Statistical Analysis Plan for the MEAT-trial

Pneumatic tourniquet versus no tourniquet in transfemoral amputation (MEAT-trial) – a randomized controlled trial

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Introduction

Major lower extremity amputations (MLEA) are common procedures, with a large variety of indications, however peripheral arterial disease and infection are the primary indications for amputation, with diabetes frequently present as a comorbid condition. The patient group is frail, with a high morbidity and mortality with a 1-year mortality at 48 % for transfemoral amputations (2).

Tourniquets are commonly used during surgery to minimize intraoperative bleeding, and general guidelines for safe tourniquet use in surgery exist (3). Tourniquet application helps maintain a bloodless surgical field, enhances anatomical visualization, and may reduce the surgery time. Given that many MLEA patients are frail and have multiple comorbidities, excessive blood loss can compromise their already limited physiological reserves, increasing the risk of postoperative anemic complications, prolonged hospitalization, and the need for additional interventions such as blood transfusions (4).

Despite these potential benefits, the use of tourniquets in MLEA remains a subject of debate due to concerns about ischemic injury and compression-related complications (5, 6). There is a theoretical risk that tourniquet-induced ischemia and the mechanical pressure, often on an already ischemic extremity, could impair wound healing in the stump, potentially leading to wound breakdown and the need for re-amputation.

Objectives

Besides the primary and secondary outcomes the protocol does also describe explorative outcomes. These will be reported separately and will not be included in this statistical analysis plan.

Reporting of harms are included in the secondary objectives and therefore not reported separately in a table.

Primary objective:

The primary objective of this trial was to investigate the effect of using a pneumatic tourniquet during a transfemoral amputation on the total calculated blood loss in mL using Nadler's approach. The primary null hypothesis was no difference between the calculated blood loss in the tourniquet group vs. the non tourniquet group i.e. $(\mathrm{H}_0 \colon \mu_{\mathrm{Tourniquet}} = \mu_{\mathrm{non-tourniquet}})$.

The description of Nadler's Equation from the full protocol (1):

Nadler's approach consists of and estimation of blood volume based on weight, height and gender and calculation of total BL based on estimated blood volume, combined with hemoglobin level before surgery and on the third postoperative day. If blood transfusion is received after surgery, this will be added.

$$Blood\ Volume(l)_{men} = height(m)^3 \cdot 0{,}367 + weight(kg) \cdot 0{,}032 + 0{,}604$$

$$Blood\ Volume(l)_{women} = height(m)^3 \cdot 0{,}356 + weight(kg) \cdot 0{,}033 + 0{,}183$$

$$Hgb_{loss} = Blood\ Volumen\ (l) \cdot \left(hgb_{pre-op} - hgb_{Final}\right) + hgb_{trans}$$

$$Total\ blood\ loss\ (mL) = \frac{Hgb_{loss}}{hgb_{pre-op}} \cdot 1000$$

Where $hgb_{pre\text{-}op}$ (g/L) is the hemoglobin measured before surgery (period 4 week to day of surgery), and hgb_{final} (g/L) is the final hemoglobin measured on the third postoperative day#. Hbg_{trans} is the amount of hgb(g) in the blood transfusions given before Hgb_{final} (g/L) is measured.

In Denmark, Hgb values are measured in mmol/L as standard. To convert to g/L the value is multiplied with 16,1.

The amount of Hgb (g) in one blood transfusion is estimated to 55g per portion.

#Due to the clinical setting of the study we expect that not all blood samples will be conducted on the third postoperative day. We will report in the text on the frequency of blood sample on each day, but will include all post-operative blood samples between days 1-5 in the analysis.

Secondary objectives:

Key secondary objectives will be to compare the effect of tourniquet use, relative to no tourniquet use on the following outcome measures:

- Blood transfusion during primary admission, Yes/No, Rate (%). Defined as any red blood cell transfusion.
- Number of transfused units: Numerical count of red blood cell units
- Intraoperative blood loss (mL): Estimated from swab weight difference and suction volume.
- Surgical duration (minutes): Time from first incision to final closure. Excludes prep, positioning, and dressing.
- Postoperative hospital stay (days): Nights from surgery to discharge home or to rehabilitation. Discharge destination recorded but not an outcome.
- In hospital complications yes/no, rate(%)*
- Mortality ≤ 30 days postoperatively, rate (%).
- All cause unplanned readmissions ≤ 30 days post discharge, rate(%)
- All cause unplanned readmissions ≤ 90 days post discharge, rate(%)
- All stump related re-operation ≤ 30-days postoperatively, rate(%).
- Alle stump related re-operation ≤ 90-days postoperatively, rate(%).

