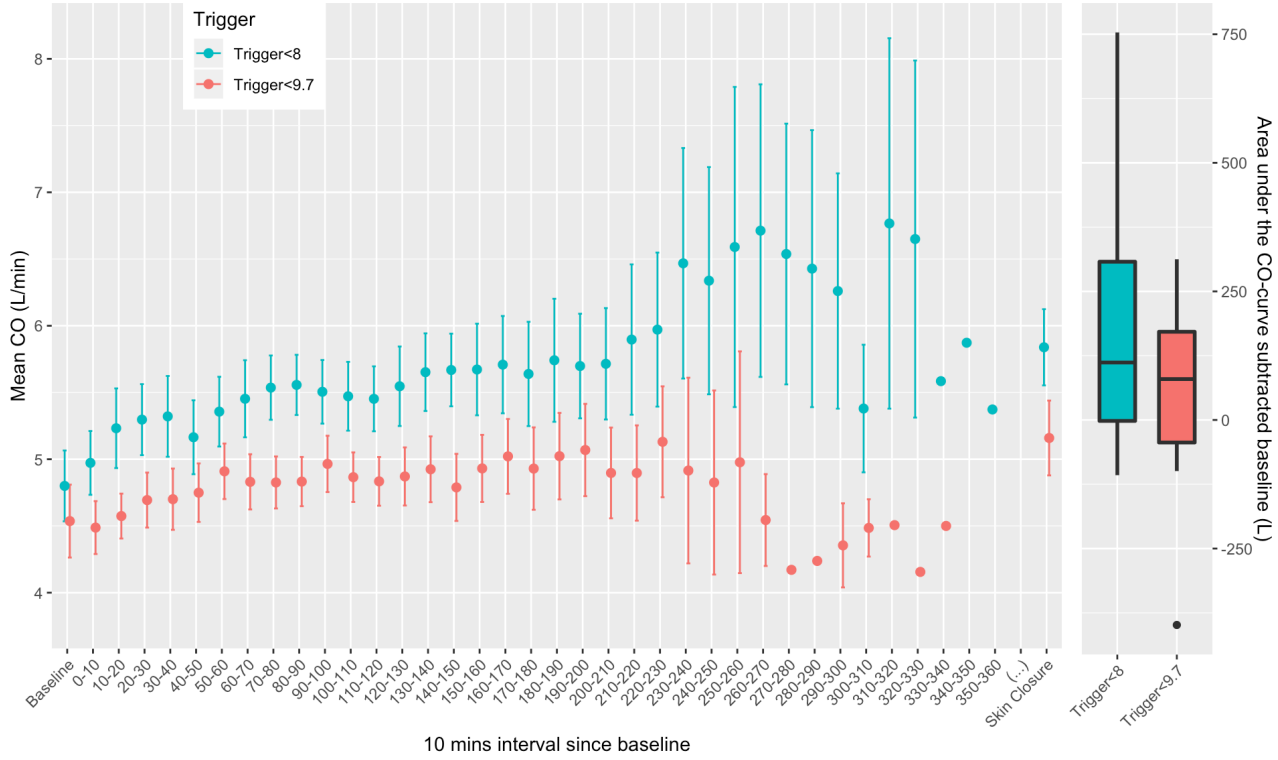


Figure S2. Longitudinal plot of mean cardiac output in the two trial groups from baseline to end of surgery – full duration of monitoring plotted.



