

# **CRYOPRECIPITATE TRANSFUSION: ASSESSING APPROPRIATENESS AND DOSING IN TRAUMA**

by

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## Abstract

Cryoprecipitate is commonly used outside guidelines. In trauma, the appropriate cryoprecipitate dose and its impact on plasma fibrinogen levels are unclear.

This retrospective study aims to evaluate: (1) the appropriateness of cryoprecipitate transfusion in trauma; and (2) the plasma fibrinogen response to cryoprecipitate transfusion during massive transfusion in trauma.

Fibrinogen levels of  $< 1.0$  g/L within 2 and 6 hours of cryoprecipitate transfusion were used for assessing appropriateness. Out of 394 events, 238 (60%) and 259 (66%) were considered appropriate using 2 and 6 hour criteria, respectively. A dose of  $8.7 (\pm 1.7)$  units caused a mean increase in fibrinogen levels of  $0.55 (\pm 0.24)$  g/L, or 0.06g/L per unit.

In our hospital, where transfusion guidelines and policies for rapid blood product and laboratory turnaround times exist, it is possible to achieve high rates of appropriateness for cryoprecipitate transfusion in trauma. The current recommended dose causes a modest increase in fibrinogen levels.

# Acknowledgments

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## 6. List of Abbreviations

**CRYO**, cryoprecipitate

**vWD**, von Willebrand's disease

**UK**, United of Kingdom

**US**, United States

**HIV**, human immunodeficiency virus

**HBV**, human B hepatitis virus

**HCV**, human C hepatitis virus

**FFP**, fresh frozen plasma

**FP**, frozen plasma

**ADP**, adenosine diphosphate

**TxA<sub>2</sub>**, thromboxane A<sub>2</sub>

**ESR**, erythrocyte sedimentation rate

**SHSC**, Sunnybrook Health Sciences Centre

**DDAVP**, desmopressin

**DIC**, disseminated intravascular coagulation

**PPV**, positive predictive value

**RBC**, red blood cells

**AAGBI**, Association of Anaesthetists of Great Britain and Ireland

**FDP**, fibrin degradation products

**PT**, prothrombin time

**RID**, radial immunodifusion

**ELISA**, enzyme-linked immunoaborbent

## 6. List of Abbreviations, cont.

**TBI**, traumatic brain injury

**DCR**, damage control surgery

**HES**, hydroxyethyl starch

**PAI-1**, plasminogen activator inhibitor – type 1

**TRALI**, transfusion-related acute lung injury

**TACO**, transfusion-associated circulatory overload

**TEG**, thromboelastography

**ROTEM**, rotational thromboelastometry

**INR**, international normalized index

**PTT**, partial thromboplastin time

**ISS**, injury severity score

**AIS**, abbreviated injury scale

# Cryoprecipitate Transfusion: Assessing Appropriateness and Dosing in Trauma

## 7. Literature Review

### 7.1 Introduction

The indications for cryoprecipitate (CRYO) use have changed considerably since its development many decades ago. Pool's cryoprecipitate was originally developed to provide factor VIII for patients with Congenital Factor VIII Deficiency in the mid-1960's (Pool, 1964; Pool *et al.*, 1965). The use was subsequently expanded to the treatment of patients with von Willebrand's disease (vWD) and hypofibrinogenemia with some benefit (Bennet & Dormandy, 1966). Nowadays, the most common indication of the product is for the replacement of low serum fibrinogen levels in patients with acquired hypofibrinogenemia and hemorrhage (O'Shaughnessy *et al.*, 2004). However, the fibrinogen content of a unit of CRYO may vary widely (range, 120 – 796mg) (Callum *et al.*, 2009), with each unit containing 30 – 50% of the original fibrinogen in the source plasma (Klein & Anstee, 2005). Factor VIII and von Willebrand factor correspond to approximately 5 % of the total pool of proteins in this concentrate (Allain, 1984). Other components of CRYO include fibronectin, factor XIII, immunoglobulins (IgG and IgM), albumin and platelet microparticles (Callum *et al.*, 2009). In some European countries, the use of CRYO solely for fibrinogen replacement has been recently challenged and increasingly substituted by the use of virally inactivated fibrinogen concentrate, which has a more standardized concentration of fibrinogen and safer profile (decreased risk of pathogen transmission and immune-mediated complications) (Fenger-Eriksen *et al.*, 2008; Bundesaertzkammer, 2009; Sorensen & Bevan, 2010). Fibrinogen concentrate is currently

licensed for use in congenital bleeding throughout Europe, USA, China and Japan, and for use in acquired bleeding in over 15 countries globally.

