Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Supplementary Appendix for

Lower versus Higher Hemoglobin Threshold for Transfusion in Septic Shock

The TRISS trial investigators

Steering Committee: Lars B. Holst, Nicolai Haase, Jan Wernerman, Anne B. Guttormsen, Sari Karlsson, Për I. Johansson, Anders Åneman, Jørn Wetterslev and Anders Perner (Chair).

Management Committee: Lars B. Holst (Chair), Jørn Wetterslev and Anders Perner.

Scientific Committee: Tim Walsh, Jacques Lacroix and Dean Fergusson

Data Monitoring and Safety Committee: Daniel De Backer (Chair), Kathy Rowan and Jørgen H. Petersen.

Trial Site Investigators (in Denmark unless otherwise specified): *Rigshospitalet*: L.B. Holst, A. Perner, L.H. Andersen, U. G. Pedersen, N. Reiter, J. Wiis, J.O. White, L. Russell, K.J. Thornberg, L. Quist, M. Ibsen, P.B. Hjortrup, R.G. Müller, M.H. Møller, M. Steensen, C. Claudius, M.N. Kjær, K.R. Uhre, V. Christiansen, L.H. Nislev, F. Sjøvall, I.L. Jarnvig, J. Nielsen, H.C. Thorsen, P. Bredahl, *Randers Hospital*: M. Vang, H. Bundgaard, M.A. Thyø, M. Villumsen, *Karolinska University Hospital, Huddinge, Sweden*: J. Wernerman, K. Kilsand, I. Tjäder, S. O. Wernerman, Herning Hospital: R. Winding, D. Lodahl, R. Mærkedahl, N. Dey, S., S. Haubjerg, *Hvidovre Hospital*: L. Nebrich, C. Albeck, A. Christensen, *Aarhus University Hospital*: H. Nibro, D. Keld, K. Larsen, S.E. Dyrskog, D. Illum, *Ålborg University Hospital*: B. Rasmussen, S.N. Granum, *Holbæk Hospital*: H. H. Bülow, J. Elkjær, J. R. M. Lauridsen, *Kolding Hospital*: J. S. Nielsen, *Haukeland University Hosptial, Norway*: A. B. Guttormsen, B. Sjøbø, *Karolinska University Hospital, Solna, Sweden*: A. Oldner, O. Friman, *Helsinki University Hospital, Finland*: V. Pettilä, L. Pettilä, S. Sutinen, *Södersjukhuset, Sweden*: M. Cronhjort, *Næstved Hospital*: B. K. Kristensen, P. Kirkegaard, *Bispebjerg Hospital*: A. Lindhardt, D. G. Strange, *Horsens Hospital*: Elsebeth Haunstrup Krogh, Omar Rian, *Hjørring Hospital*: M. Kruse, S. Aagaard, M.B. Pawlowicz, J. Madsen, *Køge Hospital*: L. M. Poulsen, Vejle Hospital: P. Berezowicz, *Halmstad Hospital, Sweden*: M. Chew, F. Abdeen, W. Müller, G. Sternheden, S. Holmstrøm, *Gentofte Hospital*: K. Thormar, N. Mtchedlishvili, *Stavanger University Hospital, Norway*: K. Strand, *Tampere University Hospital, Finland*: S. Karlsson, S. Varila, A. Kukkurainen, *Glostrup Hospital*: A. Bendtsen, *Joensuu Hospital, Finland*: M. Reinikainen, *Slagelse Hospital*: S. A. Iversen, *Sønderborg Hospital*: S. Leivdal, *Akershus University Hospital, Norway*: P. M. Bådstøløkken, *Ålesund Hospital, Norway*: J. Å. Longva, R. B. Johansen, *Helsingborg Hospital, Sweden*: N. Nielsen, *Växjö Hospital, Sweden*: J. M. Breider

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Trial criteria for septic shock

(1) ***AT LEAST TWO SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS) CRITERIA****:*[1]

1. ***CORE TEMPERATURE >38°C or <36°C****. (Core temperature was rectal, urinary bladder, central line, or tympanic). If oral, inguinal or axillary temperatures were used, we added 0.5°C to the measured value. Hypothermia <36°C was confirmed by core temperature. We used the most deranged value recorded* ***in the 24 hours before randomization****.*
2. ***HEART RATE ≥90 beats/minute****. If patient had an atrial arrhythmia, we recorded the ventricular rate. If patients had a known medical condition or were receiving treatment that would prevent tachycardia (for example, heart block or beta blockers), they had to meet two of the remaining three SIRS criteria. We used the most deranged value recorded* ***in the 24 hours before randomization****.*
3. ***MECHANICAL VENTILATION*** *for an acute process or respiratory rate ≥ 20 breaths per minute or a $PaCO_2$ < 4.3 kPa (32 mmHg). We used the most deranged respiratory rate or $PaCO_2$ recorded* ***in the 24 hours before randomization****.*
4. ***WHITE BLOOD CELL COUNT ≥12 x 10^9/l or ≤ 4 x 10^9/l****. We used the most deranged value recorded* ***in the 24 hours before randomization****.*

AND

(2) ***SUSPECTED OR VERIFIED FOCUS OF INFECTION*** *as either:*

(i) An organism grown in blood or sterile site

OR

(ii) An abscess or infected tissue (e.g. pneumonia, peritonitis, urinary tract, vascular line infection, soft tissue, etc).

