TRansfusion strategies in Acute brain INjured patients: TRAIN Study
A Prospective Multicenter Randomized Interventional Study

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Key Words: anemia; blood transfusion; brain injury; outcome; clinical study
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List of abbreviations
APACHE II = Acute Physiology and Chronic Health Evaluation
ARDS = Acute Respiratory Distress Syndrome
AVM = artero-venous malformation
CNS = Central Nervous System
CRF = Case Report Form
DSMC = Data and Safety Monitoring Committee
EC = Ethics Committee
ESICM = European Society of Intensive Care Medicine
GCS = Glasgow Coma Scale
eGOS = Extended Glasgow Outcome Scale
Hb = Hemoglobin
ICH = Intracranial Hemorrhage
ICP = Intracranial Pressure
ICU = Intensive Care Unit
mRS = modified Rankin scale
NCS = Neurocritical Care Society
PbtO2 = Brain Tissue Partial Pressure of Oxygen
PI = Principal Investigator
RBC = Red Blood Cells
SAE = Serious Adverse Event
SAH = Subarachnoid Hemorrhage
SIZ = Belgian Society of Intensive Care
SOAP = Sepsis Occurrence in Acutely Ill Patients
SOFA = Sequential Organ Failure Assessment
TACO = Transfusion Associated Circulatory Overload
TBI = Traumatic Brain Injury
TRALI = Transfusion-Related Acute Lung Injury
Abstract

Background: Although blood transfusions can be lifesaving in severe hemorrhage, they could also result in several potential complications. As anemia has also been associated with poor outcome in critically ill patients, optimal transfusion trigger is a real challenge for clinicians. This is even more important in patients with acute brain injury who were not specifically evaluated in previous large randomized clinical trials dealing with the optimal transfusion threshold. Neurological patients may be particularly sensitive to anemic brain hypoxia because of the exhausted cerebro-vascular reserve, which adjust cerebral blood flow to tissue oxygen demand.

Methods/Design: We describe herein the methodology of a prospective, multicenter, randomized, pragmatic trial comparing two different strategies to initiate red blood cells transfusions in patients with acute brain injury: a “liberal” strategy, which aims to maintain hemoglobin (Hb) concentrations above 9 g/dL and a “restrictive” approach to blood transfusion that maintains hemoglobin concentrations above 7 g/dL. Target population includes patients suffering from traumatic brain injury (TBI), subarachnoid hemorrhage (SAH) and intracranial hemorrhage (ICH). The primary outcome is neurological outcome, evaluated using extended Glasgow Outcome Scale (eGOS), at 180 days after the initial injury. Secondary outcomes include, amongst all, 28-day survival, intensive care unit (ICU) and hospital length of stay, the occurrence of extra-cerebral organ dysfunction/failure and the development of any infection or thromboembolic events (either venous or arterial). The estimated sample size to demonstrate a reduction in the primary outcome between groups from 50% to 45% is 4610 patients (2305 for each arm). The study will be initiated in 2015 within several European ICUs and conducted over 4 years.

Expected outcomes/Discussion: This trial will assess the impact of two different strategies to administer blood transfusions in a large cohort of critically ill patients with a primary brain injury. The results of this trial may help to better manage blood products and transfusion thresholds in this specific patients’ population and will provide additional data in some sub-groups of patients at high-risk of brain ischemia, such as those with an intracranial hypertension or developing cerebral vasospasm.

NCT: Pending
**TRansfusion strategies in Acute brain INjured patients (TRAIN Study): A Prospective Multicenter Randomized Interventional Study**

**General information**

- **Short study title:** TRAIN study
- **Protocol:** version 1.0
- **Version date:** 1st February 2015
- **Coordinators:** Fabio Silvio TACCONE, Hôpital Erasme, Brussels, Belgium  
  Mauro ODDO, CHUV Lausanne, Switzerland

**Support:**
The study is endorsed (but not funded) by the European Society of Intensive Care Medicine (ESICM) and the Belgian Society of Intensive Care (SIZ)

- **Principal Investigator:** Fabio Silvio TACCONE (Brussels, Belgium)
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**Database and data analysis platform:** Coordinated by the European Society of Intensive Care Medicine (ESICM)