*Complications assessed according to the OrthoSaves guildelines – Appendix 1 (7, 8).

Study Methods

Design: Randomized, prospective, blinded, two-arm, single center trial. Superiority-design.

Group 1: Transfemoral amputation performed *with* tourniquet application

Group 2: Transfemoral amputation performed *without* tourniquet application

Inclusion was conducted from October 2022 to December 2024.

Population: Patients with indication for primary transfemoral amputation (intact femoral bone).

Randomization details: Randomization is performed internet-based using REDCap Randomize, allocation 1:1. The randomization itself takes place in the period 4 weeks prior to surgery to immediately before surgery. The randomization is performed as a block randomization and will be stratified for age (>70 years / ≤70 years) and hemoglobin value pre surgery (>6mmol/L / ≤6 mmol/L). Two stratifies are acceptable for the calculated sample size.

Blinding: Participants and staff not attendant in the room will be blinded. The use of tourniquet will not be visible in the patient records but recorded directly in the REDCap database. A standard phrase to describe the surgical procedure will be used in the patient record. Deviations and adverse events will be described. Participants will be able to be informed about the procedure after 6 months. The statistical analysis will be conducted blinded.

Sample size: From the pilot series we observed a mean of 429 ml and standard deviation (SD) of 199 ml in 11 intervention procedures (with tourniquet) and a mean of 730 ml and SD of 446 ml in 12 control procedures (without tourniquet). The calculated blood loss in each group was normally distributed according to quantile-quantile plots.

We assumed that a mean difference of 200 ml would be lower than any reasonable clinically important difference, and hence chose this as the difference for our sample size calculation. From this, we calculated the need of 49 participants in each group for a two-sample t-test for a superiority trial. To take into account for up to 20% drop-out (e.g. due to invalid data/protocol violation/intraoperative mortality/participant wish/investigator indication), we decided to include 62 participants in each group (49/0.8=61.25).

The sample size calculation was performed in Stata/IC 16.1 with help from OPEN Statistics, OUH.

Screening data: The total number of patients screened for eligibility will be collected and presented in a CONSORT flowchart to describe representativeness of the trial sample (Figure 1). Furthermore, the number of ineligible patients randomized by mistake, if any, will be reported including reason for ineligibility.

Interim analysis: The study group monitored the safety of the trial on an ongoing basis. If the number of re-operations and 30 days mortality in one group becomes twice as high (and statistically significantly higher), as in the other group, the trial would be discontinued. This

applied after inclusion of minimum half of the sample size, and was monitored continuously throughout the rest of inclusion period. The trial was not discontinued.

Patient population:
Inclusion criteria

- Speak and understand Danish and able to give informed consent
- ≥18 years of age
- Indication for first transfemoral amputation (intact femoral bone)*

Exclusion criteria

- Bilateral amputation in same procedure
- Malignant disease as main cause of amputation
- Not possible to place tourniquet correctly (surgeon assessment)
- Acute trauma
- Planned surgery with surgeon charged less than second year resident.

*see Derivations section

Statistical principles

The primary null hypothesis is based on the comparison of total calculated blood loss between participants randomised to tourniquet use no matter the inflation pressure or the duration of tourniquet (H_0: $\mu_{\text{Tourniquet}} = \mu_{\text{non-tourniquet}}$).

Outcomes on primary and secondary objectives will be analysed in March – May 2025

The primary analyses will be based on the intention-to-treat (ITT) population of those patients who were included, randomized and amputated. Per-protocol analysis will be performed as a sensitivity analysis.

All analyses will be carried out as a superiority, and p-values below 0.05 will be considered statistically significant.

We will report descriptive statistics of patient characteristic as mean and standard deviation (SD) for normally distributed data, median and interquartile range (IQR) for non-normally distributed numerical data, and counts and proportions for categorical characteristics.