AND

(3) ***HYPOTENSION*** *(Systolic blood pressure ≤ 90 mmHg or mean arterial pressure ≤ 70 mmHg) despite fluid therapy OR* ***VASOPRESSOR/INTROPE*** *infusion to maintain blood pressure.*

Trial exclusion criteria

- Documented wish against transfusion **OR**

- Previous serious adverse reaction with blood products, excl. transfusion-associated circulatory overload **OR**

- Presence of acute myocardial ischemia **OR**
  (defined as: patients diagnosed with ***acute myocardial infarction*** *(ST-elevation myocardial infarction or non-ST elevation myocardial infarction) or* ***unstable angina pectoris*** during current hospital admission, according to the criteria in the clinical setting in question (e.g. elevated biomarkers, ischemic signs on ECG, clinical presence) **AND** the patient has received treatment, initiated during current hospital admission, as a consequence of this (reperfusion strategies (PCI/thromobolysis) or initiation/increased antithrombotic treatment)).

- Life-threatening bleeding **OR**
  (defined as: (1) Presence of hemorrhagic shock, as judged by research or clinical staff. OR (2) the need for surgical procedure, incl. endoscopy to maintain hemoglodin levels).

- Red cell transfusion during current ICU admission **OR**

- Withdrawal from active therapy or brain death **OR**

- Acute burn injury - regardless of degree and burn surface area **OR**

- Lack of informed consent

Trial criteria for serious adverse reactions and ischemic events

Serious adverse reactions after blood transfusion were defined as either

Allergic reactions defined by the clinician on the basis of muco-cutaneous signs and symptoms (e.g. urticaria, pruritus, localized angio-edema) occurring within 6 hours of red cell transfusion

Severe hemolytic complications defined by the clinician on the basis of hemoglobinuria or increased free plasma hemoglobin occurring within 24 hours of transfusion.

Transfusion-associated acute lung injury (TRALI) defined as: I. Acute or worsening hypoxemia ((PaO_2/FiO2 < 40 (PaO_2 in kPa) or <300 (PaO_2 in mmHg) regardless of PEEP) OR > 50% relative increase in FiO_2 AND II. Occurrence within 6 hours after red cell transfusion AND III. Acute or worsening pulmonary infiltrates on frontal chest x-ray OR clinical signs of overt pulmonary edema.

Transfusion-associated circulatory overload (TACO) defined as: I. Acute or worsening hypoxemia (PaO_2/FiO_2 < 40 (PaO_2 in kPa) or <300 (PaO_2 in mmHg) regardless of PEEP) OR > 50% relative increase in FiO_2 AND II. Occurrence within 6 hours after red cell transfusion AND III. Acute or worsening pulmonary infiltrates on frontal chest x-ray OR clinical signs of overt pulmonary edema AND IV. Increased blood pressure AND VI. Positive fluid balance.

Ischemic events were defined as either

Cerebral ischemia defined as any form of cerebral ischemia on a CT- OR MRI scan

Acute myocardial ischemia defined as patient diagnosed with acute myocardial infarction (ST-elevation myocardial infarction or non-ST elevation myocardial infarction) or unstable angina pectoris according to the criteria in the clinical setting in question (e.g. elevated biomarkers, ischemic signs on ECG and clinical presentation) AND the patient received treatment as a consequence of this (reperfusion strategies (PCI/thrombolysis) or initiation/increased antithrombotic treatment).

Intestinal ischemia was defined as ischemia verified by endoscopy OR open surgery.

Limb ischemia defined as clinical signs AND need of open/percutaneous vascular intervention, amputation OR initiation/increased antithrombotic treatment.

Point-of-care testing devices
One of the following point-of-care testing devices was used to assess hemoglobin concentrations in the TRISS trial: ABL 625, 700- and 800-series or ABL90 from Radiometer, Copenhagen, Denmark (31 ICUs) or Cobas b 221 from Roche Diagnostics, Rotkreuz, Switzerland (one ICU).[2, 3]

Protocol suspension criteria
The attending doctor could temporarily suspend the protocol and transfuse an individual patient at a hemoglobin value that differed from the allocated one during any of the following events:

- The presence of life-threatening bleeding defined as the presence of hemorrhagic shock, as judged by research or clinical staff.
- Ischemic events defined as:
    - Acute myocardial ischemia: Defined as ***acute myocardial infarction*** *(ST-elevation myocardial infarction and non-ST elevation myocardial infarction) or* ***unstable angina pectoris***, according to the criteria in the clinical setting in question (e.g. elevated biomarkers, ischemic signs on ECG, clinical presence) AND the patient had received treatment as a consequence of this (reperfusion strategies (percutaneous cardiac intervention/thrombolysis) or initiation/increased antithrombotic treatment).
    - Cerebral ischemia: Verified by CT- or MRI scan
    - Intestinal ischemia: Verified by endoscopy or surgery.
    - Acute limb ischemia: Clinical signs AND need of open or percutaneous vascular intervention, amputation or initiation/increased antithrombotic treatment.
- The use of extracorporeal membrane oxygenation (not originally protocolized, but added during trial)

The attending doctor decided when the patient again was to be transfused at the allocated hemoglobin threshold. Suspension was not considered a breach of protocol, and data collection continued during the suspension. These patients were analyzed according to their assigned group on an intention-to-treat basis.