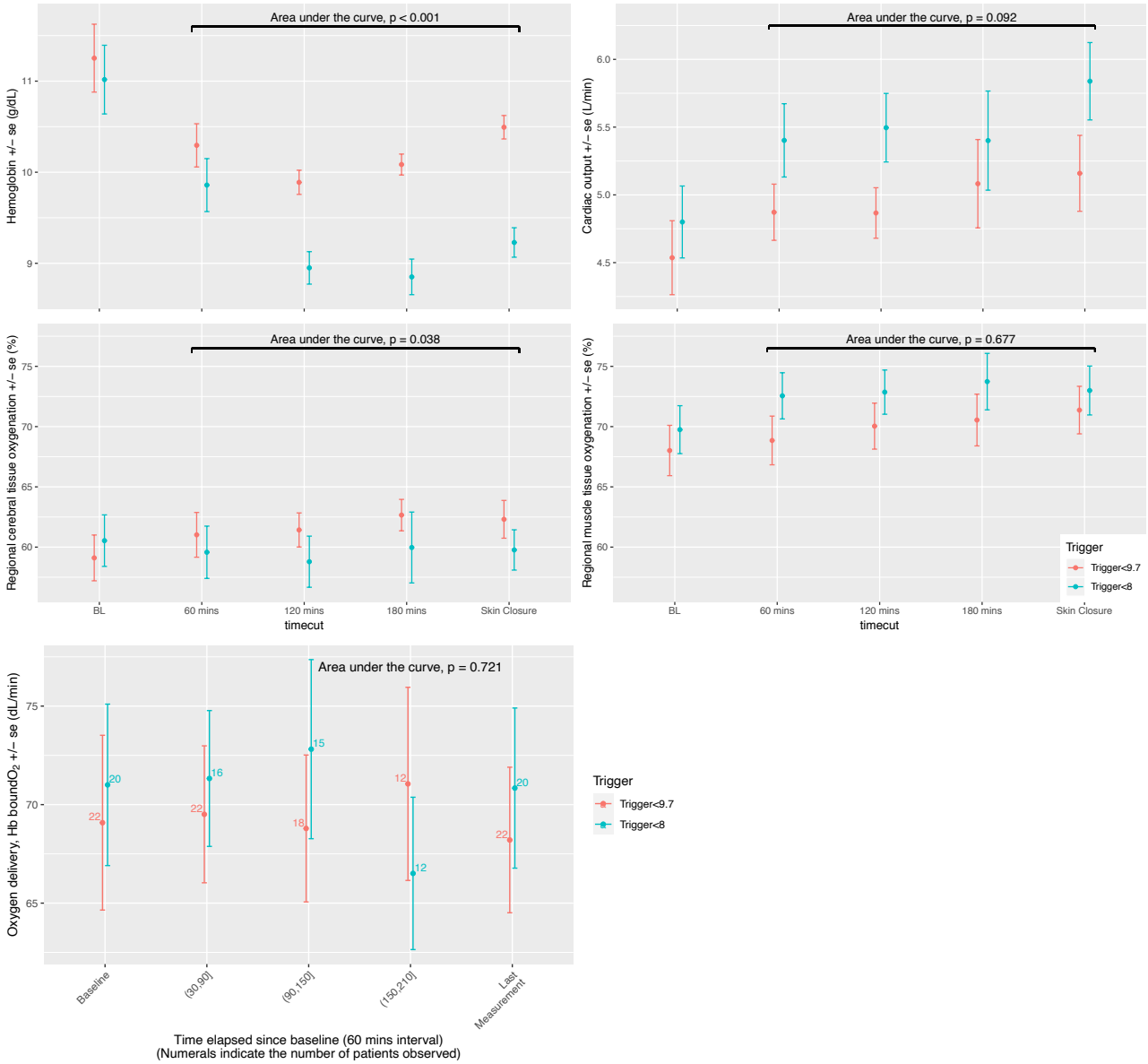
Number of patients with available cardiac output-data in each 10-minute interval:

	BL	0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280	290	300	310	320	330	340	350	skin_cl
Trigger<a	20	20	20	20	20	20	20	20	20	20	20	20	19	17	16	16	15	15	14	14	10	9	8	7	5	5	4	4	4	4	4	3	2	2	1	1	1	20
Trigger<b	22	22	22	22	21	22	22	22	22	22	22	22	22	20	19	18	18	17	15	13	12	10	9	7	4	4	4	3	2	2	2	2	1	1	1	0	0	22

“trigger a” corresponds to “trigger 8” and “trigger b” corresponds to “trigger 9.7”

Baseline was defined as the time point when the patient’s stroke volume was unresponsive to further fluid administration, i.e. was considered normovolaemic ahead of surgery.

Figure S3. Absolute change in mean Hb, CO, ScO_2, SmO_2 and DO_2 at baseline, at 1, 2 and 3 hours after baseline, and at skin closure.