**Study registration at Clinicaltrials.gov:** NCT (PENDING)
## Protocol Summary

<table>
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<th><strong>Primary outcome</strong></th>
<th>Neurological outcome at 180 days after brain injury</th>
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| **Secondary outcomes** | 1. 28-day survival; eGOS and mRS changes over time  
2. ICU and hospital length of stay  
3. Extra-cerebral organ dysfunction/failure  
4. Infection rate  
5. Composite outcome (death and/or organ dysfunction/failure) |
| **Design** | Prospective, randomized, multicenter, pragmatic, interventional study |

### Target population

**Inclusion criteria**
1. Age ≥18 years and ≤ 80 years  
2. Acute Brain Injury: Traumatic Brain Injury; Subarachnoid Hemorrhage; Intracranial Hemorrhage  
3. Glasgow Coma Score (GCS) on randomization < 12  
4. Expected ICU stay > 72 hours  
5. Hemoglobin (Hb) concentration < 9 g/dL

**Exclusion criteria**
6. Post-anoxic coma; status epilepticus without underlying brain injury; central nervous system (CNS) infections (community-acquired; hospital-acquired; ventriculitis; post-operative)  
7. Known previous neurological disease, causing significant cognitive and/or motor handicap  
8. ICH due to artero-venous malformation (AVM) or brain tumor  
9. Inability (religious reasons) or reduced ability (lack of compatible blood) to receive blood products  
10. Active and uncontrolled bleeding at the time of enrollment  
11. GCS of 3 with both fixed and dilated pupils; Brain death or imminent death (within 24 hours)  
12. Pregnancy  
13. Medical need to correct anemia (e.g. active coronary disease or severe cardiac disease) with target Hb levels > 9 g/dL  
14. DNE (do not escalate) orders  
15. Previous adverse event with transfusion

### Sample size

4610 patients; 2305 in each study arm

### Interventions

Patients will be randomized into two groups
1. **Restrictive transfusion** group = Patients will receive red blood cell transfusion when Hb is below 7 g/dL  
2. **Liberal transfusion** group = Patients will receive red blood cell transfusion when Hb is below 9 g/dL

### Study duration

4 years
Background

Although blood transfusion can be lifesaving in extreme circumstances, in the absence of life threatening hemorrhage, the indications for transfusion are somewhat controversial. Blood transfusions have well-recognized problems, including the need to type and cross match, and the potential transmission of diseases, or the development of transfusion-related complications (such as transfusion-related acute lung injury – TRALI – or transfusion-associated circulatory overload – TACO) and immunosuppression [1-3]. However, anemia too has its own problems and is associated with increased morbidity and mortality among critically ill patients [4, 5]. Determining who and when to transfuse this patients’ population is thus a challenge and recent years have seen continuing debate and discussion regarding the optimal transfusion ‘trigger’.

In a landmark multicenter Canadian trial, Hebert and colleagues [6] randomized 838 critically ill patients to either a liberal protocol where transfusions were administered to maintain hemoglobin levels above 9 g/dl or a restricted strategy where hemoglobin levels were kept between 7 and 9 g/dl. Overall, the 30-day mortality rate was 19 % in the restricted group and 23 % in the liberal transfusion group (p=NS), with a significant difference in outcome among younger patients (i.e. with age less than 55 years: 6% vs. 13 %, respectively - p=0.02) and less sick patients (with APACHE II < 20: 9% vs. 16%, respectively - p=0.03). The overall hospital mortality was also significantly lower in the restricted than in the liberal transfusion group (22% vs. 28%, p = 0.05). These results had a definite influence on ICU practice, encouraging intensivists to limit the use of transfusions. The ABC study [7], an epidemiological survey of 3534 patients conducted in 146 ICUs of West-Europe, confirmed an increased mortality rate (both ICU and hospital) in transfused patients. The increased mortality rates were maintained in a propensity analysis with patients matched for age, sex, disease severity, hemoglobin (Hb) level on admission, a recent history of hemorrhage or anemia and hospital length of stay. In particular, the 28-day mortality was 23% in transfused patients and 17% in those who did not receive a transfusion (p =0.02). Moreover, in a multivariate analysis, receipt of a blood transfusion increased the risk of dying by a factor of 1.4. Nevertheless, a more recent study on the Sepsis Occurrence in Acutely Ill Patients (SOAP) database (n=3147) found that 1040 (33%) patients received a blood transfusion. Also, there was a direct relationship between the number of blood transfusions and the mortality rate, but in the multivariate analysis, blood transfusion was not significantly associated with a worse mortality rate [8].
Importantly, most of these studies did not consider the presence of acute brain injury as a specific target population in whom transfusion threshold could be critical. Even isovolemic anemia (Hb of 5 g/dL) induced in healthy volunteers, resulted in some alterations in memory and motor skills [9]; however, these Hb levels are not currently allowed in critically ill patients. Interestingly, in acute brain injury, the Hb threshold associated with potential cerebral hypoxia may be higher, because of the exhausted cerebro-vascular reserve, i.e. cerebral vasodilation that adapts cerebral blood flow to tissue oxygen demand [10]. As such, several studies have shown that Hb levels below 9 g/dL were associated with a poorer outcome in patients with from traumatic brain injury (TBI) or subarachnoid hemorrhage (SAH) [11, 12]. On the other hand, the administration of red blood cells (RBC) transfusion was also associated with an increased risk for complications or mortality in this setting [13, 14]. A recent meta-analysis showed that studies aiming to compare two different transfusion thresholds in these patients’ populations were largely underpowered to identify the best Hb levels [15]. Accordingly, the effects of transfusion need to be better assessed in acute brain injury.

