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Title: Assessing Response to Changing Plasma:Red Cell Ratios in a Bleeding Trauma Patient

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Abstract: N/A

Response to Reviewers: April 4, 2009

Douglas White MD, MPH, MBA
Medical College of Virginia/VCU, Richmond

Dear Dr. White:

Thank you very much for sending your reviewer's comments. We found them very useful and revised our manuscript accordingly. We are pleased to re-submit our article: Assessing Response to Changing Plasma:Red Cell Ratios in a Bleeding Trauma Patient for consideration by the American Journal of Emergency Medicine as a Case Report.

With regard to Reviewer#1's comments:

i) We changed the title and are resubmitting the manuscript as a case report.

ii) We removed the Abstract.

iii) For methods, we added quite a bit to methods. First, we explicitly stated that "we assessed the dose:response of increasing the ratio of plasma to red blood cells transfused …" as recommended by the reviewer. To help clarify the measurements, we then explicitly stated that we were measuring INR, PTT, thrombolelastograms and clotting factor levels at four different time points, and we labeled those consistently throughout the manuscript (Points A, B, C, D).

iv) As suggested, we then reported the results of all measures of coagulation at these four points in time. We included these time points in all three figures.

v) We revised our conclusions in the Discussion section. Specifically, we stated that increasing the plasma:RBC ratio appeared to correct the hemostatic mechanism as measured in our case report.

vi) We removed the term "paradigm shift" in the Introduction, with regards to damage control resuscitation.

vii) We outlined Methods before the Case Report.

viii) Limitations: we included a discussion that changes in the measurements of coagulation in this patient are only associated with a change in FFP:RBC ratios, and do not demonstrate cause/effect. We added a statement that a randomized controlled trial is required to definitively demonstrate efficacy of this approach in reversing coagulopathy.

ix) We added the missing references.

We did keep the headings, however. Because there is a technical component to describing how the clotting factor assays and thromboelastography are done, we felt that the case report was

better organized with the headings. Reviewer#1 only suggested that we change the order of “Methods” and “Case Report”.

Thank you again for forwarding the comments. Thank you also for re-considering our manuscript.

HC Tien, MD MSc FRCSC FACS
Lieutenant Colonel, Canadian Forces
Assistant Professor
Universitiy of Toronto

April 4, 2009

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Case Report

Title: Assessing Response to Changing Plasma:Red Cell Ratios in a Bleeding Trauma Patient

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Running Head: Plasma Transfusion and Trauma

Introduction

Damage control resuscitation (DCR) is a novel resuscitation strategy that may increase survivability of combat casualties [1, 2]. One important aspect of DCR is transfusing fresh frozen plasma (FFP) and packed red blood cells (PRBCs) in a 1:1 ratio to patients at risk for coagulopathy. DCR has also been adopted by many civilian trauma centres [3-9], despite the lack of supporting prospective trials. DCR is only supported by retrospective studies which may have been affected by survivorship bias [10], and other studies have found no benefit [11, 12]. Caution is needed in recommending DCR, considering the potential risks arising from increased FFP use [3-6, 10]. We report a massively transfused gunshot victim, whose case highlights a possible novel method for assessing a patient's response to different FFP:PRBC transfusion ratios.

Methods

The patient was enrolled in a prospective study on thromboelastography in trauma patients. In brief, two extra 1.8mL tubes with 0.109M trisodium citrate additive were drawn each time routine coagulation tests were obtained. One tube was centrifuged, the plasma was frozen, and then sent to an outside laboratory (Haemostasis Reference Laboratory, Hamilton, Canada) to perform clotting factor activity assays [13, 14]. From the second tube, thromboelastography (TEG®) was performed on whole blood within 30 minutes of collection. We assessed the dose:response of increasing plasma:red cell transfusions on INR (international normalized ratio), PTT (partial thrombolplastin time), thromboelastograms, clotting factor assays and the clinical situation at four different time points during the first 20 hours of this patient's hospital admission. The study was

approved by our institutional review ethics board, with delayed consent (within 48 hours) where required. The patient's family also consented to having his case reported.