We will compare the primary outcome (calculated blood loss) by two-sample t-test with unequal variance and report mean and SD for each group as well as the mean difference with a 95% normal confidence interval (CI). If the variable is not as normally distributed as expected, or if many outliers are evident, we will conduct a Wilcoxon rank-sum test as the primary analysis, and the two-sample t-test as a sensitivity analysis. The result of the sensitivity analysis will be reported in the text.

Negative data: in case of negative values of the total calculated blood loss these will be set to 0 in the main analysis. It is not possible to gain blood during an amputation, and a negative loss of blood is unrealistic. A sensitivity analysis will be conducted with the exacts value.

Missing data: Missing data is common in randomized controlled trials. We will use the 4. step framework from White et al(9) to handle missing data, using multiple imputation. We will define the imputation values adjusting for the applied stratifications (age and pre-operative hemoglobin) and randomization group. A sensitivity analysis without imputation will be performed.

We will report the dichotomous secondary and exploratory outcomes as counts and proportions, compare these with chi-squared test (or Fisher's exact test, if any counts are below five) and report odds ratios with 95% CI from crude logistic regression.

We will report numerical secondary and exploratory outcomes either as means and SD and compare those by two-sample t-test, if deemed normally distributed by quantile-quantile plots, or otherwise, as medians and IQR and compared by Wilcoxon rank-sum test if not deemed normally distributed.

The evaluability of each participant in the statistical analyses will be assessed before unblinding. A detailed account of excluded participants, as well as missing, unused, or false data, will be provided.

Derivations

Protocol Derivations: Protocol deviations—defined as failures to adhere to the study protocol, such as wrong intervention, incorrect data collection or documentation, errors in applying inclusion/exclusion criteria, or missed outcomes—will be classified as either major or minor. To prevent bias, protocol deviations will be predefined before data unblinding, ensuring appropriate consideration of participants in the analysis populations. All protocol derivations will be described in detail in the article.

Derivations from outcomes reported at ClinicalTrials.gov

The number of blood transfusion units was added as a secondary outcome.

Duration of surgery in minutes was added as a secondary outcome.

All cause unplanned readmissions ≤ 30 days post discharge was added as a secondary outcome

Other derivations from the clinical trials registration

The weight of the leg was not measured, this was decided early on, due to limited use of this variable.

The inclusion period was extended due to a period with low inclusion rates. The original protocol stated inclusion would be done in September 2024, but the inclusion period was extended to September 2025. The inclusion ended in December 2024.

Known general derivations from the study protocol, at the time of the finalization of the SAP:

Minor deriviation: In the study protocol the pressure of the tourniquet is stated to always be 250 mmHg, however the actual pressure was 250mmhg or 300 mmHg, decided by the surgeon.

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Minor deriviation: Redefinition of the inclusion criteria “intact femur”. This criteria was designed to avoid inclusion of revisions of a prior femoral amputation. However the wording led to some misunderstandings, leading to redefinition of this criteria, so patients that did not have an “intact” femur due to previous surgery, e.g. a total knee arthroplasty, a total hip arthroplasty or osteosynthesis material was still included, if eligible. Some patients had a prior transtibial amputation, but was converted to a femoral amputation, and were included due to the “intact femur” principle.