Only one recent randomized clinical trial compared in a factorial design the effects of erythropoietin and two Hb transfusion thresholds (7 g/dL vs. 10 g/dL) on neurological recovery after TBI (n=200) [16]. Favorable neurological outcome was 43% for the hemoglobin transfusion threshold of 7 g/dL and 33% for 10 g/dL (p = 0.28). Moreover, there was a higher incidence of thromboembolic events for the transfusion threshold of 10 g/dL (22% vs. 8%; p = 0.009) than the other. Nevertheless, the number of patients included in the study was relatively small and the two groups of patients showed mean Hb levels much higher than those associated with the treatment arm in which they were randomized.

Thus, the aim of the current study is to determine whether a “liberal” strategy of maintaining Hb concentrations above 9 g/dL would result in a different neurological outcome when compared to a “restrictive” approach to red-cell transfusion to avoid hemoglobin concentrations < 7 g/dL in critically ill anemic patients (i.e. Hb< 9 g/dL) with acute brain injury.

**Methods/Design**

**Study Design**
This is a prospective, multi-center, randomized, pragmatic, controlled study conducted in different European intensive care units (ICUs). Patients are randomized to two different thresholds of Hb to initiate RBC transfusions (< 7 g/dL vs. < 9 g/dL).

Study sites

The complete list of recruiting sites is shown in Appendix 1. The selection of participating ICUs has been initiated within the Neuro-Intensive Care (NIC) Section of the European Society of Intensive Care Medicine (ESICM), with the identification of national investigators which have thereafter selected any potential ICUs with neurosurgical facilities and an adequate numbers of patients (>50) with an acute brain injury admitted per year.

Screening and Inclusion/Exclusion criteria

All patients admitted to the ICU because of a TBI, SAH or intracranial hemorrhage (ICH) will be screened for study eligibility during the first 10 days after the initial injury. Anemia is defined as Hb < 9 g/dL and should occur within this time period (Figure 1).

A log list will be kept of all patients who are screened for the study. If a patient is considered as ineligible for the study, the reason will be recorded as for eligible patients who decide not to participate to the study. No other particular information for such patients will be recorded. Inclusion and exclusion criteria are to be checked by the attending physician and confirmed by the local investigator.

Inclusion criteria are:
1. Age ≥ 18 and ≤ 80 years
2. Glasgow Coma Score (GCS) on randomization < 12 (see Appendix 2)
3. Expected ICU stay > 72 hours
4. Hb concentration < 9 g/dL within 10 days from brain injury

Exclusion criteria are:
1. Post-anoxic coma; status epilepticus without underlying brain injury; central nervous system (CNS) infections (community-acquired; hospital-acquired; ventriculitis; post-operative)
2. Known previous neurological disease, causing significant cognitive and/or motor handicap
3. ICH due to artero-venous malformation (AVM) or brain tumor
4. Inability (religious reasons) or reduced ability (lack of compatible blood) to receive blood products
5. Active and uncontrolled bleeding at the time of enrollment
6. GCS of 3 with both fixed and dilated pupils; Brain death or imminent death (within 24 hours)
7. Pregnancy
8. Medical need to correct anemia (e.g. active coronary disease or severe cardiac disease) with target Hb levels > 9 g/dL
9. DNE (do not escalate) orders
10. Previous adverse event with RBC transfusion

**Randomization**

Eligible patients (whenever possible) and the next of kin should be informed about the rationale and the aims of the study and potential risks of blood transfusion. Local ethical regulations should be otherwise followed. A written informed consent must be obtained prior to the randomization to the study. Considering the difficulties to predict the exact moment for anemia occurrence during the ICU stay, it is strongly recommend to obtain a written informed consent since ICU admission in order to initiate randomization whenever necessary.