In the Hb panel, recovery room Hb and not skin closure reading is plottet.
In the DO_2 panel, last measurement and not skin closure reading is plottet.

Plot stratified by type of surgery

Figures S4. Cardiac output relative to baseline stratified by type of surgery

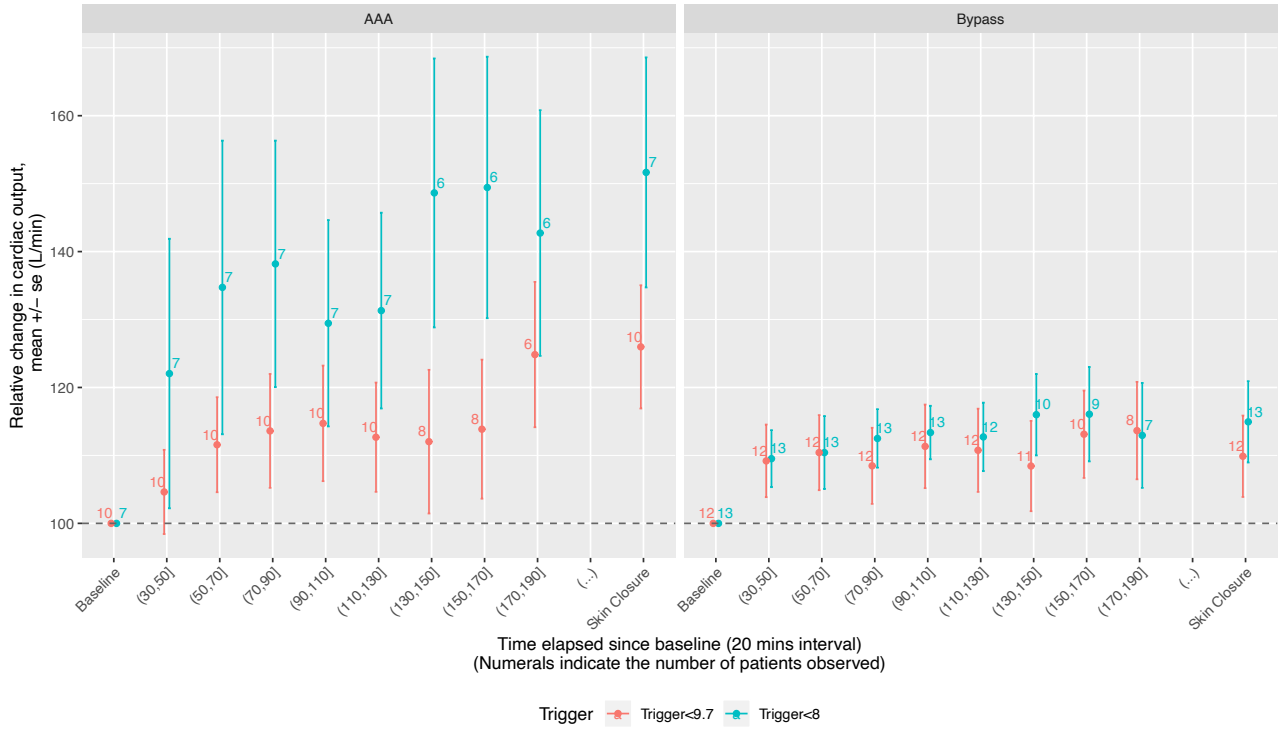


Figure S4.1. Individual changes in cardiac output from baseline (T0) to the mean value (T0-T2) by trigger (paired t-test)

