Appendix 1: Supplementary methods, results and tables (as supplied by the authors)

METHODS

RANDOMIZATION

Blood bank personnel were notified of a potential eligible patient by either an automatic alarm which sounded if the blood refrigerator in the trauma room was accessed for uncrossmatched RBCs or by the trauma team leader. A study research coordinator was then notified by blood bank to review the inclusion and exclusion criteria prior to enrolment of a patient. The study research coordinator would also answer any questions and obtain informed consent from the patient/ substitute decision maker, if possible, prior to randomization. S/he was also available to answer any questions about the treatment or control arms, if requested by any of the clinical attending staff. Eligible patients were then randomized by the blood bank.

The randomization was stratified by type of trauma (blunt versus penetrating), and with an allocation sequence derived from blocks of four for the control and fixed-ratio groups. Type of trauma may be associated with the likelihood of receiving massive transfusion and the development of coagulopathy; therefore it might influence outcomes. In addition, penetrating trauma represents only approximately 15 - 20% of all trauma admissions at our institution.

STUDY PROCEDURES

Canadian Blood Services (CBS) supplied all red blood cells (RBCs), frozen plasma (FP) and platelets (PLTs) administered in this trial. FP is used to indicate plasma frozen within 24 hours of collection, as oppose to FFP, which has a shorter processing time (eight hours) from collection to freezing. All RBCs and PLTs were prestorage and leukoreduced. During the study time period, a male-predominant plasma policy was in effect and all PLTs were from male apheresis donors units or buffy coat pools resuspended in male plasma.

Crystalloid and colloid administration was not standardized, but intentionally limited. Cryoprecipitate was transfused in pools of 10 units to keep fibrinogen above 1·0 g/L in both arms. During the study period, thromboelastometry/graphy were not available for transfusion decisions.

STUDY SECONDARY END POINTS

Briefly, other secondary outcomes included, but are not limited to, bleeding-free hours up to 24 hours, coagulation competence defined by correction of standard coagulation assays and clotting factor activities, ventilator-free days, hospital and ICU length of stay, transfusion requirements, and thromboembolic event rates. After transfusion protocol termination, we also evaluated the need for additional transfusions and further resuscitation during the following 24 hours for each patient.

Cause of death was adjudicated by an independent physician and one of the principal investigators, blinded to the treatment allocation. Acute respiratory distress syndrome was assessed by an independent physician who performed clinical assessment and gathered reports for chest radiograph from blinded radiologists.

DATA MONITORING AND SAFETY BOARD

During the study, an independent Data Monitoring and Safety Board (DMSB) committee was provided with blinded data on death and complications to ensure the safety of study participants. In addition, this committee reviewed study cases considered for post-randomization exclusions. These were patients that met inclusion criteria, were randomized but later were found to have exclusion criteria not identified due to the narrow window preceding enrolment. The DMSB committee was blinded to the randomization status of each patient.

Appendix to: Nascimento B, Callum J, Tien H, et al. Effect of a fixed-ratio (1:1:1) transfusion protocol versus laboratory-guided transfusion in patients with severe trauma: a randomized feasibility trial. *CMAJ* 2013. DOI:10.1503/cmaj.121986.

RESULTS

CO-INTERVENTIONS

Regarding co-interventions during transfusion protocols, the group allocated to lab-guided received more crystalloid than the fixed-ratio intervention arm (Table A). In addition, the lab-guided arm required more cryoprecipitate than the fixed-ratio group (Table A). Similar frequencies and times to urgent trauma surgery or angioembolization; percentages of decompressive craniectomy; and the utilization of pro-hemostatic agents were observed between both groups (Table A).

MISSED CASES AND POST-RANDOMIZATION EXCLUSIONS

During the first six months of the study, the trauma team was primarily responsible for notifying research personnel of eligible patients; and five eligible patients were missed. The study steering committee then requested the Blood Bank staff to notify the research team of all emergency use of uncrossmatched RBC for trauma patients, which resulted in a reduction in the number of missed cases (to two misses for the remainder of the study). Patients were randomized at a median of 55 min (interquartile range, 35 to 110) after hospital arrival. After reviewing data on randomization errors, the DMSB agreed to exclude six patients post-randomization, three from each group. One patient with non-hemorrhagic shock (cardiac tamponade), one with unsalvageable TBI and another older than 90 years of age were excluded from the fixed-ratio group. One patient with unsalvageable TBI, one on Coumadin and another enrolled more than six hours of injury were excluded from the lab-guided group. In addition, three patients in the lab-guided control arm were excluded after refusing to continue in the study, including one Jehovah's Witness. Therefore, 69 patients were included in the analysis and had complete follow-up.