Trial populations

Intention-to-treat population: All randomized patients except those who
- Withdrew consent for the use of data
OR
- Were not eligible for randomization according to the inclusion/exclusion criteria AND never had the intervention (decision to transfuse based on the allocated hemoglobin threshold)

Per-protocol population no. 1: All randomized patients except patients having one or more major protocol violations defined as:

1. One or more blood transfusions given despite a hemoglobin level above 7.0 g/dl in patients assigned to the lower Hb-threshold group
OR

2. One or more blood transfusion not given within 24 hours after a hemoglobin level less than 9.0 g/dl in patients assigned the higher Hb-threshold group
OR

3. Monitoring revealed that one or more in- or exclusion criteria were violated
OR

4. One or more transfusions of red cell unit(s) destined for another patient
OR

5. One or more transfusions given despite lack of cross-match between donor and recipient. Administration of 0-neg blood without cross-match between donor and recipient was not regarded as a protocol violation
OR

6. Any protocol suspension defined as transfusions administered when hemoglobin level was above the allocated threshold level on days with the presence of ischemic events, life-threatening bleeding events
OR

7. Patients stopped or withdrawn

Per-protocol no. 2: All randomized patients except patients having one or more protocol violations defined as

1. One or more transfusions *given* despite the patients hemoglobin level being *above the threshold* the patient was assigned to
OR

2. *O*ne or more transfusions *not given* within a period of 24 hours after the patient documented a hemoglobin level *below the threshold* that the patient was assigned to

Per-protocol no. 3: All randomized patients except patients having one or more:

1. Bleeding episodes
OR

2. Ischemic episodes

Handling of missing data

SAPS II in the 24 hours prior to randomization
This score is based on 17 variables, which were registered in the baseline case report form from source data. We had missing source data for one or more of the 17 variables in 176 patients. These values were not included in the baseline characteristics and we imputed best/worst case scenarios in the analyses adjusting for design variables (Table S12).

SOFA score in the 24 hours prior to randomization
Missing Glasgow Coma Scale (GCS) score: No missing values, because investigators recorded 15, if the score was missing in source data. If the patient was sedated, the GCS score estimated before sedation was used; if missing the GCS score was registered as 15.

Missing cardiovascular component: Missing values were imputed using data from the screening form (if the patient had a mark for hypotension he/she was given the cardiovascular SOFA score 1 and with a mark for vasopressor or inotropic agent the score 3 was given). After this imputation we had no missing values.

Missing PaO_2/FiO_2-ratio: No missing values.

Missing renal component: No missing values.

Missing platelet count: 17 missing values.

Missing plasma bilirubin: 109 missing values.

We handled the missing SOFA scores as we did for the missing SAPS II.

Missing outcome data
For the primary outcome measure and all mortality endpoints we had full data sets on all 998 patients in the intention-to-treat population.

There were missing data for 21 patients for the following secondary outcome measures, because the patient or the surrogate decision-maker did not want continued data registration: life support at days 5, 14 and 28 after randomization, serious adverse reactions in the ICU, ischemic events in the ICU, percent of days alive without vasopressor/inotropic therapy, mechanical ventilation or renal replacement therapy, and percent of days alive out of hospital.

We did not impute any data for these outcome measures, because they only represented 21/998 (2%) of the patients.

Table S1. Additional baseline characteristics

Characteristic	Lower Hb-threshold (N=502)	Higher Hb-threshold (N=496)
Estimated body weight – kg	75 (64-87)	75 (62-87)
Chronic cardiovascular disease – no. (%)		
Previous myocardial infarction	33 (7)	30 (6)
Previous angina pectoris	26 (5)	7 (1)
Severe chronic heart failure (NYHA group 3-4)	13 (3)	9 (2)
Previous coronary intervention	38 (8)	32 (6)
Previous vascular intervention (non-coronary)	16 (3)	18 (4)
Previous ischemic stroke or transitory cerebral ischemia	40 (8)	43 (9)
Organ failures – no. (%) †		
Cerebral failure ‡	120 (24)	132 (27)
Respiratory failure	456 (91)	442 (89)
Circulatory failure	502 (100)	496 (100)
Hepatic failure	88 (20)	70 (16)
Kidney failure	219 (44)	232 (47)
Coagulation failure	144 (29)	122 (25)
Renal replacement therapy - no. (%)		
Acute	55 (11)	42 (8)
Chronic	13 (3)	11 (2)

Values with ranges are medians (interquartile ranges). NYHA denotes New York Heart Association

† Defined as Sepsis-related Organ Failure Assessment score of 2 or more in the given organ system at randomization except for circulatory failure which was defined as a score of 1 or more (Table S15).[4] Most patients had 2 or more organ failures.

‡ If the patient was sedated, the Glasgow Coma Scale (GCS) score estimated before sedation was used; if missing the GCS score was registered as 15.

Table S2. Protocol suspensions

Suspension	Lower Hb-threshold (N=488)	Higher Hb-threshold (N=489)
Myocardial ischemia – no. (%)	6 (1.2)	0 (0)
Transfusions given during suspensions for myocardial ischemia – no. (%)	10/1545 (0.7)	0/3088 (0)
Other ischemia – no. (%) *	6 (1.2)	0 (0)
Transfusions given during suspensions for other ischemia – no. (%)	16/1545 (1.0)	0/3088 (0)
Life-threatening bleeding – no. (%)	18 (3.7)	9 (1.8)
Transfusions given during suspensions for life-threatening bleeding – no. (%)	52/1545 (3.4)	13/3088 (0.4)
ECMO therapy – no. (%) †	1 (0.2)	2 (0.4)
Transfusions given during ECMO – no.	55	10 and 15

Values with ranges are medians (interquartile ranges). Four patients had 2 suspension periods based on different criteria, all in the lower Hb-threshold group.