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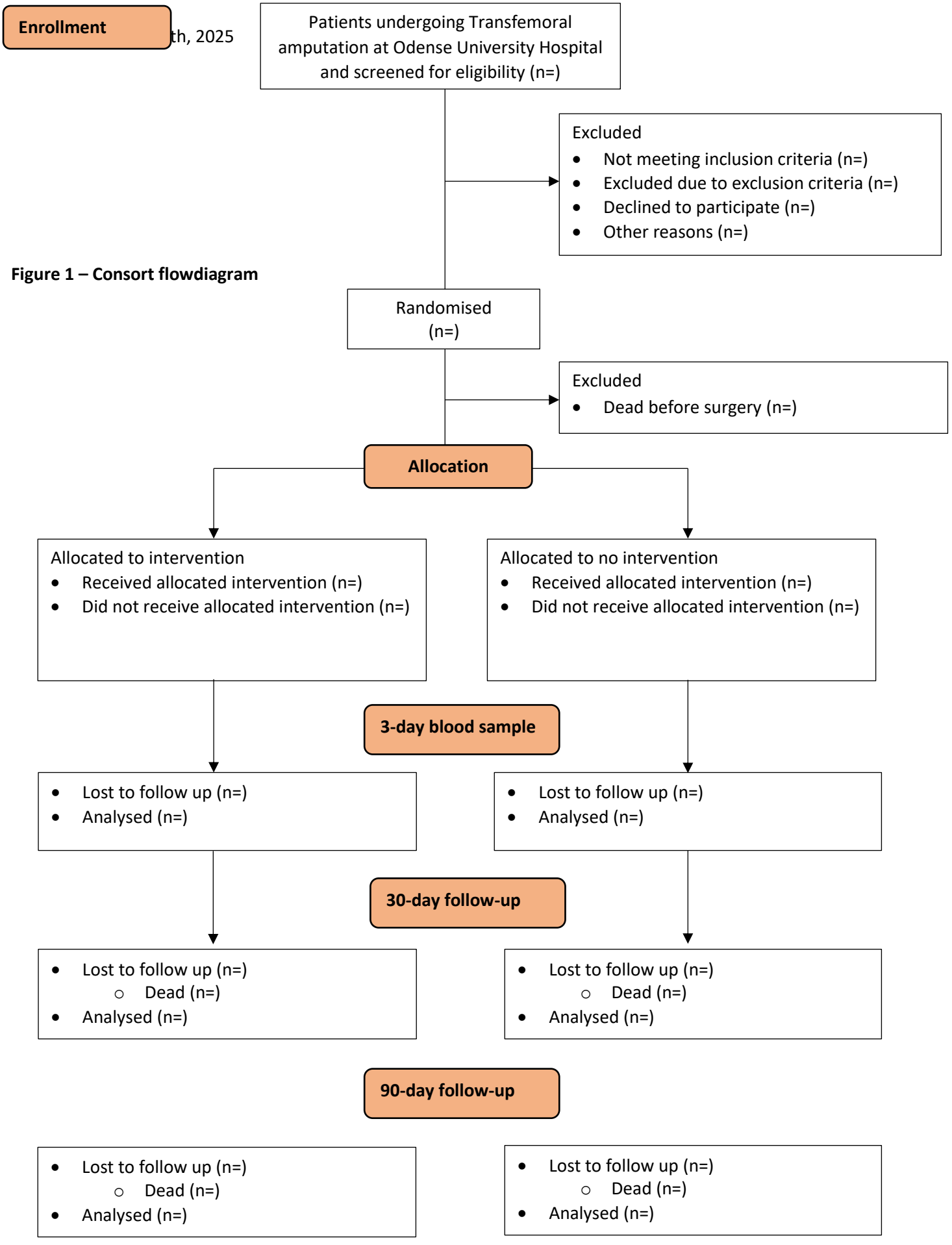

Figure 1 – Consort flowdiagram

Table 1 – Demographic and clinical characteristics of the patient at baseline in the ITT population

Characteristics	No-tourniquet group (n=)	Tourniquet group (n=)	Total (n=)
Male sex – no(%)			
Age – yr, median (iqr)			
Height – m			
Weight - kg			
Body mass index – Kg/m^2			
Current smoker			
Alcohol consumption above 7 units/week no(%)			
Married, No(%)			
Nursing home/institution, No%			
Working y, no(%)			
Pre-surgery details			
ASA-score			
I, no (%)			
II, no (%)			
III, no (%)			
IV, no (%)			
Site of surgery dxt no(%)			
*Acute procedure, y no(%)			
Assessed by vascular surgeon before amputation, y no(%)			
Indication of amputation			
Ischemic, no(%)			
Infection, no(%)			
Combined, no(%)			
Other, no(%)			
Previously major amputated on same site, y no(%)			
Previously major amputated on opposite site, y no(%)			
¤Comorbidities			
Non-insulin depended diabetes no (%)			
Insulin depended diabetes, no (%)			
Peripheal arterial disease, no (%)			
Cardiovascular disease, no (%)			
Pulmonary disorder, no (%)			
Renal failure, no (%)			
Hypertension, no (%)			
Dyslipidemia, no (%)			
Active cancer, no (%)			
Psychic disorder, no (%)			
Inflammatory/autoimmune disorder, no (%)			
Lever insufficiency, no (%)			

*Acute procedures: defined as procedures that were not pre-scheduled (The patient was not seen in the outpatient clinic) but amputation was decided during an admission.