FLUID REQUIREMENT AFTER STUDY INTERVENTIONS

During the 24 hours following the end of transfusion protocols, the fixed-ratio group required less crystalloid than the lab-guided arm (2645 ml [interquartile range, 1800 to 4300] vs. 3950 ml [interquartile range, 2325 to 5299]; $P = 0.05$). However, no differences were noted with respect to blood, blood products and colloid requirements (data not presented).

CLINICAL END POINTS

Three cases of deep venous thrombosis and one case of abdominal compartment syndrome were diagnosed in the fixed-ratio group, and none in the control arm.

TABLES

Table A: Co-interventions during Study Transfusion Protocols*

	Fixed-ratio (N = 37)	Control (N = 32)
Protocol duration, median (IQR), hr	5 (2.3-6)	4.7 (3-7.2)
Urgent operation/angioembolization, no, %	22 (59)	21 (67)
Time to operation/angioembolization, no, %, min†	99 (66-148)	100 (67-167)
Decompressive craniectomy, no, %	2 (5)	2 (6)
Crystalloid, median (IQR), ml	4900 (3000-7150)	6050 (4000-8781)
Colloid, median (IQR), ml	0 (0-0)	0 (0-625)
Cryoprecipitate units, median (IQR)	0 (0-0)	0 (0-10)
Recombinant factor VIIa	0	0
Tranexamic acid, no, %	5 (13.5)	6 (18.7)

Abbreviation: IQR, interquartile range.

* Except for crystalloid utilization, there were no significant differences between the two groups in any of other baseline characteristics.

Data are number (% of the group), mean (SD) and median (IQR).

† Denotes time from hospital admission to either urgent operation or angioembolization.

Table B: Coagulation Tests at the End of Study Transfusion Protocols and Bleeding-free Hours*

	Fixed-ratio (n = 37)	Control (n = 32)	p value‡
International normalized ratio, median (IQR)	1.19 (1.1-1.3)	1.34 (1.2-1.5)	0.01
Fibrinogen, mean (SD), g/L	1.69 (0.6)	1.45 (0.5)	0.23
Partial thromboplastin time, median (IQR), s	30 (27-38)	33 (28-44)	0.28
Platelet count, median (IQR), x 10^9/L	113 (95-142)	93 (71-136)	0.25
Hemoglobin, mean (SD), g/L	90 (23)	89 (21)	0.90
Bleeding-free hours, median (IQR), h∫∫	15 (0-20)	19 (9-21)	0.26

Abbreviations: IQR, interquartile range; SD, standard deviation.

* Blood work performed within 2 hours after study transfusion protocols.

‡ All two-sided P values were calculated with the use of chi-square test or Fisher's exact test to compare proportions, and the Wilcoxon rank-sum test or Student's t-test to compare distributions, as appropriate.

∫∫ Denotes numbers of hours between cessation of bleeding and 24 hours after study enrolment; a score of zero is assigned to patients who died by exsanguination.

Table C: Secondary End Points

	Fixed=ratio (n = 37)	Control (n = 32)	p value*
Intensive care admission, no, %†	26 (70)	30 (94)	0.03
Died in operating room, no, %†,‡	8 (22)	1 (3)	0.03
Ventilator-free days, median (IQR), days∫∫	18 (0-26)	20.5 (3-25)	0.84
ICU-free hospital days, median (IQR), days¶	23 (12-26)	20 (5-24)	0.27
Hospital-free days, median (IQR), days**	0 (0-15)	1.5 (0-12)	0.39

Abbreviation: IQR, interquartile range.

* All two-sided P values were calculated with the use of chi-square test or Fisher's exact test to compare proportions, and the Wilcoxon rank-sum test to compare distributions, as appropriate.

† Data are numbers and proportions of total for each group.

‡ Denotes patients who died during surgery and thus never admitted to intensive care.

∫∫ Defined as the number of days between liberation from mechanical ventilation and day 28 after enrolment.

¶ Defined as the number of days between discharge from intensive care unit and day 28 after enrolment.

** Defined as the number of days alive between hospital discharge and day 28 after enrolment.

∫∫, ¶, ** A score of zero is assigned to patients who died between getting off ventilator; being discharged from intensive care or hospital, and day 28 after enrolment.