Randomization will be performed using a computer generated random sequence (variable blocks of 4, 6 and 8), stratified by center, by disease (TBI, SAH or ICH) and by the GCS at the moment of randomization (3-5; 5-8; 8-11). The ICU or hospital personnel will be not blinded to the treatment assignment, since patients are easily distinguishable from routine daily assessment of Hb concentrations. However, the final neurological evaluation of the patient will be performed by doctors/nurses who are blinded to patient’s group assignment.

**Trial conduct**

The trial will be conducted in adherence to the current Helsinki Declaration and to the standards of good clinical practice. Screening of patients will only start after approval by the ethics committees (EC) in the countries of the trial sites. No deviation from the protocol will be implemented without the prior review and approval of the ECs. Trial details and the trial protocol will be entered into a public database prior to randomization of the first participant (www.clinicaltrials.gov).
**Consent/Ethics**

The practical modalities of the informed consent will be left to each institution according to National Laws: patient inclusion is not considered valid if written consent is not obtained from the patient or their legal representative. For patients who are unable to consent, their legal representative will be informed of the study as soon as possible and must sign for participation to the trial before randomization. Subjects who will show neurological recovery will be informed of their study participation and be asked to provide their consent for the use of their data. Patients or next of kin must have the option to withdraw consent at any time during the study and without giving a specific reason. Withdrawal should not influence patients’ standard of care, with an Hb threshold for RBC transfusion that will be then decided by the attending physician. Patients withdrawing from the study should consent for the inclusion of data collected before withdrawal. The Site Investigator may withdraw a subject from the study for safety reasons (i.e. acute myocardial infarction needing higher Hb levels). In these cases, data surrounding the event leading to subject withdrawal will be retained for safety analyses.

Obtaining approval by local ECs is the responsibility of each investigator. However, national investigators will be designed to facilitate the process of submission. The material provided by the steering committee and the principal investigator (PI) could be used to obtain this approval.

The PI may decide to close a study site when one of the following occurs: a) the Site Investigator at an individual site fails to enroll patients into the study at an acceptable rate ($\leq$ 10 patients/year); b) Site Investigator fails to adhere sufficiently to protocol requirements (i.e. protocol deviation or violation); c) Site Investigator knowingly submits false information from the research facility to the Principal Investigator, Steering Committee or appropriate regulatory authority; d) Site Investigator does not provide clinical and follow-up information on the included patients within an acceptable time-frame (i.e. one week from randomization for demographics; one month from ICU discharge or death for daily data collection; one month from 6-month assessment for survivors). If the study is terminated early, all specified follow-up data on subjects enrolled prior to termination will be collected and reported.

**Study Treatment**

All patients should preferably receive one unit of RBC at a time; no particular recommendation is given concerning the “age” of RBC (i.e. length of storage time, which should be reported whenever possible). Patients randomized to the Hb threshold for
transfusion of < 7g/dL will have Hb concentrations maintained at least 7g/dl with transfusion of packed RBCs whenever the patient’s hemoglobin level is found to be less than 7g/dl; similarly, patients randomized to the threshold of <9 g/dL will be managed accordingly whenever the patient’s hemoglobin level is found to be less than 9 g/dl. Transfusions thresholds will be maintained until a maximum of 28 days after randomization or hospital discharge/death, whichever occurs first. Daily Hb concentrations will be recorded at least every 12 hours to avoid protocol violation. Patients are allowed to be included in the study only once. Previously included patients who are readmitted to the ICU after the 28 days since randomization are managed according to the attending physician. No data will be recorded for readmitted patients.