¤The comorbidities were defined though patient records and prescription lists. A comorbidity were defined present if the patient had an active medical treatment for it at the time of amputation or it was evident through the patient record that the patient had an active.

Table 2 Primary and secondary outcomes in the ITT population

Outcomes	No-tourniquet group (n=)	Tourniquet group (n=)	Difference (95% CI)	p-value
Primary Outcome				
^Calculated blood loss on day 3, mmol/L, mean (SD)				
Secondary Outcomes				
Any blood transfusions during primary admission y, no(%)				
Number of blood transfusions per patient in transfered units (mean SD or median IQR)				
Intraoperative blood loss, mL				
Duration of surgery, minutes				
Postoperative length of stay, days				
*Complications during primary admission, y no(%)				
30-day mortality postoperatively (%)				
Readmission ≤ 30-days post discharge, rate (%)				
Readmission ≤ 90-days post discharge, rate (%)				
Re-operation ≤ 30-days, postoperatively, rate (%)				
Re-operation ≤ 90-days postoperatively, rate(%)				

^Calculated using Nadlers Equation.

*Grouping of complications: pneumonia xx vs xx, e.g. for all relevant complications using the Orthosaves guidelines.

SUPPLEMENTARY Table 1, sensitivity analysis - Primary and secondary outcomes in the per protocol population

Outcomes	No-tourniquet group (n=)	Tourniquet group (n=)	Difference (95% CI)	p-value
Primary Outcome				
^Calculated blood loss on day 3, mmol/L, mean (SD)				
Secondary Outcomes				
Any blood transfusions during primary admission y, no(%)				
Number of blood transfusions per patient in transfered units (mean SD or median IQR)				
Intraoperative blood loss, mL				
Duration of surgery, minutes				
Postoperative length of stay, days				
*Complications during primary admission, rate y no(%)				
30-day mortality postoperatively (%)				
Readmission ≤ 30-days post discharge, rate (%)				
Readmission ≤ 90-days post discharge, rate (%)				
Re-operation ≤ 30-days, postoperatively, rate (%)				
Re-operation ≤ 90-days postoperatively, rate(%)				

^Calculated using Nadlers Equation.

*Grouping of complications: pneumonia xx vs xx, e.g. for all relevant complications using the Orthosaves guidelines.

SUPPLEMENTARY Table 2, sensitivity analysis - Primary and secondary outcomes without missing data imputation in the ITT population

Outcomes	No-tourniquet group (n=)	Tourniquet group (n=)	Difference (95% CI)	p-value
Primary Outcome				
^Calculated blood loss on day 3, mmol/L, mean (SD)				
Secondary Outcomes				
Any blood transfusions during primary admission y, no(%)				
Number of blood transfusions per patient in transfered units (mean SD or median IQR)				
Intraoperative blood loss, mL				

Duration of surgery, minutes				
Postoperative length of stay, days				
*Complications during primary admission, rate y no(%)				
30-day mortality postoperatively (%)				
Readmission ≤ 30-days post discharge, rate (%)				
Readmission ≤ 90-days post discharge, rate (%)				
Re-operation ≤ 30-days, postoperatively, rate (%)				
Re-operation ≤ 90-days postoperatively, rate(%)				

^Calculated using Nadlers Equation.
*Grouping of complications: pneumonia xx vs xx, e.g. for all relevant complications using the Orthosaves guidelines.