Major guidelines on blood component therapy in the United Kingdom (UK), United States (US) and Canada, where fibrinogen concentrates are not available, suggest transfusion of CRYO when plasma fibrinogen level is  $< 1.0\text{g/L}$  in the context of bleeding and/or disseminated intravascular coagulation (American Society of Anesthesiologists, 1996; O'Shaughnessy *et al.*, 2004; Droubatchevskaia *et al.*, 2007; Levi *et al.*, 2009). Typically, an adult dose of 8 – 12 units (one unit per 5 - 10 kg of body weight) is administered (Canadian Blood Services, 2006; Ketchum *et al.*, 2006). These recommendations are extrapolated from limited scientific evidence. In 1987, Ciavarella *et al.* reviewed 36 massively transfused patients and observed that microvascular bleeding occurred in all 4 patients whose fibrinogen levels were below 0.5 g/L and only occurred in 2 out of 10 patients with fibrinogen levels 0.5 – 1.0 g/L of whom both were severely thrombocytopenic (Ciavarella *et al.*, 1987). The authors suggested a threshold of 1.0 g/L as a trigger for CRYO transfusion. Lately, this “historical” 1.0g/L threshold was considered inadequate, being associated with excessive blood loss during cardiac surgery and severe postpartum bleeding (Charbit *et al.*, 2007; Karlsson *et al.*, 2008). In trauma, a recently formed European multidisciplinary Task Force for Advanced Bleeding Care in Trauma (Rossaint *et al.*, 2010), recognizing the pivotal role of fibrinogen in the trauma-induced coagulopathy, recommends the replacement of fibrinogen (using fibrinogen concentrate or CRYO) for plasma fibrinogen level of less than 1.5 – 2.0 g/L (Rossaint *et al.*, 2010). This guideline also suggests increased doses of CRYO (15 -20 units in a 70 Kg adult). However, the impact of CRYO transfusion on plasma fibrinogen levels, particularly in the context of major blood loss, is unclear and mostly based on limited evidence (Faringer *et al.*, 1993) and expert opinion (Callum *et al.*, 2009). Furthermore, the risk of bleeding when fibrinogen level is  $< 1.0\text{g/L}$  in trauma patients is unclear (Callum *et al.*, 2009). Durante the acute resuscitation of a

trauma patient, fibrinogen is most commonly replaced by plasma transfusion. The content of a standard dose of CRYO is equivalent to the amount of fibrinogen found in an average adult dose of FFP (4 units), where each unit of FFP contains 0.5 g of fibrinogen (Callum *et al.*, 2011; Nascimento *et al.*, 2010). There is little published literature in trauma patients to guide when CRYO is required and what the effective dose may be.

Notwithstanding the lack of randomized control trials demonstrating its efficacy, CRYO is largely used for the management of acquired hypofibrinogenemia following hemorrhage. Rates of CRYO transfusion in the UK and Canada have been estimated to be 1.7 and 1.5 units per 1000 people annually, respectively (Callum *et al.*, 2009). A recent Canadian audit observed that the commonest indication for CRYO transfusion was for the treatment of bleeding after cardiac, noncardiac surgery and trauma (Alport *et al.*, 2008). Despite this widespread practice, previous audits have documented a wide variation in clinical practice, with rates of inappropriateness, as defined by expert opinion, ranging from 24 to 62% (Schofield *et al.*, 2003; Pantanowitz *et al.*, 2003; Alport *et al.*, 2008).

This study reviews the practice of CRYO transfusion at large academic hospital during a period of 10 years, with the aim to assess the appropriateness of and the plasma fibrinogen response to CRYO transfusion in trauma patients. This review will provide further information to assist in determining the appropriate dose for the use of CRYO in the context of major blood loss.

## 7.2 Cryoprecipitate History

The first description of CRYO preparation dates back in 1964. In that year, Judith Graham Pool (1919-1975), working at Stanford University, prepared the first unit of CRYO (Pool *et al.*, 1964; Pool & Hink, 1964). The method described the thawing of frozen plasma

over a period of 18 to 24 hours to temperatures of 4°C - 0°C. This was followed by a centrifugation step when the product was placed in a refrigerated centrifuge set at 2°C and at 2,000 x g for twenty minutes (Pool, 1965). Then, gravity drainage was used to remove the supernatant plasma (named “exhausted” plasma), leaving only 10-15 mL of plasma in the preparation. Finally, the product underwent a re-freezing process to temperatures around -20°C.

The first reported clinical use of the product happened one year after the development of the product. In October of 1966, three patients with vWD were reported to have successfully received CRYO (Bennett & Dormandy, 1966). In the same year, Bennett and Dormandy reported on the successful use of “exhausted” plasma for a patient with factor XI deficiency (Bennett & Dormandy, 1966). Subsequently to the first positive experiences with the product, in January of 1967, Barrett and colleagues reported on the successful use of cryoprecipitate for hemophilia in 7 patients (Barrett *et al.*, 1967). CRYO thus gained popularity for the management of hemophilia. The product had great impact on the quality of life of patients with hemophilia. However, some significant risks of infectious disease transmission were associated with the introduction of this pooled blood product and many recipients of CRYO transfusion were exposed to Human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV).