Protocol Violation

Protocol violation is defined as one of the following:

- The inability to maintain the daily max Hb values within <7.8 g/dL in the restrictive group or > 9 g/dL in the liberal group for two consecutive days
- One or more transfusions given contradictory to the trigger level of assignment
- One or more transfusions given despite the occurrence of previous adverse event related to transfusion
- Lack of matching between donor and recipient or transfusion of RBC unit scheduled for another patient

Patients’ management

Management of the underlying disease (i.e. TBI, SAH or ICH) will be at discretion of the attending physicians; the use of international guidelines for the monitoring and the adequate therapeutic interventions are recommended in all these patients.

Patients with severe TBI should be managed according to the Brain Trauma Foundation (BTF) guidelines; the third edition of these recommendations has been published in 2007 [17-34] and underlined the importance of early stabilization of the patient, if still unstable, the need for monitoring and prevention of intracranial hypertension, the maintenance of an adequate and stable cerebral perfusion pressure (CPP), the avoidance of systemic secondary brain insults (i.e. hypoxemia, hyperglycemia, hyponatremia etc.) and the optimization of cerebral hemodynamic and oxygenation.
For patients with SAH, recent recommendations (including prevention of re-bleeding; including early aneurysm treatment; glucose, blood pressure and temperature control; oral nimodipine for prevention of delayed cerebral ischemia; hypertensive and/or hyperdynamic therapy for delayed ischemic neurological deficit) will be considered in the management of such patients [35]. Finally, treatment of patients suffering from ICH will be in accordance with recent European guidelines [36], which suggest avoiding hemostatic therapy for acute ICH when it is not associated with antithrombotic drug and using blood pressure lowering for secondary prevention, early surgery for patients with a GCS score between 9 and 12 and avoidance of corticosteroids.

For those centers in which a cerebral multi-modal monitoring (in particular, brain tissue oxygen monitoring – $P_{btO_2}$) has been implemented in patients with TBI, SAH or ICH, RBC therapy cannot be driven by those monitoring devices and should strictly follow the randomization targets.

**Data collection**

Data collection on admission include: demographic characteristics; comorbidities; source of admission; primary and secondary admission diagnoses; APACHE II score (the worst of the first 24 hours); SOFA score on admission; GCS immediately after injury; GCS on hospital admission after initial resuscitation; initial Hb concentration; sodium (mEq/L) and glucose (mg/dL) levels.

For patients with TBI, the following data will also be collected: Marshall score on cerebral CT-scan (the worst of the first 24 hours); presence of traumatic SAH AND/OR epidural mass on CT-scan; pupillary reactivity; mechanisms of injury; hypoxemia ($SpO_2<90\%$) AND/OR hypotension (systolic blood pressure < 90 mmHg) before or on hospital arrival; ICP monitoring within the first 48 hours; previous therapy with antiplatelet drugs or anticoagulants.

For patients with SAH, the following data will also be collected: WFNS score; Fisher CT scale; ICP monitoring within the first 48 hours; pupillary reactivity on arrival; hydrocephalus; diagnosis of vasospasm (either using trans-cranial Doppler [TCD], contrast CT-scan and/or angiography); development of delayed neurological ischemic deficit (DNID); therapies for DNID; occurrence of DCI. TCD-vasospasm was defined as a mean flow velocity in any vessel >200 cm/sec or >120 cm/sec AND a Lindegaard ratio above 3 [37]. Angiographic or contrast CT-scan vasospasm was defined by a neuroradiologist as moderate-to-severe arterial narrowing (>50%) on specific imaging not attributable to atherosclerosis, catheter-induced
spasm or vessel hypoplasia [38]. Definition of DNID was based on the development of new focal neurological signs, deterioration in level of consciousness, or both, when the cause was felt to be ischemia attributable to vasospasm after other possible causes of worsening (i.e. hydrocephalus, seizures, metabolic derangement, infection or excessive sedation) are excluded [38]. Finally, DCI was defined as the appearance of new infarction on cerebral CT-scan or magnetic resonance imaging (MRI), when the cause was attributed to vasospasm [39].

For patients with ICH, the following data will also be collected: ICH volume above 30 ml on the initial CT-scan; presence of intra-ventricular hemorrhage; location (deep, cortical, infratentorial); ICP monitoring within the first 48 hours; pupillary reactivity on arrival.