SUPPLEMENTARY Table 3- Sensitivity analysis, Primary and secondary outcomes with exact values in the ITT population

Outcomes	No-tourniquet group (n=)	Tourniquet group (n=)	Difference (95% CI)	p-value
Primary Outcome				
^Calculated blood loss on day 3, mmol/L, mean (SD)				
Secondary Outcomes				
Any blood transfusions during primary admission y, no(%)				
Number of blood transfusions per patient in transfered units (mean SD or median IQR)				
Intraoperative blood loss, mL				
Duration of surgery, minutes				
Postoperative length of stay, days				
*Complications during primary admission, y no(%)				
30-day mortality postoperatively (%)				
Readmission ≤ 30-days post discharge, rate (%)				
Readmission ≤ 90-days post discharge, rate (%)				
Re-operation ≤ 30-days, postoperatively, rate (%)				
Re-operation ≤ 90-days postoperatively, rate(%)				

^Calculated using Nadlers Equation.
*Grouping of complications: pneumonia xx vs xx, e.g. for all relevant complications using the Orthosaves guidelines.

Appendix 1 – OrthoSAVES guidelines

Orthopedic Surgical Adverse Events Severity System	
Grade	Definition
I	Adverse event does not require treatment and has no adverse effect
II	Adverse event requires simple or minor invasive treatment (e.g., antibiotics, Foley catheter, nasogastric [NG]) tube) and has no long-term effect on patient outcome
III	Adverse event requires invasive (e.g., surgery) or complex treatment (e.g., monitored bed) and is most likely to have a temporary (less than 6 months) adverse effect on outcome
IV	Adverse event requires invasive (e.g., surgery) or complex treatment (e.g., monitored bed) and is most likely to have a prolonged (more than 6 months) adverse effect on outcome[1]
V	Sentinel or significant life or limb threatening event[2]
VI	Adverse event resulting in death

1. Any adverse event with functionally significant (i.e., patient-reported or objective) and most likely prolonged (>6 months) adverse effect on outcome should be graded as severity grade 4, regardless of treatment complexity (or scenario where there is no possibility of treatment).
2. A sentinel event is an unexpected serious life or limb-threatening event and/or an event that necessitates institutional investigation and review to determine the root cause. For example, the wrong surgical site should automatically be graded at severity 5.

Orthopedic Surgical Adverse Events Severity System Categories	
Intraoperative	Postoperative
(1) Airway/ventilation (2) Allergic reactions (3) Cardiac arrest/failure/arrhythmia (4) Compartment syndrome (5) Cutaneous injury (e.g., pressure sore) (6) Dural tear (7) Hypotension (clinically relevant) (8) Implant/instrumentation related (a) Instrumentation/fixation/implant/mal positioning requiring revision (b) Peri-implant fracture (9) Incorrect operative site (10) Blood loss >5 L in 24 hrs or >2 L in 3 hrs (11) Neural injury (a) Spinal cord (b) Nerve root (c) Peripheral nerve (12) Soft-tissue injury/failure (a) Ligament/tendon injury requiring additional surgery	(15) Airway/breathing (16) Cardiac arrest/failure/arrhythmia (17) Cerebrovascular event (18) Compartment syndrome (19) Cutaneous injury (e.g., pressure sore) (20) Delirium/altered mental state (21) Dysphagia/dysphonia (22) Fall (23) Gastrointestinal bleeding (24) Hematoma (25) Ileus/bowel obstruction (26) Implant/instrumentation-related (a) Loss of reduction/alignment/correction (b) Peri-implant fracture (c) Joint instability/dislocation (d) Aseptic loosening (27) Infection (a) Superficial wound (b) Deep wound (c) Urinary tract (d) Systemic

(b) Soft-tissue reconstruction/repair failure requiring revision (13) Vascular injury (14) Other	(28) Myocardial infarction (29) Neurological deterioration (a) Cord (≥1 motor grade in American Spinal Injury Association [ASIA] motor scale) (b) Nerve root/peripheral nerve ≥1 Medical Research Council (MRC) grade (c) Cauda equina syndrome (30) Nonunion/malunion (31) Pain – new onset (e.g., neuropathic pain/reflex sympathetic dystrophy/pain disorder) (32) Pneumonia (33) Renal insufficiency (34) Thromboembolic event (a) Deep vein thrombosis (b) Pulmonary embolism (35) Soft-tissue reconstruction/repair failure (36) Wound dehiscence (37) Urinary retention (38) Wound drainage (clinically significant) (a) Cerebrospinal fluid leak/meningocele (b) Serous (requiring treatment) (39) Other