### 7.3 Manufacturing Procedure

The process of manufacturing CRYO remains essentially unchanged since its original description many decades ago (Pool *et al.*, 1964) and only minor variations may be encountered in different jurisdictions. CRYO can be considered a sub-product of fresh frozen plasma (FFP) or frozen plasma (FP). The latter two blood components differ only with respect to the time to

freezing after collection and consequently to the content of clotting factors recovered in each unit. While FFP is frozen within 8 hours from collection, the time from drawing to freezing in FP can reach up to 24 hours (Callum & Pinkerton, 2005). Due to the longer time to freezing, FP contains 15–20% lower factor VIII levels than FFP (O'Neill *et al.*, 1999; Smith *et al.*, 2000)

FP or FFP is thawed at 1 to 6°C for the preparation of CRYO (Brecher, 2005). After thawing, the product is centrifuged at 5,000 x g for about 6 minutes and then the supernatant is removed. The original bag is left with only 5-15 mL of plasma and the cold insoluble precipitate. This residual material, the cryoprecipitate, is re-frozen within one hour of thawing and stored at -18°C or colder. Under ideal storage conditions, the final product has a shelf-life of 12 months.

It has been estimated that each unit of CRYO contains approximately 100-250 mg of fibrinogen (Poon, 1993). However, recent fibrinogen testing reveals concentrations of fibrinogen in current preparations of cryoprecipitate higher than previously reported, with a median value of 388 mg per bag (range 120-796 mg) (Robert Romans. Canadian Blood Services. Personal communication). The American Association of Blood Banks standards for fibrinogen content in CRYO, requires a minimum of 150 mg per bag (American Association for Blood Banks Standards for Blood Bank and Transfusion Services, 2008). Each unit contains 30-50% of the original fibrinogen in the source plasma (Klein & Anstee, 2005).

In the final CRYO preparation, factor VIII and von Willebrand factor represents only 5% of its total protein content, which corresponds to about 40-70% of the original factor VIII/vWF that was present in the original plasma (Allain, 1984). The *in vivo* recovery of factor VIII can be improved by a series of measures during the manufacturing process: continuous mixing of blood during collection; minimizing the time from donation of plasma to freezing; rapid re-freezing of CRYO on dry-ice; and limiting the duration of CRYO storage



(Kasper *et al.*, 1975). Higher donor baseline levels of factor VIII are also associated with improved in vivo recovery (Kasper *et al.*, 1975). Despite increased levels of factor VIII in exercised donors, this recommendation failed to translate into superior post-infusion in vivo recovery of factor VIII due to the rapid clearance of the “exercise” factor VIII (van Gastel *et al.*, 1973).

CRYO is considered a potential rich source of fibronectin, which represents 20 -25% of the total protein in the product (Allain, 1984; Reilly *et al.*, 1983). It also contains several other proteins as follow: albumin (5-8%), IgG (5-8%), IgM (1-2%) (Allain, 1984).