* Other ischemia includes cerebral, intestinal and limb ischemia.

† ECMO denotes extracorporeal membrane oxygenation.

Table S3. Blood hemoglobin concentrations before and after randomization

	Lower Hb-threshold (N=488)	Higher Hb-threshold (N=489)
Baseline †		
Lowest hemoglobin – g/dl	8.4 (7.8-8.7)	8.4 (7.7-8.7)
Highest hemoglobin – g/dl	9.8 (9.2-10.5)	9.8 (9.2-10.6)
Day 1 ‡		
Number of measurements	5 (3-8)	6 (4-8)
Lowest hemoglobin – g/dl	8.1 (7.4-8.5)	8.5 (8.1-8.7)
Highest hemoglobin – g/dl	8.7 (8.2-9.2)	9.8 (9.5-10.2)
Day 2		
Number of measurements	8 (6-10)	8 (6-10)
Lowest hemoglobin – g/dl	7.7 (7.1-8.3)	9.0 (8.8-9.5)
Highest hemoglobin – g/dl	8.7 (8.2-9.2)	10.3 (9.9-10.7)
Day 3		
Number of measurements	7 (5-10)	8 (6-10)
Lowest hemoglobin – g/dl	7.7 (7.1-8.2)	9.3 (8.9-9.8)
Highest hemoglobin – g/dl	8.7 (8.1-9.3)	10.3 (9.8-10.8)
Day 4		
Number of measurements	8 (6-9)	7 (5-9)
Lowest hemoglobin – g/dl	7.6 (7.1-8.2)	9.3 (9.0-10.0)
Highest hemoglobin – g/dl	8.5 (8.1-9.2)	10.4 (10.0-11.0)
Day 5		
Number of measurements	7 (5-9)	7 (5-9)
Lowest hemoglobin – g/dl	7.7 (7.3-8.2)	9.3 (9.0-10.0)
Highest hemoglobin – g/dl	8.5 (8.1-9.2)	10.3 (10.0-11.0)
Day 6		
Number of measurements	7 (6-9)	7 (5-9)
Lowest hemoglobin – g/dl	7.7 (7.1-8.2)	9.4 (8.9-9.9)
Highest hemoglobin – g/dl	8.7 (8.2-9.3)	10.5 (10.0-11.0)
Day 7		
Number of measurements	7 (6-9)	7 (5-9)
Lowest hemoglobin – g/dl	7.6 (7.1-8.1)	9.3 (8.9-9.8)
Highest hemoglobin – g/dl	8.5 (8.1-9.1)	10.3 (10.0-11.0)

Values are medians (interquartile ranges).
† In the 24 hours prior to randomization
‡ The first day was from the time of randomization to the next start of the specific ICU's 24-hour observation chart and lasted median 15 (9-19) hours in the lower Hb-threshold group and 14 (9-19) hours in the higher Hb-threshold group

Table S4. Blood transfusion before and after randomization

	Lower Hb-threshold (N=488)	Higher Hb-threshold (N=489)
Baseline †		
No. of patients transfused (%)*	48/487 (10)	58/488 (12)
Number of units per patient	2 (2-4)	2 (1-3)
Volume – ml	600 (395-900)	490 (290-800)
Day 1 ‡		
No. of patients transfused (%)*	81/488 (17)	455/489 (93)
Number of units per patient	1 (1-2)	2 (1-2)
Volume – ml	250 (240-480)	480 (240-512)
Day 2		
No. of patients transfused (%)*	93/472 (20)	208/473 (44)
Number of units per patient	1 (1-2)	1 (1-2)
Volume – ml	250 (240-500)	250 (240-480)
Day 3		
No. of patients transfused (%)*	86/430 (20)	134/419 (32)
Number of units per patient	1 (1-1)	1 (1-1)
Volume – ml	241 (240-295)	240 (240-283)
Day 4		
No. of patients transfused (%)*	64/366 (17)	103/375 (27)
Number of units per patient	1 (1-1)	1 (1-1)
Volume – ml	240 (240-264)	240 (240-270)
Day 5		
No. of patients transfused (%)*	54/328 (16)	72/326 (22)
Number of units per patient	1 (1-1)	1 (1-1)
Volume – ml	240 (240-276)	240 (240-290)
Day 6		
No. of patients transfused (%)*	56/287 (20)	82/288 (28)
Number of units per patient	1 (1-1)	1 (1-1)
Volume – ml	240 (240-286)	240 (240-290)
Day 7		
No. of patients transfused (%)*	45/260 (17)	71/248 (27)
Number of units per patient	1 (1-1)	1 (1-2)
Volume – ml	240 (240-274)	250 (240-480)

Values with ranges are medians (interquartile ranges) of the patients transfused on that day.

† In the 24 hours prior to randomization

* Where the denominator is below all patients allocated to the group this was due to death, ICU discharge or missing source data.

‡ The first day was from the time of randomization to the next start of the specific ICU's 24-hour fluid chart and lasted median 15 (9-19) hours in the lower Hb-threshold group and 14 (9-19) hours in the higher Hb-threshold group.