Clotting Factor assays were done as follow: extrinsic factor assays were performed by mixing patient plasma with plasma controls known to be deficient in factors II, V, VII or X (Precision BioLogics). The degree of correction of the Prothrombin Time (PT) (Dade-Behring Innovin) is proportional to the factor activity. Similarly, intrinsic factor assays were done by mixing patient plasma with plasma known to be deficient in factors VIII, IX, XI or XII (precision BiolLogicis). Again, the degree of correction of the partial thromboplastin time (PTT) (Date-Behring Actin FSL) is proportional to the factor activity. Clotting factor activity was considered critically low if any factor level was below 30%, which is often cited as the threshold level for hemostasis [15, 16]. In calculating transfusion ratios, we assumed that five units of platelets is equivalent to one unit of FFP [2].

The TEG® 5000 Hemostasis Analyser (Haemoscope Corporation, Illinois, USA), was used to produce thromboelastograms. TEG® can be useful in assessing coagulation status in trauma patients [17-21], and the basic TEG® principles have been previously described [22, 23]. One mL of whole citrated blood was mixed with buffered stabilizers and a blend of phospholipids (Kaolin®). A 340 uL sample was then warmed and mixed with calcium chloride. Measurements were made for no less than 40 minutes

Case

A 26 year-old male arrived to our trauma room with a transpelvic gunshot wound and pulseless electrical activity. Resuscitation commenced with crystalloid (2 liters) and 2 units of uncross-matched blood. He went directly to the operating room for a laparotomy, where his right internal iliac artery and vein were ligated for bleeding. He was massively transfused intra-operatively, and was hypothermic (30.8^0C). The abdomen was packed and temporarily closed.

Re-warming and further transfusions were administered in the intensive care unit (ICU). Transfusions were aimed at normalizing standard laboratory coagulation tests and hemodynamic indices according to our institutional massive transfusion protocol. Platelet transfusions were aimed at restoring platelet count to above $50x10^9$/L, FFP to keep International Normalized Ratio (INR) below 1.5, and cryoprecipitate to keep fibrinogen above 0.8g/L. PRBC transfusions were aimed at normalizing tissue perfusion and at maintaining hemoglobin levels above 70g/L. He initially continued to bleed, averaging 500mL/hour from his abdominal dressing. A second laparotomy failed to identify surgical bleeding. His coagulopathy was finally controlled seven hours later and abdominal bleeding stopped.

Results

During the first 20 hour period in hospital, three distinct phases of care were evident: the Resuscitation phase, ICU phase 1 and ICU phase 2 (See Figure 1). The Resuscitation phase included treatment administered in the trauma room and operating room, and lasted for 3.5 hours. During this time, the patient received FFP:PRBC in a 1:2 ratio. The second phase (ICU phase 1) consisted of the overnight resuscitation that occurred in the ICU, and lasted for eight hours. During this phase, the patient received FFP:PRBC in a 4:5 ratio. The final phase (ICU phase 2) phase commenced with the arrival of the day-time ICU staff and lasted for seven hours. During this phase, the patient received FFP:PRBC in a 7:5 ratio. Also, during this period, the patient underwent a bedside laparotomy to look for missed bleeding.

We obtained the full complement of coagulation testing at four different time points during this 20 hour period. Testing at Time Point A assessed the patient's status on arrival in the trauma room at the beginning of the Resuscitation Phase. Laboratory testing at Time Point B occurred two hours after arrival into the ICU after the first surgery (ICU Phase 1). Testing at Time C occurred 2 hours after the arrival of the day-time ICU staff (ICU phase 2), during the second-look laparotomy. Testing at Time D occurred at the end of this study's observation period, 18.5 hours after arrival in the trauma room.

Time Point A: On arrival to the trauma room, the patient's INR was greater than 13, and PTT was greater than 150 seconds. His platelet count was 16 ($x10^9$/ L) and his fibrinogen level was 0.5 grams/L. His TEG® (Figure 2) showed almost no clot formation and all clotting factor activity was critically low (Figure 3).

Resuscitation Phase: During this phase, he was transfused 8 units of FFP, 15 units of platelets, and 20 units PRBCs (FFP:PRBC of 1:2). As well, he received 8 units of cryoprecipitate, 2.4 mg of recombinant activated factor VII (rFVIIa), 5 liters of isotonic crystalloid and 1 liter of colloid.