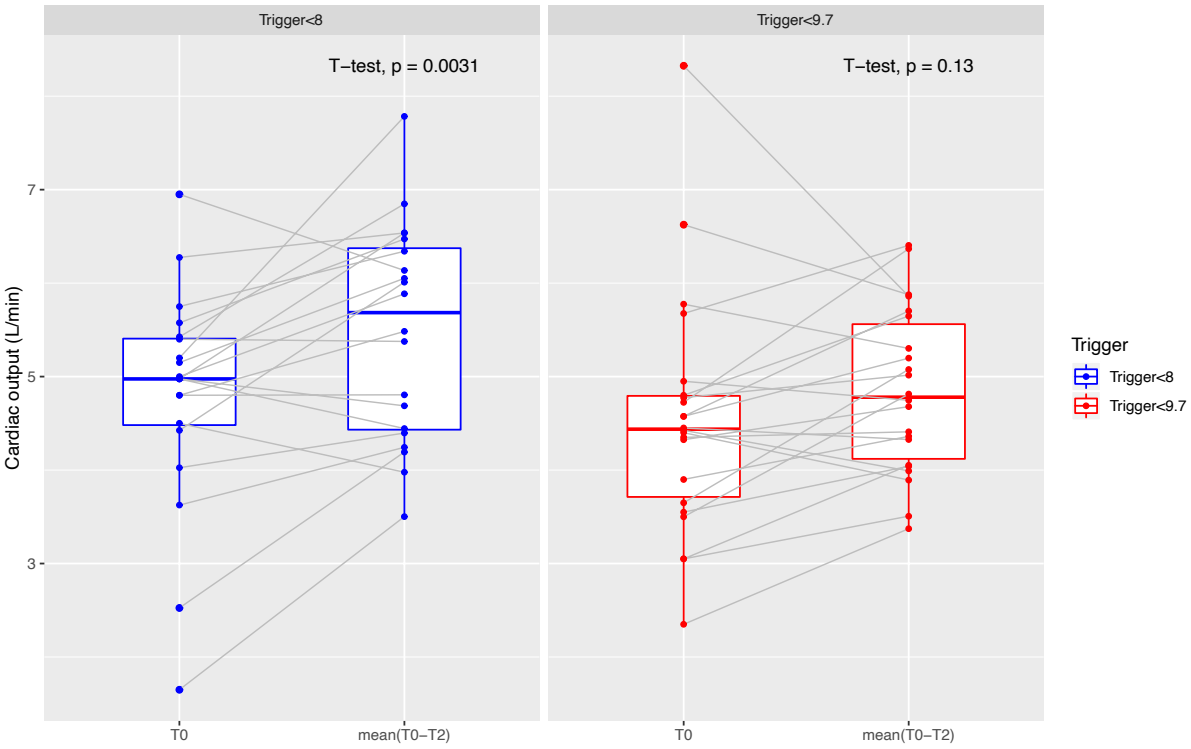
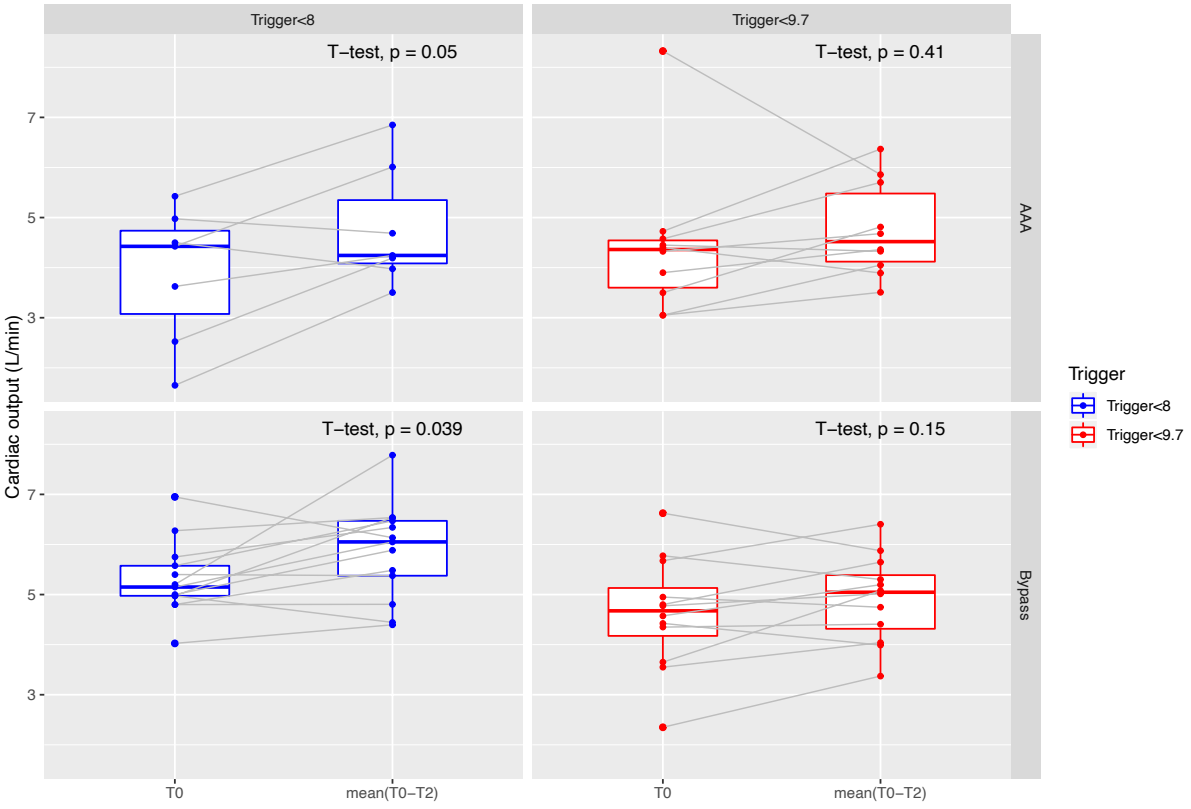