Table S5. Number of patients stratified by the number of units transfused

	Lower Hb-threshold (N=488)	**Higher Hb-threshold (N=489)**
	no./total no. (%)	
0 units	176/488 (36)	6/489 (1)
1 unit	88/488 (18)	70/489 (14)
2 units	57/488 (12)	85/489 (17)
3 units	47/488 (10)	80/489 (16)
4 units	27/488 (6)	53/489 (11)
5 or more units	93/488 (19)	195/489 (40)

Table S6. Bleeding and surgery after randomization

	Lower Hb-threshold (N=488)	**Higher Hb-threshold (N=489)**
Overt bleeding - no. (%)*	147 (30)	148 (30)
Upper GI tract	36 (7)	34 (7)
Lower GI tract	33 (7)	31 (6)
Lower airway	46 (9)	27 (6)
Urinary tract	17 (3)	9 (2)
Wounds	37 (7)	61 (12)
During surgery	41 (8)	51 (10)
Other	46 (9)	41 (8)
Severe bleeding - no. (%) †	30 (6)	52 (11)
Surgery		
No. of patients having one or more days with surgery (%)	148 (30)	166 (34)
No. of days with surgery in these patients - median (interquartile range)	2 (1-3)	1 (1-3)
No. of patients transfused above their allocated threshold during surgery - (%) ‡	28 (6)	15 (3)

* Any bleeding from any of the anatomical sites given below.

† Overt bleeding and the use of 3 units of red blood cells on that day.

‡ These were not violations as transfusion was not protocolized during surgery.

Table S7. Patients with do not resuscitate orders

	Lower Hb-threshold (N=488)	Higher Hb-threshold (N=489)
No. of patients with one or more days with DNR order – (%)	125/488 (26)	154/489 (31)
No. of days with DNR order for the patients who had these – median (IQR)	2 (1-4)	2 (1-4)
No. of patients transfused on a day with DNR order – (% of patients transfused)	41/312 (13)	73/483 (15)
No. of transfusions given on days with DNR order – (% of transfusions)	127/1545 (8)	210/3088 (7)

DNR denotes do not resuscitate and was defined as any written order limiting cardio-pulmonary resuscitation in the case of cardiac arrest. IQR denotes interquartile range.

Table S8. Use of plasma, platelets and fluids and fluid balances

Variable	Lower Hb-threshold (N=488)		Higher Hb-threshold (N=489)	
	No. receiving / No. at risk (%)*	Volume (ml)	No. receiving / No. at risk (%)*	Volume (ml)
Fresh frozen plasma				
Day -1 ¶	61/487 (13)	0 (0-0)	62/488 (13)	0 (0-0)
Day 1 ‡	41/488 (8)	0 (0-0)	43/489 (9)	0 (0-0)
Day 2	43/472 (9)	0 (0-0)	43/473 (9)	0 (0-0)
Day 3	20/430 (5)	0 (0-0)	19/419 (9)	0 (0-0)
Total §	113/488 (23)	0 (0-0)	127/489 (26)	0 (0-264)
Platelets				
Day -1 ¶	32/487 (7)	0 (0-0)	29/488 (6)	0 (0-0)
Day 1 ‡	26/488 (5)	0 (0-0)	23/489 (5)	0 (0-0)
Day 2	34/472 (7)	0 (0-0)	42/473 (9)	0 (0-0)
Day 3	30/430 (7)	0 (0-0)	24/419 (6)	0 (0-0)
Total §	79/488 (16)	0 (0-0)	96/489 (20)	0 (0-0)
Albumin ‡‡				
Day 1 ‡	132/487 (27)	0 (0-100)	126/489 (26)	0 (0-100)
Day 2	141/472 (30)	0 (0-194)	125/473 (26)	0 (0-100)
Day 3	99/430 (23)	0 (0-0)	98/419 (23)	0 (0-0)
Total §	306/487 (63)	250 (0-1000)	303/489 (62)	250 (0-950)
Synthetic colloids ††				
Day 1 ‡	7/487 (1)	0 (0-0)	5/489 (1)	0 (0-0)
Day 2	5/472 (1)	0 (0-0)	3/473 (1)	0 (0-0)
Day 3	0/430 (0)	0 (0-0)	1/419 (0)	0 (0-0)
Total §	16/487 (3)	0 (0-0)	15/489 (3)	0 (0-0)
Other fluids ¶¶				
Day 1 ‡	479/485 (99)	1944 (977-3430)	486/487 (100)	2027 (990-3403)
Day 2	466/469 (99)	2523 (1590-3726)	467/471 (99)	2351 (1498-3559)
Day 3	425/428 (99)	2017 (1188-3103)	416/417 (100)	2100 (1285-2992)
Total §	438/440 (99)	14128 (6745-27853)	449/449 (100)	14778 (6741-26756)
Fluid balance	No. with data / No. at risk**	Volume (ml)	No. with data / No. at risk**	Volume (ml)
Day 1 ‡	485/488	890 (-46-2156)	487/489	1328 (394-2629)
Day 2	469/472	813 (-223-2183)	467/471	724 (-342-1901)
Day 3	428/430	291 (-629-1281)	417/419	259 (-650-1094)
Total §	431/488	2649 (-195-7021)	447/489	3351 (276-8037)

Values are medians (interquartile ranges) of all patients who had data registered on that day(s).