## 7.4 Cryoprecipitate Content & Physiology

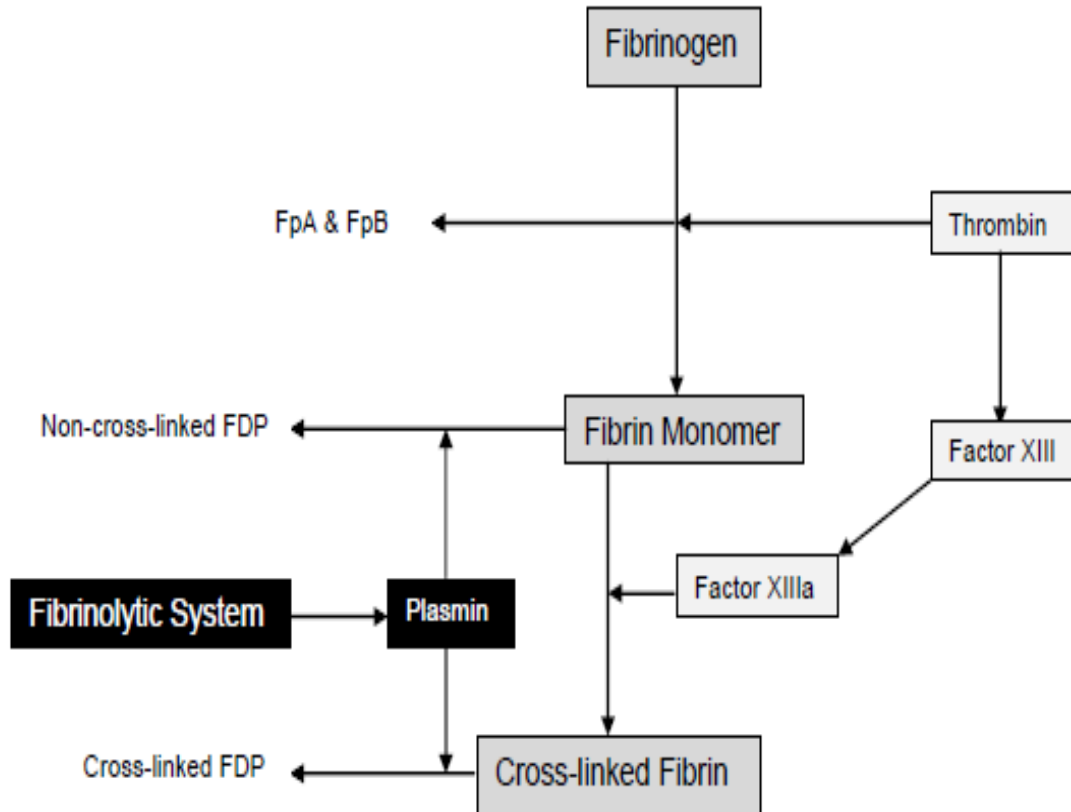
### **Fibrinogen**

Fibrinogen is a major soluble plasma glycoprotein of 340,000-Da containing three pairs of protein chains that are interconnected by disulfide bonds (Hantgan *et al.*, 2006). Its concentration in the plasma, when measured as a clottable protein varies from 1.5 – 4.5 g/L. This glycoprotein is synthesized by the hepatocytes and has a long half-life in the plasma of approximately 4 days (Kreuz *et al.*, 2005). Due to the fibrinogen’s diffusion phase half-life of 12 hours, it is recommended that CRYO infusions be administered every 12 hours for patients with congenital fibrinogen deficiency where fibrinogen concentrates are not available. As oppose to the reduced factor VIII recovery, fibrinogen activity remains stable with 87% of the original content at time of thawing of fibrinogen recovered at 24 hours of liquid storage (Saxena *et al.*, 1990).

Fibrinogen plays a critical role in hemostasis and thrombosis. It is the precursor of fibrin and promotes platelet aggregation (Hantgan *et al.*, 2006). Thrombin cleaves the polypeptide chains ( $A\alpha$  and  $B\beta$ ) of the fibrinogen molecule, releasing fibrinopeptides A and B

from the amino-terminal ends (Fig 1). The cleavage of fibrinogen results in the formation of fibrin monomers, which subsequently undergo polymerization to form an insoluble fibrin clot (Fig 1). The formation of fibrin is regulated by the endogenous fibrinolytic system (Hantgan *et al.*, 2006). Fibrin is lysed by the activity of plasmin and then fibrin degradation products are formed (Fig 1). Besides being a substrate for fibrin formation, fibrinogen works on platelet aggregation by linking activated platelets (Colman *et al.*, 2006). Activated platelets express on their surface the glycoprotein receptor GP IIb/IIIa, which binds fibrinogen (either plasma fibrinogen or fibrinogen that is secreted from intraplatelet granules following platelet activation) and link platelets together.

**Fig 1. Fibrinogen Cleavage by Thrombin and Formation of Fibrin**



FpA, Fibrinopeptide A; FpB, Fibrinopeptide B; FDP, fibrin degradation products.

Hemostasis is a physiologic mechanism where a well-balanced and complex response to bleeding is expected. Shortly, blood vessel wall, platelets, coagulation and fibrinolysis interact in order to stop bleeding while preserving vessel patency. Circulating platelets are activated by thrombin and subendothelial collagen after vessel wall injury. Once activated, platelets, in a positive feed-back, produce and secrete other stimulants of platelet aggregation, such as adenosine diphosphate (ADP), thromboxane A<sub>2</sub> (TxA<sub>2</sub>) and serotonin. In the initial step to secure hemostasis (primary hemostasis), platelets adhere (platelet adhesion) to injured vessel walls and to other platelets (platelet aggregation), forming a plug at the site of bleeding (Colman *et al.*, 2006). Fibrinogen and von Willebrand Factor can bind to exposed membrane glycoproteins IIb and IIIa on activated platelets, acting as a ligant between platelets for primary hemostasis (Colman *et al.*, 2006). The initial platelet-rich plug is considered friable and is stabilized by the secondary hemostasis process, which is characterized by a subsequent formation of fibrin. Finally, the activity of endogenous fibrinolytic enzymes such as plasmin and neutrophil elastase, associated with other processes of tissue repair, is responsible for the digestion of the fibrin plug over a period of several days (Colman *et al.*, 2006).

Fibrinogen is also a positive acute-phase reactant protein, which is elevated in response to a variety of acute clinical conditions, including injury (Lowe *et al.*, 2004). Normally, plasma fibrinogen is synthesized in the liver, with a steady-state rate of 1.7 – 5.0 g per day. However, in patients with peripheral consumption of fibrinogen, the production rates of fibrinogen can reach up to 20-fold increase from baseline rates (Colman *et al.*, 2006). Its elevation in acute phase response might affect plasma viscosity and the erythrocyte sedimentation rate (ESR) (Lowe, 1987). Fibrinogen, plasma viscosity and ESR have been shown to be independent predictors of coronary heart disease events (Danesh *et al.*, 1998; Lowe & Rumley, 1999; Danesh *et al.*, 2000).

## **Factor VIII and von Willebrand factor (vWF)**

Factor VIII is present in plasma mostly as a noncovalent complex with vWF and its coagulation role is to hasten factor IXa conversion of factor X to Xa. vWF has important participation in primary hemostasis. It promotes the adhesion of platelets to exposed subendothelium and also stabilizes coagulation factor VIII in the plasma. Factor VIII and vWF correspond to approximately 5% of the total protein in CRYO (Allain, 1984). The final CRYO preparation retains 40% - 70% of the original VIII/vWF that was present in the original plasma (Allain, 1984).