Time Point B: Testing at this time point reflected the coagulation response of the patient to the 1:2 resuscitation strategy used in the trauma room and operating room (Resuscitation Phase). His INR had normalized to 1.2 and clotting factor VII activity corrected (Figure 3). This was likely a direct response to the recombinant activated factor VII given in the operating room. However, PTT remained elevated at 100 seconds and his TEG® showed no clotting activity (Figure 2). As well, all other factors remained critically low (Figure 3). His hemoglobin had fallen to 43 g/L .

ICU Phase 1: During this phase, he received 12 units of FFP, 20 units of platelets, and 23 units of PRBCs (FFP:PRBC of 4:5). He also received 3 liters of isotonic crystalloid, 1 liter of colloid, and 24 units of cryoprecipitate. His temperature was corrected to 35.8^0C.

Time Point C: This time point reflected the coagulation response of the patient to the 4:5 transfusion strategy used during ICU Phase 1. His INR and factor VII activity remained normal, likely because of residual rFVIIa activity. However, his PTT remained elevated (50.4 seconds). His TEG® still showed hypocoagulability (Figure 2), and clotting factor V remained at critical levels (5%). Most importantly, his hemoglobin dropped to 23 g/L. A second laparotomy failed to identify surgical bleeding.

ICU Phase 2: This phase started with the arrival of the day-time ICU staff, and lasted seven hours. During this period, he received 16 units FFP, 30 units of platelets, and 18 units of PRBCs (FFP:PRBC of 7:5). He also received 16 units of cryoprecipitate, and more rFVIIa (9.6 mg).

Time Point D: Testing occurred 7 hours into ICU phase 2, and reflected the coagulation response to the 7:5 transfusion strategy adopted during ICU phase 2. His TEG® corrected to within normal limits (Figure 2). INR was 1.15 and PTT was 42.4 seconds. Also, all clotting factor activity was above 30% activity (Figure 3), with the exception was factor V (13%). His hemoglobin stabilized and bleeding stopped. The patient had a difficult ICU course, and unfortunately, died a month later from multiple organ failure after a septic event.

Discussion

We described the changes in coagulation measures that occurred in association with changes to FFP:PRBC transfusion ratios in an exsanguinating trauma patient. This patient initially had critically low clotting factor activity, TEG® evidence of coagulopathy and abnormal standard coagulation parameters. These did not correct until almost 20 hours after admission. Correction occurred after FFP and PRBCs were transfused at a 7:5 ratio. Only at this point did clinical hemostasis also occur.

Clotting factor V activity was the only coagulation parameter that remained difficult to correct. One possible reason for this is activation of protein C. Brohi and colleagues reported that shock leads to activation of protein C via thrombomodulin expression, and subsequent down-regulation of factor V [24, 25]. For this patient, Factor V only corrected to 13% activity, but bleeding did stop. Although 20-30% activity level is considered the threshold for hemostatic activity [15, 16], we have no data on the level required for hemostasis in patients with massive injuries.

Standard resuscitation practice [26] withholds plasma transfusions until after infusing crystalloid and red cells. The need for FFP transfusion is assessed by laboratory tests. When required, four units of FFP are transfused at a time so as to raise clotting factor activity by 20-30% [27]. However, retrospective studies [28, 29] and mathematical models [30, 31] suggest this approach is inadequate for exsanguinating trauma patients. We showed that increasing FFP:PRBC transfusion ratios appeared to correct the

hemostatic mechanisms in this coagulopathic, hemorrhaging trauma patient. Evidence of correction included improvement of almost all clotting factor activity to above 30% normal activity, normalization of thromboelastography, and normalization of INR and PTT laboratory parameters. Most importantly, the patient stopped bleeding clinically.

Limitations

We only demonstrated an association between a high FFP:PRBC ratio and correction of coagulopathy in an exsanguinating patient. One interpretation is that our patient was initially under-resuscitated with FFP, and would have benefited from a DCR approach from the outset. Another distinct possibility, however, is that a separate pathologic process such as hypo-perfusion caused coagulopathy [32]. However, as this process was reversed by resuscitation, clotting factor activity became easier to correct. In either case, clotting factor activity remains a useful measure of the adequacy of resuscitation with fresh frozen plasma.