* No. receiving is those patients who did receive the specific solution on the given day(s). No. at risk is those patients who had registered data on that day(s). Where the no. is below all patients allocated to the group this is due to death, ICU discharge or missing data. We had the following missing data: data on single day forms were missing for one patient for each of the synthetic colloid (starch, dextran and gelatin) and for one patient for each of the albumin solutions (5% and 20%) and data on 1.1% and 1.2% of day forms for total inputs and total outputs (used to calculate other fluids), respectively. Complete cases are given here.

¶ In the 24 hours prior to randomization.

‡ The first day was from the time of randomization to the next start of the specific ICU's 24-hour fluid chart and lasted median 15 (IQR, 9-19) hours in the lower Hb-threshold group and 14 (9-19) hours in the higher Hb-threshold group.

§ Cumulative data for the full trial period in ICU to a maximum of 90 days after randomization

‡‡ Total volumes of 5 and 20% albumin.

†† Total volumes of hydroxyethyl starch, dextran and gelatin solutions.

¶¶ Including crystalloids, dextrose (10% or below), water and fluids given with medications.

** No. with data is those patients where data were registered for that day(s). No. at risk is those patients who were in the ICU on that day(s). Where the no. is below the no. allocated to the group this is due to death or ICU discharge. Data were missing on 1.1% and 1.2% of day forms for total inputs and total outputs (used to calculate fluid balances), respectively. Complete cases are given here.

Table S9. Circulatory parameters from baseline to day 7 after randomization

Variable	Lower Hb-threshold (n=488)		Higher Hb-threshold (n=489)	
	No. assessed†	Value	No. assessed†	Value
Lowest $ScvO_2$ – % ‡				
Baseline	230	69 (58-76)	228	68 (60-76)
Day 1	191	69 (61-75)	185	70 (62-76)
Day 2	175	66 (60-74)	191	71 (64-77)
Day 3	143	69 (61-75)	135	71 (64-76)
Day 4	128	66 (58-72)	107	69 (64-77)
Day 5	88	65 (60-72)	88	71 (65-77)
Day 6	80	67 (60-75)	81	71 (63-76)
Day 7	68	66 (58-74)	67	69 (63-75)
Highest lactate – mmol per liter ‡				
Baseline	485	2.4 (1.6-4.2)	486	2.7 (1.6-4.5)
Day 1	481	2.1 (1.4-3.3)	487	2.1 (1.4-3.3)
Day 2	467	1.9 (1.4-2.9)	469	2.0 (1.4-3.0)
Day 3	419	1.8 (1.3-2.6)	412	1.8 (1.4-2.6)
Day 4	360	1.7 (1.3-2.5)	370	1.7 (1.3-2.5)
Day 5	318	1.7 (1.3-2.4)	318	1.7 (1.3-2.4)
Day 6	282	1.7 (1.3-2.4)	279	1.6 (1.3-2.3)
Day 7	254	1.6 (1.2-2.3)	243	1.7 (1.3-2.4)

Values are medians (interquartile ranges).

$ScvO_2$ denotes central venous oxygen saturation, which was sampled from a central line with the tip in the superior caval vein.

† Number of patients where the measurements were documented in source data.

‡ Where more measurements were documented within the time period the lowest value of $ScvO_2$ and the highest value of lactate were registered.

Table S10. Protocol violations*

Violation	Lower Hb-threshold (N=463) †	Higher Hb-threshold (N=470) †
One or more transfusions *given* despite the patients Hb-level being *above the assigned Hb-threshold* – no. (% of patients)	45/463 (10)	16/470 (3)
No. of transfusions *given* despite the patients Hb-level being *above the assigned Hb-threshold* – (% of transfusions)	80/1323 (6)	18/3005 (1)
Transfusion *not given* on a day where the patient had at least one Hb-measurement *below the assigned Hb-threshold* – no. (% of patients)	42/463 (9)	104/470 (22)
One or more transfusions destined for another patient – no.	0	0
One or more transfusions given despite lack of cross-match between donor and recipient – no.	0	0

* In addition to the transfusions registered as violations, 43 patients were transfused above their allocated threshold during surgery. These patients are not included here; the details appear in Table S6.
Hb denotes hemoglobin

† This population consisted of 933 patients (all 998 patients minus the 62 patients who discontinued the trial protocol (see Fig 1) and the 3 patients who received extracorporeal membrane oxygenation).

Table S11. Results of the unadjusted outcome analyses

Outcome	Lower Hb-threshold	Higher Hb-threshold	Relative Risk* (95% CI)	P-value*
Primary outcome measure	*no./total no. (%)*			
Dead at day 90	216/502 (43.0%)	223/ 496 (45.0%)	0.96 (0.83 - 1.10)	0.54
Secondary outcome measures †	*no./total no. (%)*			
Use of life support ‡				
Day 5	278 / 432 (64.4%)	267/ 429 (62.2%)	1.03 (0.93 -1.15)	0.52
Day 14	140 / 380 (36.8%)	135 / 367 (36.8%)	1.00 (0.83 - 1.21)	0.99
Day 28	53 / 330 (16.1%)	64 / 322 (19.9%)	0.81 (0.58 - 1.12)	0.20
Ischemic events in the ICU ¶	35 / 488 (7.2%)	39 / 489 (8.0%)	0.90 (0.58 - 1.39)	0.64
Severe adverse reactions §	0 / 488 (0.0)	1 / 489 (0.2)	-	1.00

* Results of Chi^2 analyses

† Some patients did not wish a part of the follow-up, so there were missing data for 14 patients in the lower Hb-threshold group and 7 patients in the higher Hb-threshold group for the secondary outcome measures.