## **Factor XIII**

Factor XIII is present in the blood as a tetramer composed of 2 A subunits (enzymatic portion) and 2 B subunits (carrier portion). When activated, Factor XIII loses its 2 B carrier proteins (Board *et al.*, 1993). It plays a pivotal role in promoting clot stability. Factor XIII improves the mechanical strength of the fibrin clot and protect it from proteolytic degradation by forming covalent bonds between fibrin monomers and by cross-linking alpha-2 antiplasmin, fibrinogen, fibronectin, collagen, and other proteins (Lovejoy *et al.*, 2006). Approximately 20-30% of factor XIII in the original plasma remains in CRYO (Klein & Anstee, 2005).

## **Fibronectin**

Fibronectin is a high molecular weight (440-530 kD) adhesive glycoprotein, which contains two almost identical subunits disulfide-bridged close to their C-terminal ends (Mitrovic S *et al.*, 1995). It is present as a soluble glycoprotein in plasma and as an insoluble protein in the extracellular matrix of many tissues (Lucena *et al.*, 2007). In human plasma, it is present at a concentration of 300 ug/mL (Mosher, 1984) and is involved in mechanisms of immune defense, immune surveillance and hemostasis. Among many biological roles, fibronectin is believed to have opsonic activity, which assists with the phagocytosis of

particulate debris by the reticuloendothelial system (Saba & Jaffe, 1980). CRYO is an important source of fibrinectin, containing approximately 1500 ug/mL (Reilly *et al.*, 1983). Human derived factor VIII concentrates (excluding the high purity preparations), which are considered rich potential sources of fibrinectin; contain 2100-3600 ug/mL (Reilly *et al.*, 1983).

### **Platelet Microparticles**

Microparticles can be defined as phospholipid microvesicles with size that usually range from less than 100 nm derived from blood cells, platelets, endothelial cells, and several other types of cells (Horstman & Ahn, 1999; Freyssinet, 2003; Simak & Gelderman, 2006) . These microvesicles contain certain receptors and other proteins innate of their parental cells. The identification of cell-specific antigens allows inferences about their origin. Microparticles are present in circulating blood and in plasma and cellular blood products; and possess a wide spectrum of biological activities. They may facilitate the interactions between cells, promote cell signaling and transfer of functional receptors between different types of cells (Mack *et al.*, 2000; Simak & Gelderman, 2006). It has been suggested that microparticles can play active roles in various tissue defense physiological processes and has also been implicated in the pathophysiology of thrombosis, inflammation, vascular reactivity, cancer metastasis and response to pathogens (Simak & Gelderman, 2006; Ardoin *et al.*, 2007).

The first official description of platelet microparticles dates back in 1967, when Wolf (Wolf, 1967) demonstrated that activated platelets released membrane fragment which he called *platelet dust*. However, the search for platelet microparticles in CRYO did not start until the detection of anti-platelet antibodies in hemophilia patients after the exposure to CRYO more than a decade later (McVerry & Machin, 1979). The content of platelet microparticle in plasma and CRYO are measured using an antibody to the platelet membrane receptor glycoprotein IIb (George *et al.*, 1986). Due to freezing and thawing, plasma used in the

process of preparing CRYO further concentrates the platelet membrane microparticle content. Plasma processed to produce frozen plasma and CRYO contains a high number of platelets and elevated concentration of platelet membrane microparticles is particularly found in CRYO. It is estimated that each dose of CRYO (10 units) contains approximately  $4 \times 10^9$  platelets in microparticle form. The concentration of platelet membrane microparticles in CRYO is many folds greater than the cryosupernant plasma and the original plasma (Callum *et al.*, 2009). It has been shown that the microparticle content of plasma prepared from platelet-rich plasma was greater than that of plasma prepared directly from whole blood. However, it is unknown whether the platelet membrane microparticles retain hemostatic capacity after processing and freezing. Further understanding about the role of platelet microparticles in hemostasis, the content of these biologically active particles in CRYO and their potential participation in thrombosis and transfusion reactions (alloimmunization) is greatly required.

## 7.5 Pre-transfusion Compatibility Testing

Due to the small volume of plasma transfused, ABO-compatible CRYO is not required, although this may be significant in patients receiving large volumes of cryoprecipitate relative to their red blood cell mass (Brecher, 2005). In neonates, where the volume of plasma transfused via CRYO can be proportionally large, ABO-compatible CRYO is recommended by some (Klein & Anstee, 2005).

In patients receiving non-ABO compatible CRYO, a positive direct antiglobulin test and hemolysis have only rarely been reported (Burman *et al.*, 1973). The selection of this product for transfusion usually does not require consideration of Rh compatibility.

At Sunnybrook Health Sciences Centre (SHSC), no compatibility test for CRYO transfusion is required according to current hospital transfusion guidelines.