Daily data collection (only during ICU stay) will include: best GCS score (days 1, 2, 3, 5, 7, 10, 14, 21, and 28/or the day of ICU discharge); Hb concentration (minimum and maximum); sodium (mEq/L) and glucose (mg/dL) levels at 8am (or the first value of the day); ICP levels at 8 am (or the first value of the day); maximum ICP levels during the day; SOFA score; the presence of infection (location, pathogen, treatment); sepsis syndromes (i.e. sepsis, severe sepsis or septic shock; the occurrence of serious adverse events (see paragraph on adverse events); the need for second-tier therapies for increased ICP (hypothermia; barbiturates; decompressive craniectomy) or seizures (convulsive or non-convulsive).

Also, data on each transfused RBC unit during the ICU stay, premature study termination (documenting the reason and time of termination) will be recorded. In case of death, reasons for withdrawal of care will be collected. Glasgow Coma Outcome scale will be evaluated on ICU discharge and hospital discharge, as well as ICU and hospital length of stay, the duration of mechanical ventilation over the first 28 days (either by endotracheal tube or tracheostomy), the need for tracheostomy on ICU discharge and location of discharge after hospital stay (home vs. rehabilitation vs. nursing home). Follow-up at 180 days for primary outcome measure assessment will be performed.

Follow-up
Neurological status will be classified using the extended Glasgow Coma Outcome scale [40] and modified Rankin Score (mRS) [41]. Subjects will be followed until death or hospital discharge. If alive but not in the hospital after 180-200 days since injury, neurological status will be evaluated by a visit with a neurologist (blinded to the assignment to one of the two arms of therapy); if not possible, the GOS will be completed with the General Practitioner
(GP) or after a 5-min telephone interview performed by a nurse or doctor not involved in the study and patient’s management.

**Study Outcomes**

The primary outcome measure is the proportion of patients with good neurological outcome at 180 days after randomization, assessed by the extended Glasgow Outcome Scale (eGOS) and dichotomized as “poor” (eGOS 1-5) and “good” (eGOS 6-8).

The secondary outcome measures are:

1. 28-day survival
2. eGOS and mRS changes over the two groups
3. ICU and hospital length of stay
4. Presence and severity of extra-cerebral organ dysfunction/failure, assessed using daily SOFA score
5. Infection Rate, except those involving only the CNS
6. Composite outcome (death and/or organ dysfunction/failure)
7. For centers using monitoring of brain tissue oxygenation (PbtO2) catheters, time spent with P_btO2 < 20 mmHg (brain hypoxic burden)
8. Serious adverse events (SAE)

**Serious Adverse Events**

A complete summary of the adverse events reported in each group and the total number of adverse events of each type will be reported each 6 months to the Data Safety Monitoring Committee (DSMC). In particular, the following SAEs will be reported:

a) Severe Hypertension = MAP > 130 mmHg in absence of vasopressor agents and increased intracranial hypertension and needing specific therapy

b) Venous Thrombotic Events = deep vein thrombosis (formation of a blood clot within a deep vein in the legs or arms, that could be associated with non-specific signs, such as pain, swelling, redness, warmness, and engorged superficial veins – it can diagnosed either by echography, venography or CT imaging); pulmonary embolism (formation of a clot within pulmonary arterial circulation, that is diagnosed by contrast pulmonary CT-scan or echocardiography)
c) Acute Myocardial Ischemia = acute myocardial infarction (ST-elevation and non-ST elevation myocardial infarction) or unstable chest pain diagnosed during current hospital admission, according to specific criteria (i.e. elevated biomarkers of myocardial injury, ischemic signs on ECG, clinical suspicion) AND the patient has received a specific treatment (reperfusion strategies such as percutaneous coronary intervention [PCI] or thrombolyis) or initiation/increase of antithrombotic drug treatment during current ICU admission.

d) Cerebral ischemia = new cerebral ischemic areas on cerebral CT-scan or MRI.

e) Intestinal ischemia = ischemic lesions confirmed by endoscopy AND/OR open surgery.

f) Acute peripheral limb ischemia = clinical signs AND the need of open or percutaneous vascular intervention, amputation or initiation/increased antithrombotic treatment.

g) Anaphylactic reaction to RBC transfusion = cutaneous signs (i.e. urticaria, pruritus) AND/OR hemolytic anemia within 24 hours from transfusion.

h) ARDS = acute hypoxemia with bilateral infiltrates according to recent definitions [42].

i) TRALI = ARDS occurring within 6 hours after RBC transfusion.