A second limitation is that the critically low levels of factor activity observed in this case may be due to laboratory error, as clotting factors may have degraded. This is unlikely, however. Many studies have reported on the stability of clotting factors in citrated blood despite transportation at ambient temperatures and despite freezing [13, 14]. These studies also studied factor V activity, which remained stable for up to 24 hours at ambient temperatures in citrated whole blood. Randomized controlled trials are required to definitively assess the efficacy of high FFP:PRBC ratios in controlling coagulopathy in exsanguinating trauma patients.

Conflicts of Interest

SR receives salary support from Novo Nordisk.

Acknowledgements

We would like to acknowledge the Surgeon General of the Canadian Forces Health Services (BGen H. Jaeger) for her support of this project, and the financial support from a Military Health Services Research Grant from the Canadian Forces Health Services. As well, we would like to acknowledge the support of Defence Research and Development Canada (DRDC) for their financial support as well.

Both HT and SR had full access to all data in this study, and take responsibility for the integrity of the data and for the accuracy of its analysis.

SR receives salary support from a combined partnership of the Canadian Institute of Health Research (CIHR) and from Novo Nordisk.

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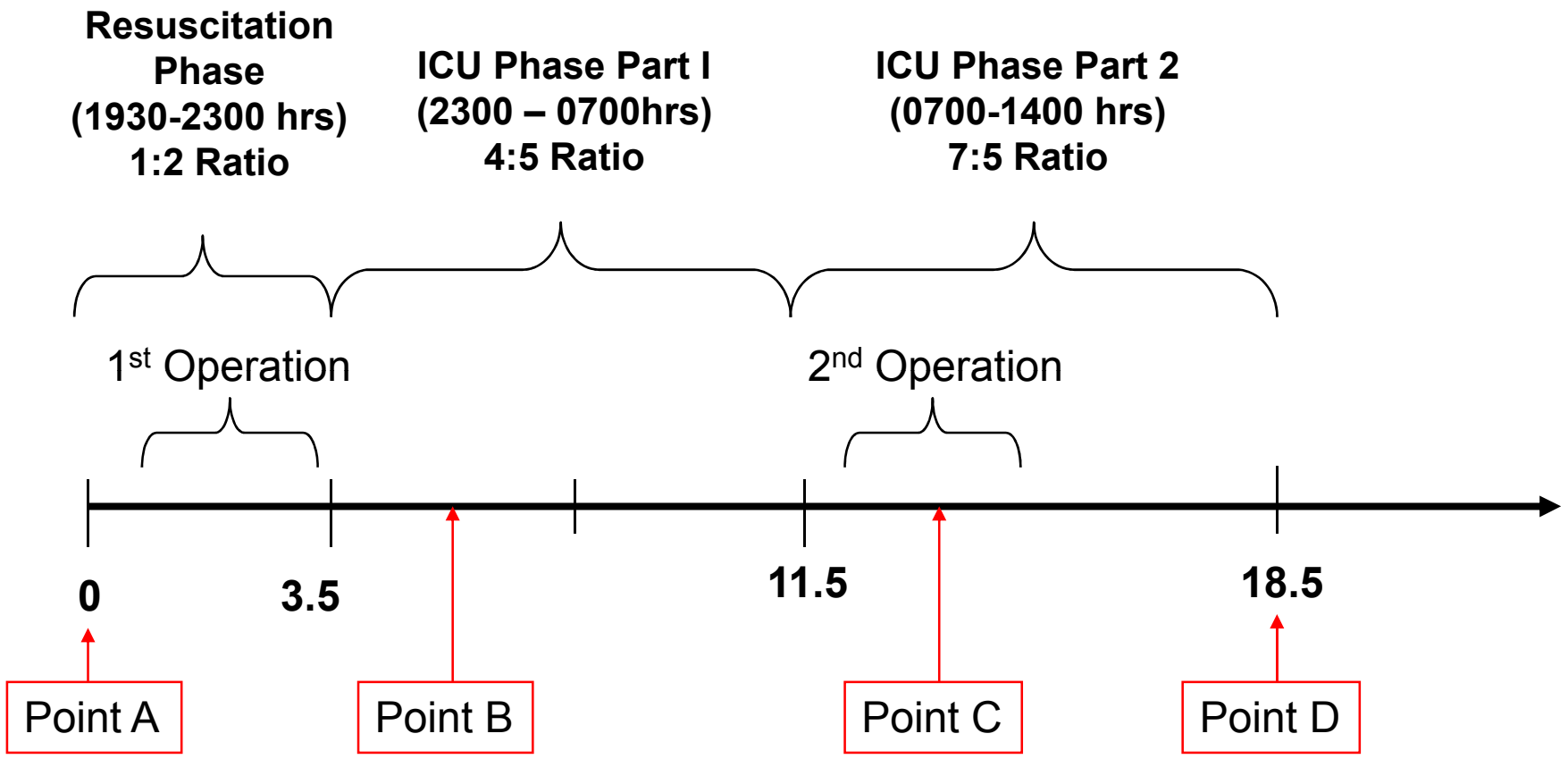
Figure(s)