## 7.6 Pre-transfusion Preparation

### **Thawing**

There are two approved methods for thawing of frozen CRYO bags. In an approved temperature-controlled water bath, the product should be thawed in a plastic ‘over bag’ at 30 to 37°C for no more than 15 minutes. Alternatively, the product can be thawed with an approved microwave device. The water bath method is currently the only thawing technique used at SHSC.

### **Use and Storage after Thawing**

After the thawing, CRYO should be infused immediately. In case the product is not used immediately, it should be stored at 20-24°C and, varying according to local regulations in different jurisdictions, the product must be used within 4-6 hours (American Association of Blood Banks, 2002; Canadian Blood Services, 2005). In Canada, if the indication is for fibrinogen replacement and the processing closed system is used, the Canadian Standards allow for storage for up to 24 hours at 20 – 24°C (Canadian Standards Association, 2004). The product cannot be re-frozen.

### **Pooling**

Currently, pooling of CRYO is not a routine practice in many transfusion services. Pooling of CRYO within the transfusion laboratory is estimated to happen only in approximately 20% of laboratories in the United Kingdom (UK Blood Transfusion & Tissue Transplantation Services, 2004). The rate of pooling in other jurisdictions is unknown. In the European Union, all transfusion services must be licensed under the European Union Blood Directive to pool CRYO. These services are also subject to regular inspections. In addition, a unique identifier must be assigned to pools of CRYO, allowing tracing to the original donor



unit numbers. These requirements lead many facilities in Europe to abandon the practice of pooling CRYO (Salter & Lloyd, 2005).

In order to maintain sterility throughout the pooling process, approved biosafety cabinets are recommended. Furthermore, in high volume hospitals where CRYO is used usually in the context of emergent bleeding for cardiac and trauma patients (Alport *et al.*, 2008), pooling within the blood transfusion service can ensure the product is available in an expedited fashion. On the other hand, pooling at the bedside may cause delays in infusing the product. Pooling involves opening of multiple single bags, diluting the bags with saline, mixing the fluid and then serially infusing the final product.

During pooling, each bag of CRYO should be mixed with 10-15 mL of 0.9% Sodium Chloride solution to ensure complete removal of all the material from the bag. After pooling, CRYO must be used within 4 hours. As mentioned previously, the product cannot be re-frozen.

At SHSC, the preparation of a CRYO dose is performed at the blood bank by qualified blood bank technologists.

## 7.7 Standards for Cryoprecipitate Use

Major recommendations from regulatory agencies are in place with respect to processing, compatibility and the content of fibrinogen in CRYO. The American Association of Blood Banks Standards affirm that CRYO shall be prepared by a method known to separate the cold insoluble portion from FFP and result in a minimum of 150 mg of fibrinogen and 80 IU of coagulation factor VIII (American Association of Blood Banks, 2008).

The Canadian Standards for Transfusion Medicine recommend the use of ABO-compatible CRYO, with a policy required for situations where ABO-compatible CRYO is not

available (Canadian Society of Transfusion Medicine, 2007). Additionally, in keeping with recommendations from the American Association of Blood Banks, the Canadian Society of Transfusion Medicine recommends that product should be thawed in a protective overbag at 30-37°C in an approved warming device.

The Council of Europe Recommendation requires that a unit of cryoprecipitate contain a minimum of 140 mg of fibrinogen and 70 IU of factor VIII (Council of Europe, 2006).

## 7.8 Clinical Guidelines for the Use of Cryoprecipitate

Clinical practice guidelines are important sources of information for health care providers and are increasingly common in medicine. The field of transfusion medicine is not different, with a series of guidelines addressing blood and blood product transfusion published in the last two decades (College of American Pathologists, 1994; American Society of Anesthesiologists, 1996; O'Shaughnessy et al., 2004; Stainsby et al., 2006; American Society of Anesthesiologists, 2006; Ketchum *et al.*, 2006; Droubatchevskaia *et al.*, 2007; Hedges *et al.*, 2007; Spahn *et al.*, 2007; Levi *et al.*, 2009; Rossaint *et al.*, 2010; Thomas *et al.*, 2010). These guidelines compile the available evidence in the area of transfusion medicine, providing recommendations to help the provision of care for patients necessitating transfusion.

The major guidelines containing recommendations on CRYO transfusion are discussed individually below. Overall, these recommendations are derived from low-quality scientific evidence (observational studies or case series). There is a lack of randomized controlled trials in the area of CRYO transfusion. Most of these guidelines suggest a fibrinogen cut-off of 1.0 g/L as a trigger for CRYO transfusion with a couple of exceptions. This cut-off is likely based primarily on expert opinion, although a study by Ciavarella et al. in 1987 reviewed 36 massively transfused patients and observed that no microvascular bleeding occurred in patients

with fibrinogen levels above 1.0 g/L (Ciavarella *et al.*, 1987). Typically, an adult dose of 8 – 12 units (one unit per 5 - 10 kg of body weight) is administered. However, there is a growing body of evidence that supports a fibrinogen level above 1.5 g/L and increased doses of CRYO or fibrinogen according to the most recent guidelines (Rossaint *et al.*, 2010; Thomas *et al.*, 2010). In Canada, the number of units of CRYO issued by the Canadian Blood Service increased 7.2%, from approximately 41,000 in 2007/08 fiscal year to over 46,000 in 2010/11 fiscal year (Mr. David Howe, Canadian Blood Services, personal communication). Regarding the measurement of plasma fibrinogen, few guidelines recommend the Clauss assay as the method of choice. The impact of CRYO transfusion on plasma fibrinogen levels, particularly in the context of major blood loss, is unclear and based on expert opinion.