‡ Defined as infusion of vasopressor or inotropic agents or use of invasive or non-invasive mechanical ventilation or renal replacement therapy on those days. The total no. declined because of patients dying.

¶ Defined as one or more events of acute myocardial, cerebral, intestinal or limb ischemia (the details appear in Table S13 in the Supplementary Appendix).

§ Defined as either allergic, hemolytic, transfusion-associated acute lung injury or transfusion-associated circulatory overload (Table S13).

Table S12. Results of the adjusted analyses of 90-day mortality

	Partially adjusted analyses (stratification variables)*		**Fully adjusted analyses (stratification and design variables) †**	
Populations			**Lowest possible relative risk**	**Highest possible relative risk**
	Relative risk (95% confidence interval) P value		Relative risk (95% confidence interval) P value	
Intention-to-treat N = 998 N-low = 502 N-high = 496	0.94 (0.78 to 1.09) 0.44		0.94 (0.80 to 1.10) 0.90	1.01 (0.68 to 1.17) 0.87
Per-protocol #1 N = 759 N-low = 402 N-high = 357	0.92 (0.75 to 1.09) 0.34		0.93 (0.75 to 1.12) 0.45	1.02 (0.84 to 1.21) 0.83
Per-protocol #2 N = 769 N-low = 400 N-high = 369	0.92 (0.77 to 1.09) 0.37		0.94 (0.77 to 1.12) 0.51	1.03 (0.85 to 1.21) 0.78
Per-protocol #3 N = 661 N-low = 334 N-high = 327	0.95 (0.78 to 1.14) 0.62		0.96 (0.77 to 1.15) 0.68	1.04 (0.85 to 1.24) 0.66

N = number of patients in the total population
N-low = number of patients in the lower Hb-threshold group
N-high = number of patients in the higher Hb-threshold group

*The primary analyses (Column 1) were adjusted for the stratification variables (hematological malignancy and site (all sites including < 10 patients were grouped into one resulting in 20 site variables instead of 32).

† The secondary analyses were adjusted for stratification and design variables (Columns 2 and 3). The design variables were the following predefined baseline risk factors: (a) previous cardiovascular disease Y/N, (b) surgery during the index hospitalization, but prior to randomization Y/N, (c) Age, (d) hemoglobin value, (e) blood transfusion given 24 hours prior to randomization Y/N, (f) binary Simplified Acute Physiology Score (SAPS) II (threshold 53) and (g) binary Sepsis-related Organ Failure Assessment (SOFA) score (threshold 10).[4, 5] These variables were forced into the model.

We had missing SAPS and SOFA scores for 176 and 115 patients, respectively. Therefore we did sensitivity analyses imputing the missing values to test the maximum range of possible results in the multiple logistic regression analysis. At one end missing values were imputed with parameters resulting in the maximum obtainable scores of SAPS and SOFA in the lower Hb-threshold group and the parameters that resulted in the minimum obtainable scores of SAPS and SOFA in the higher Hb-threshold group (Lowest possible relative risk; results in Column 2) and vice versa giving the other extreme (Highest possible relative risk; results in Column 3). As the results of these two sensitivity analyses were comparable to the primary analyses (Column 1 vs. Columns 2 and 3), we did not perform multiple imputation of the missing SAPS II and SOFA scores.

Table S13. Use of life-support, severe adverse reactions and ischemic events

	Lower Hb-threshold (N=488)	Higher Hb-threshold (N=489)
Use of life support †	*no./total no. (%)*	
Day 5*		
Vasopressor/inotropic agent	153/432 (35.4)	140/429 (32.6)
Mechanical ventilation	242/432 (56.0)	238/429 (55.5)
Renal replacement therapy	109/432 (25.2)	88/429 (20.5)
Day 14*		
Vasopressor/inotropic agent	44/380 (11.6)	45/367 (12.3)
Mechanical ventilation	116/380 (30.5)	109/367 (29.7)
Renal replacement therapy	59/380 (15.5)	46/367 (12.5)
Day 28*		
Vasopressor/inotropic agent	9/330 (2.7)	16/322 (5.0)
Mechanical ventilation	35/330 (10.6)	48/322 (14.9)
Renal replacement therapy	24/330 (7.3)	28/322 (8.7)
Severe adverse reactions to blood in ICU	*no./total no.*	
Allergic reactions ‡	0/488	0/489
Acute hemolysis §	0/488	1/489
Transfusion-associated acute lung injury ¶	0/488	0/489
Transfusion-associated circulatory overload ‖	0/488	0/489
Ischemic events in ICU	*no./total no. (%)*	
Cerebral ††	4/488 (1.0)	10/489 (2.0)
Myocardial **	13/488 (2.7)	6/489 (1.2)
Intestinal ‡‡	11/488 (2.3)	14/489 (2.9)
Limb §§	11/488 (2.3)	11/489 (2.3)

* The total numbers of patients were below the group totals because of patients dying. Beyond that we had no missing data.

† Defined as infusion of vasopressor or inotropic agents or use of invasive or non-invasive mechanical ventilation or any form of renal replacement therapy on those days.

‡ Allergic reactions after blood transfusion was defined by the clinician on the basis of muco-cutaneous signs and symptoms (e.g. urticaria, pruritus, localized angio-edema) occurring within 6 hours of red cell transfusion

§ Severe hemolytic complications after blood transfusion was defined by the clinician on the basis of hemoglobinuria or increased free plasma hemoglobin occurring within 24 hours of transfusion.