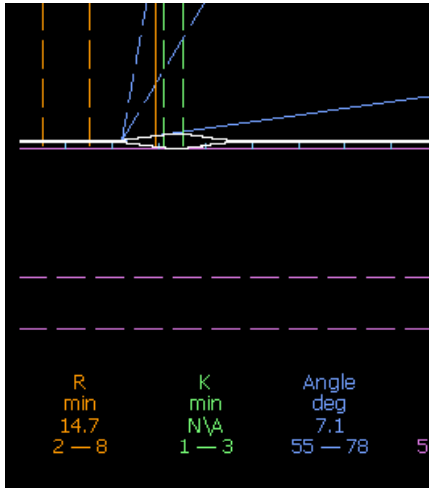
Figure 1 - FFP:RBC Transfusion Strategies Used During Different Periods



Figure(s)

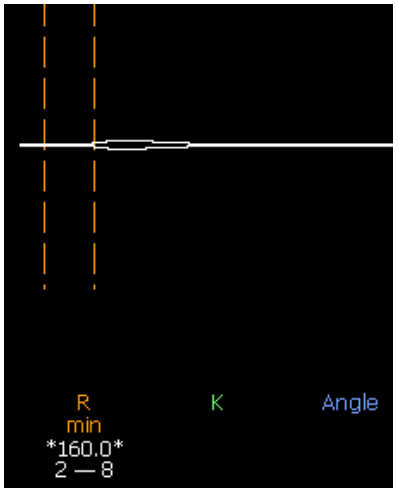
Figure 2 – Thromboelastography Results

Time Point A



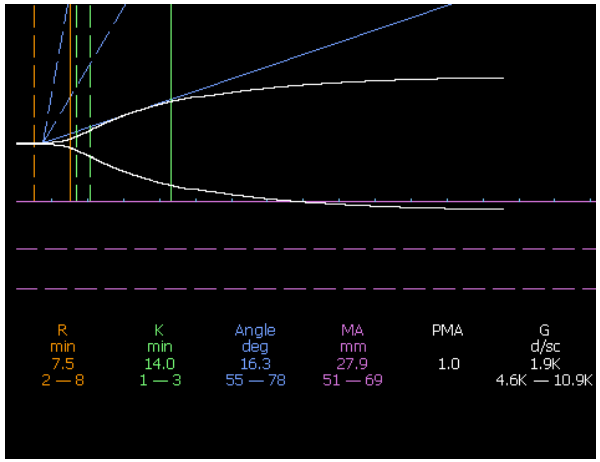
FFP:PRBC Of 1:2

Time Point B



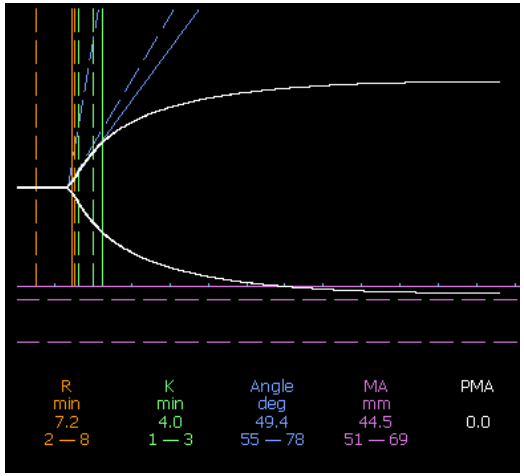
FFP:PRBC Of 4:5

Time Point C



FFP:PRBC Of 7:5

Time Point D



Figure(s)

Figure 3 – Effect of FFP/PRBC Ratio on Coagulopathy