j) TACO = Acute hypoxemia (PaO2/FiO2 < 300 regardless or positive end-expiratory pressure [PEEP] with bilateral lung infiltrates AND occurrence within 6 hours after RBC transfusion AND increased blood pressure AND Positive fluid balance.

k) Sepsis = presence of a Systemic Inflammatory Response Syndrome (SIRS) plus a suspected/confirmed infection. Sepsis with arterial hypotension despite adequate fluid resuscitation needing vasopressor therapy will be defined as septic shock [43].

l) Organ Failure = organ failure is defined as a specific organ sub-score > 2 [44].

m) Infection = infections are identified according to CDC definitions [45].

n) Brain Tissue Hypoxia = for those patients with a PbtO2 monitoring, values of < 20 mmHg for at least 1 hour identify this condition.

Statistical analysis

The primary outcome measure of this study is neurological intact survival at 180 days, evaluated by the eGOS of 6-8. To calculate the sample size, we estimated a mortality rate of
15% and a percentage of poor neurological outcome of 35% (i.e. eGOS 1-5 of 50%). Thus, a total of 2095 patients should be recruited for each arm to achieve a power of 90% at a two-sided alpha level of 0.05 or less to detect an improvement of good outcome rate at 180 days from 50 to 45% (absolute reduction of 5%, i.e. 10% relative reduction) in one of the two arms. Considering a potential 5% of follow-up lost and 5% of protocol violation, a total of 4610 (2305 for each arm) is needed to complete the study. Analysis of data will be based on "Intention-to-treat". Statistical analysis will be performed using the last version of SPSS for Windows (Chicago, USA). Continuous variables are summarized using medians and quartiles and analyzed using a Wilcoxon rank sum test. Categorical variables are analyzed using Fisher exact test. The primary outcome comparisons will be analyzed using a Chi-square analysis and will be processed by an independent statistician. Primary outcome will also be adjusted for pre-specified covariates (stratification criteria) and presented for each category of brain injury (TBI, SAH, ICH). The Cox proportional hazard model will be used to determine time-to-event hazard ratios and 95% confidence intervals.

A first interim analysis will be performed after 200 patients; conditional power for meeting the primary endpoint will be, if needed, recalculated at that time. Other interim analyses will be performed at 500 patients and then each 500 patients included. To obtain a convenient sample for each of the brain injury evaluated in this study, a maximal recruited number of patients of 2000 for TBI, 1500 for SAH and 1200 for ICH is scheduled. Early stopping for efficacy reasons or lack of efficacy (i.e. futility) will only be considered if major outcome differences are seen between the groups according to the the interim analyses and using the O’Brien-Fleming stopping boundary rule. The study is expected to last 4 years, assuming the inclusion of 75-95 patients/month in 50-70 different centers.

Stratified analyses will be performed for patients:

a) Underlying brain injury (TBI vs. SAH vs. ICH).

b) GCS at the moment of randomization (3-5; 5-8; 8-11); the same different GCS categories will be analyzed within the different forms of brain injury.

c) Presence of increased intracranial hypertension (defined as the need for specific therapies to reduce intracranial pressure [ICP] - if no ICP monitoring, then the patient is considered as not having intracranial hypertension); the same analysis will be performed within the different forms of brain injury.

d) By age (< 45 years or ≥ 45 years).

e) SOFA on randomization (<8 and ≥ 8).
f) For patients with TBI: presence of hypoxemia AND/OR hypotension before hospital arrival (yes/no)

g) For patients with TBI: presence of hypoxemia AND/OR hypotension on hospital arrival (yes/no)

h) For patients with SAH: presence of vasospasm (yes/no)

i) For patients with SAH: presence of DNID (yes/no)

j) For patients with SAH: presence of DCI (yes/no)

k) For patients with ICH: blood volume (≤30/>30 mL)

Data Safety Management

Members of the Data Safety Monitoring Committee (DSMC) are individuals free of conflicts of interest for this protocol; DSMC will analyze the safety of the study. DSMC membership is to be for the duration of the clinical trial. If any members leave the DSMC during the course of the trial, the Steering Committee will decide for replacement. Sites will record all SAEs that occur within 7 days of enrollment directly in the electronic case report forms (eCRF). Abnormal laboratory values are expected in these patients, and these are not to be recorded as SAEs. The occurrence of SAEs will be then evaluated by the DSMC at the different interim analyses.