### **The College of American Pathologists Guidelines – Practice Parameter for the Use of Fresh-frozen Plasma, Cryoprecipitate and Platelets - 1994**

The College of American Pathologist guidelines, developed by a group of experts assessing the available evidence at the time, published recommendations on the use of CRYO. These guidelines recommended that the use of CRYO should be reserved for patients with clinical bleeding or planned invasive procedures in patients with a fibrinogen level less than 1.0 g/L (College of American Pathologists, 1994). They suggested that CRYO might be considered for vWD and Hemophilia A when factor concentrates are not available. The guidelines also suggested a dose of one unit of CRYO per 5 Kg of body weight for hypofibrinogenemia, and one unit of CRYO per 10 Kg of body weight in case of vWD. For Hemophilia patients, the number of bags of CRYO can be calculated according to the following formula: number of bags = [(plasma volume in mL x % increase in factor VIII needed)/100]/80. It states that the plasma recovery of transfused fibrinogen is approximately 50%. No specific recommendations

on time to fibrinogen measurement before administration of the product or specific assay for fibrinogen measurement were made in this document.

### **The American Society of Anesthesiologists Practice Guidelines for Blood Component Therapy – 1996**

The American Society of Anesthesiologists convened the Task Force on Blood Component Therapy to develop evidence-based guidelines on the proper indications for perioperative and peripartum administration of red blood cells, platelets, fresh-frozen plasma and cryoprecipitate (American Society of Anesthesiologists, 1996). The available evidence during the time was classified by study design category, using the grading of evidence proposed by the US Preventive Services Task Forces (US Preventive Services Task Forces, 1989) as follow (from the stronger to the weaker): I – randomized controlled trials; II -1 – nonrandomized controlled trials; II-2 – controlled observational studies (e.g.: case-control, cohort studies); II-3 – uncontrolled observational studies; and III – descriptive studies and expert opinion. Based on the strength of evidence and expert opinion regarding effectiveness of the intervention, recommendations for indications were generated (category II-3 and expert opinion): CRYO is likely to be effective and is recommended for: (i) prophylaxis in nonbleeding perioperative or peripartum patients with congenital fibrinogen deficiencies or vWD unresponsive to desmopressin (DDAVP ) (whenever possible, these decisions should be made in consultation with the patient’s hematologist); (ii) bleeding patients with vWD; and (iii) correction of microvascular bleeding in massively transfused patients with fibrinogen concentrations levels less than 80 – 100 mg/dL (or when the fibrinogen levels cannot be measured in a timely fashion). No specific dose was recommended in these guidelines, but it was stated that one unit of CRYO per 10 Kg body weight raises plasma fibrinogen by approximately 50 mg/dL in the absence of continued consumption or massive bleeding.

**The British Committee for Standards in Haematology, Blood  
Transfusion Task Force – Guidelines for the Use of Fresh-frozen Plasma,  
Cryoprecipitate and Cryosupernatant - 2004**

A blood transfusion task force from the British Committee for Standards in Haematology addressed the use of CRYO and published their recommendations (O'Shaughnessy *et al.*, 2004). CRYO was considered appropriate to enhance fibrinogen levels in dysfibrinogenaemia and acquired hypofibrinogenaemia seen in the setting of severe bleeding and/or disseminated intravascular coagulation. The guidelines stated that treatment was usually indicated if plasma fibrinogen was less than 1.0 g/L, although there is no absolute threshold value for diagnosing clinically significant hypofibrinogenaemia. It was also stated that results of fibrinogen assays vary according to the method used; nevertheless no particular recommendations on fibrinogen assays and time to its measurement before CRYO transfusion were made. The guidelines did not address the dosage of CRYO to be utilized in those indications nor made any statement on the plasma recovery of fibrinogen following CRYO infusion.

**The British Committee for Standards in Haematology, Blood  
Transfusion Task Force – 2006**

In 2006, the British Blood Transfusion Committee Task Force published guidelines on the management of massive blood loss, including recommendations on the use of CRYO (Stainsby *et al.*, 2006). During massive blood loss - defined as the loss of one blood volume (Mollison *et al.*, 1997), or a 50% blood volume loss within 3 hours or a rate of loss of 150 ml/min (Fakhry & Sheldon, 1994) – CRYO should be used to maintain fibrinogen > 1.0 g/l if not corrected by fresh-frozen plasma. Two packs of pooled CRYO should be given for adults according to these guidelines. It was stated that 1 l of fresh-frozen plasma might be expected to

provide 2 – 5 g of fibrinogen, while two pools of CRYO provides 3.2 – 4 g of fibrinogen in a volume of 150 – 200 ml. They commented that fresh-frozen plasma alone, if given in sufficient quantity, will correct fibrinogen in most cases, thus CRYO is rarely needed in the context of massive blood loss except in the presence of disseminated intravascular coagulation. These guidelines also suggested that fibrinogen should be measured by the Clauss method. No reference was made to the plasma fibrinogen response to CRYO infusion.