¶ Transfusion-associated acute lung injury (TRALI) after blood transfusion defined as: I. Acute or worsening hypoxemia ((PaO_2/FiO2 < 40 (PaO_2 in kPa) or <300 (PaO_2 in mmHg) regardless of PEEP) OR > 50% relative increase in FiO_2 AND II. Occurrence within 6 hours after red cell transfusion AND III. Acute or worsening pulmonary infiltrates on frontal chest x-ray OR clinical signs of overt pulmonary edema.

‖ Transfusion-associated circulatory overload (TACO) after blood transfusion defined as: I. Acute or worsening hypoxemia (PaO_2/FiO_2 < 40 (PaO_2 in kPa) or <300 (PaO_2 in mmHg) regardless of PEEP) OR > 50% relative increase in FiO_2 AND II. Occurrence within 6 hours after red cell transfusion AND III. Acute or worsening pulmonary infiltrates on frontal chest x-ray OR clinical signs of overt pulmonary edema AND IV. Increased blood pressure AND VI. Positive fluid balance.

†† Cerebral ischemia was defined as any form of cerebral ischemia on a CT- OR MRI scan.

** Myocardial ischemia was defined as patient diagnosed with acute myocardial infarction (ST-elevation myocardial infarction or non-ST elevation myocardial infarction) or unstable angina pectoris according to the criteria in the clinical setting in question (e.g. elevated biomarkers, ischemic signs on ECG and clinical presentation) AND the patient received treatment as a consequence of this (reperfusion strategies (PCI/thrombolysis) or initiation/increased antithrombotic treatment).

‡‡ Intestinal ischemia was defined as ischemia verified by endoscopy OR open surgery.

§§ Limb ischemia was defined as clinical signs AND need of open/percutaneous vascular intervention, amputation OR initiation/increased antithrombotic treatment.

Table S14. Post-hoc analyses of number of patients with myocardial ischemia

	Lower Hb-threshold	Higher Hb-threshold	Relative Risk (95% CI)	P-value*
	no./total no. (%)			
Myocardial ischemia †	13 / 488 (2.7)	6 / 489 (1.2)	2.17 (0.83 – 5.67)	0.10
STEMI ‡	4 / 488 (0.8)	1 / 489 (0.2)	4.01 (0.45 – 35.73)	0.18
Non-STEMI/unstable angina §	9 / 488 (1.8)	5 / 489 (1.0)	1.80 (0.61 – 5.34)	0.28

* Analyzed by unadjusted chi^2-testing.
STEMI denotes ST-elevation myocardial infarction

† The number of patients having one or more myocardial ischemic events in ICU. Myocardial ischemia was defined as patient diagnosed with acute myocardial infarction (ST-elevation myocardial infarction or non-ST elevation myocardial infarction) or unstable angina pectoris according to the criteria in the clinical setting in question (e.g. elevated biomarkers, ischemic signs on ECG and clinical presentation) AND the patient received treatment as a consequence of this (reperfusion strategies (PCI/thrombolysis) or initiation/increased antithrombotic treatment).

‡ Defined post-hoc as signs of myocardial ischemia (e.g. symptoms, elevated biomarkers or clinical signs) and ST elevations on ECG.

§ Defined post-hoc as non-ST elevation myocardial infarction or unstable angina pectoris according to the criteria in the clinical setting in question (e.g. elevated biomarkers, ischemic signs on ECG and clinical presentation) AND antithrombotic treatment was initiated/increased).

Table S15. Sepsis-related organ failure assessment (SOFA) scoring in the TRISS trial

Organ System	0	1	2	3	4
GCS score	15	13-14	10-12	6-9	<6
Respiration PaO_2 / FiO_2 (in mmHg)	>400	301 - 400	<301	101 - 200 (with respiratory support*)	≤ 100 (with respiratory support*)
(in kPa)	>53	40 – 53	<40	13 – 27 (with respiratory support*)	≤ 13 (with respiratory support*)
Coagulation Platelets (x 10^9 / l)	>150	101 - 150	51 - 100	21 – 50	≤ 20
Liver Bilirubin (mg / dl)	< 1.2	1.2 – 1.9	2.0 – 5.9	6.0 – 11.9	> 12.0
(μmol / l)	<20	20 - 32	33 - 101	102 - 204	>204
Cardiovascular Hypotension	MAP > 70 mmHg	MAP < 70 mmHg	dopamine ≤ 5.0 (doses are given in μg / kg / minute)	dopamine >5.0 (doses are given in μg / kg / minute)	dopamine >15.0 (doses are given in μg / kg / minute)
			or any dose dobutamine	or adrenaline ≤0.1	or adrenalin >0.1
			or any dose milrinone or any dose levosimendan	or noradrenaline ≤0.1 or any dose vasopressin or any dose phenylephrine	or noradrenaline >0.1
Renal Creatinine (mg / dl)	< 1.2	1.2 – 1.9	2.0 – 3.4	3.5 – 4.9	> 5.0
(μmol/l)	< 110	110 – 170	171 – 299	300 – 440	> 440
OR Urine output				or < 500 ml / day	or < 200 ml / day

GCS denotes Glasgow Coma Scale. If a value was not available, the value of the latest obtained sample was used.*Respiratory support was defined as any form of invasive or non-invasive ventilation including mask CPAP or CPAP delivered through a tracheotomy.

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