Formal meeting will be held for each interim analysis to review the data related to the primary outcome, the safety findings as well as the quality of trial conduct. To enhance the integrity of the trial, the DSMC will have access only to the different results aggregated by treatment group and will remain unaware of the treatment assignment (the two groups will be encoded as A and B). Importantly, a report including data on recruitment and baseline characteristics, and pooled data on eligibility violations will be prepared by the statistician for each DSMC meeting. Only the independent statistician will have access to the whole database. A closed report will be then prepared to allow confidential discussion of clinical data and the DSMC has to prepare minutes of their meetings, with a list of recommendations for the Steering Committee (to continue, to hold or to terminate the trial). If the recommendation is to stop the trial, a final decision will be made after the analysis of all patients included at the time (including patients randomized after data collection for the DSMC meeting). The Steering Committee will be responsible for deciding whether to continue, hold or stop the trial based on the DSMC recommendations. The DSMC will be notified of all changes to the trial protocol or conduct.
Funding
The study is endorsed by the European Society of Intensive Care Medicine (ESICM) and the Belgian Society of Intensive Care (SIZ); no specific finding has been obtained. Dr. FS Taccone received the NeXT Grant at the 27th Congress of ESICM (Barcelona, 2014) to initiate this study.

Organization
Data will be recorded using pre-printed CRF by the attending intensivist or a trained research nurse. All data will be then reported in a web-based database. Data will be periodically introduced on the website by trained personnel. The study coordinator will contact each time a patient is included the local PI to ensure data collection and reporting as well as completion of patient follow-up or on premature termination of the study protocol. The individual data provided by a participating ICU are primarily the property of the ICU who generated the data. All investigators have the right to access their data at any time.

The PI of the study has the responsibility to perform periodic and spot checks visits to monitor the progress of the clinical study. Completed CRFs will be reviewed for completeness, compliance with the investigation plan, and appropriate device use and accountability. Case Report Forms will be provided to each site for each subject enrolled in the study. Completed CRFs will be uploaded on a website developed with the help of the ESICM and the independent statistician, which will be overseeing data entry and data quality management. Data on safety will be provided to the DMSC with regular time intervals. The Steering Committee will review study integrity, safety and risk/benefit issues at periodic intervals throughout the study (each 6 months). The frequency of these reviews will be dependent upon the rate of patient enrolment and relevant safety issues. Independent analyses of serious adverse events will be performed and adjudicated if the frequency or nature of serious adverse events warrants it.

Data control will involve the following levels:

a) All participants will be provided with detailed information, including exhaustive definitions of medical terms. The coordinating center will provide a rapid response for any query throughout the study period.

b) Data entry will involve trained personnel and data plausibility check will start at the entry level electronically, setting validity limits for each variable. Investigators will be queried in case of outliers or excessive numbers of missing values.
c) Random ICUs will be visited and patients’ reports will be matched to previously collected data.

The steering committee, on behalf of the investigators has the right to use all data that are pooled in the databank for scientific purposes. Investigators will be regularly informed about ongoing study activities. All participants have the right to access the data, pooled in the databank, for research purposes after the research project has been terminated, and with the approval of the steering committee. A copy of the databases generated by the project can only be provided to third-part entities after specific approval by the participating ICUs.

A copy of the electronic databank will be kept in the coordinating centers and preserved for 15 years for subsequent use by the steering committee and investigators. It is recommended that a copy of CRFs be kept at each center for future reference.

**Publication rules**

The trial will be registered on www.clinicaltrials.gov. The final protocol will be published as a design and rationale paper including the plan for analyses. Steering committee members will be all part of the writing committee and listed as authors in the final manuscript. All centers that have at least 40 patients recruited will earn an authorship in the “authors’ list”; a second author will be allowed for each 40 additional patients recruited. Authors list will take into account the number of enrolled patients; FS Taccone, JL Vincent and M Oddo will be first, second and last authors. For other co-authors, in case of a similar number of recruited patients, the participation to data analysis and contribution to the manuscript will be considered for the order authorship. Upon trial completion the main manuscript will be submitted to one of the major clinical journals regardless of the result, and the results will in any case be published at the TRAIN trial homepage.
References


Figure 1. Study flow diagram.
Appendix 1: TRAINNational Investigators and Recruiting Centers

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- Geert MEYFROIDT (Leuven)

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