Figure S4.2. Cardiac output at baseline (T0) and at the mean value (T0-T2) by trigger and operation type (paired t-test)



Figures S5. Near infrared spectroscopy determined cerebral- and biceps muscle tissue oxygenation relative to baseline, stratified for type of surgery

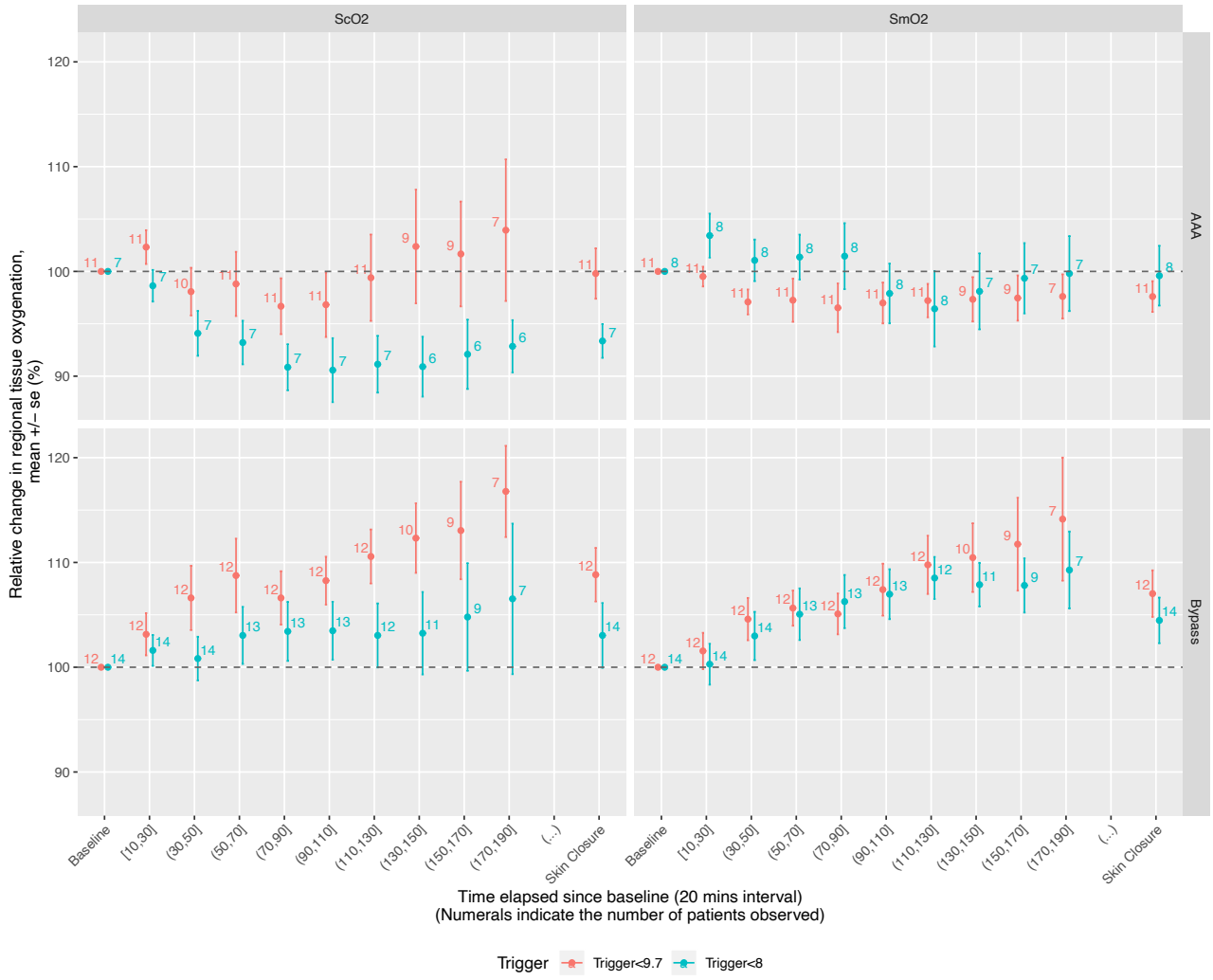


Figure S5.1. Individual changes in near infrared spectroscopy determined cerebral oxygenation (ScO_2), from baseline (T0) to the mean value (T0-T2) by trigger (paired t-test)

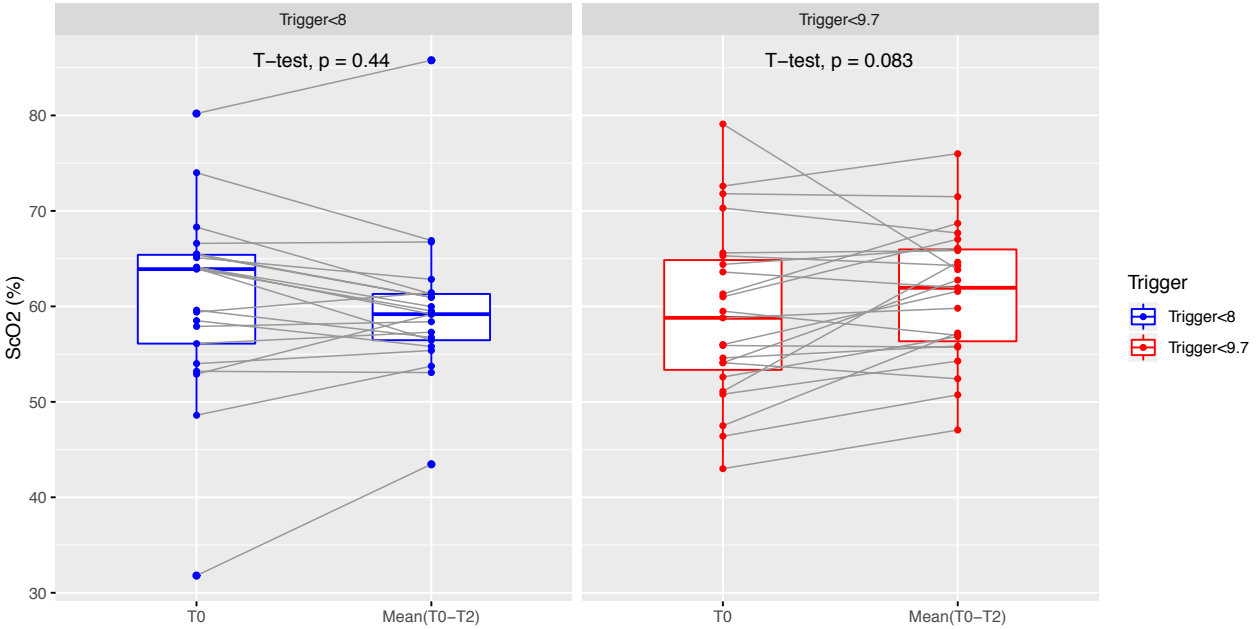


Figure S5.2. ScO_2 at baseline (T0) and at the mean value (T0-T2) by trigger and operation type (paired t-test)