**The American Society of Anesthesiologists Task Force on  
Perioperative Blood Transfusion and Adjuvant Therapies – Practice  
Guidelines for Perioperative Blood Transfusion and Adjuvant Therapies: An  
Updated Report - 2006**

In these updated report from the American Society of Anesthesiologists Task Force, the same recommendations on CRYO use, made a decade before in their first statement on perioperative blood transfusion and adjuvant therapies (American Society of Anesthesiologists, 1996), were re-iterated (American Society of Anesthesiologists, 2006). CRYO is usually indicated: (i) when fibrinogen concentration is less than 80 – 100 mg/dl in the presence of excessive microvascular bleeding, (ii) to correct excessive microvascular bleeding in massively transfused patients when fibrinogen levels cannot be obtained in a timely fashion, (iii) for patients with congenital fibrinogen deficiencies and bleeding patients with vWD if concentrates are not available.

**Trauma Guidelines (Ketchum et al.) - 2006**

A review article by Ketchum et al. has recently addressed the indications for early FFP, CRYO and platelets transfusion in trauma (Ketchum *et al.*, 2006). This review cautioned against the prophylactic administration of cryoprecipitate in trauma patients in the absence of hypofibrinogenemia. This document recognized the importance of FFP and platelets in

preserving adequate coagulation. They argued that adequate FFP administration should be enough to ensure hemostatic concentrations of all coagulation factors and thus prevent severe hypofibrinogenemia. The review stated that one unit of FFP contains approximately 0.5 g of fibrinogen, while one unit of CRYO contains 0.25 g. They observed that CRYO is usually given in 10 units, which contains 2.5 g of fibrinogen in only 100 ml of plasma, rapidly raising the plasma concentration of fibrinogen. However, the benefits of higher than normal fibrinogen concentrations in trauma patients remain unknown. The review makes no recommendation on timing to fibrinogen measurement, type of fibrinogen assay, nor on specific triggers for CRYO transfusion.

### **British Columbia Medical Journal Guidelines - 2007**

The Transfusion Medicine Advisory Group of British Columbia in Canada also developed evidence-based guidelines on the appropriate use of CRYO (Droubatchevskaia *et al.*, 2007). The levels of evidence and grades of recommendations were based on standards developed by the US Agency for Healthcare Research and Quality – formerly the US Agency for Health Care Policy and Research (United States Department of Health and Human Services, 1993). Due to the weak level of evidence available (Level IV) on the clinical application of CRYO, all recommendations in these guidelines were rated as grade C recommendations (based on expert opinion). These guidelines indicated that CRYO is usually recommended if fibrinogen levels are less than 1.0 g/L in the context of bleeding, albeit clinically significant bleeding can occur at higher levels. In the presence of DIC associated with bleeding and fibrinogen levels greater than 1.0 g/L, these guidelines advise on the use of FP instead of CRYO in order to replace multiple factor deficiencies seen in that condition. When small volume infusion is desirable for the replacement of fibrinogen, CRYO may be preferred over FP as suggested by these guidelines. This document also recommends the use of CRYO for

hypodysfibrinogenemia when recombinant or virally-inactivated concentrates are not available for von Willebrand's disease, hemophilia A, factor XIII deficiency, and thrombolytic-related hemorrhage. No recommendations on timing to fibrinogen measurement and choice of fibrinogen assay to be used were made. Finally, this document considered the use of CRYO inappropriate for the preparation of fibrin glue and for the replacement of fibronectin in sepsis.

### **Evidence-based Guideline on Uremic Bleeding - 2007**

These evidence-based guidelines included recommendations on the use of CRYO for the management of uremic bleeding in 2007 (Hedges *et al.*, 2007). Despite acknowledging that the response to CRYO administration in uremic patients can be unpredictable, CRYO was considered a reasonable therapeutic option in uremic patients at high risk for bleeding or actively bleeding. Other therapeutic options recommended in this document included dialysis, erythropoietin, DDAVP, and conjugated estrogens.

### **Management of Bleeding After Major Trauma: a European Guideline - 2007**

An international initiative, the Multidisciplinary European Task Force for Advanced Bleeding Care, developed comprehensive guidelines on multiples aspects of the management of bleeding following severe injury, including recommendations on the use of CRYO and fibrinogen concentrates (Spahn *et al.*, 2007). According to the grading system utilized in these guidelines (Guyatt *et al.*, 2006), the recommendations on CRYO use (grade 1 C) were derived from observational studies and case series, and graded as being low-quality evidence but where the benefits clearly outweighed the risks, thus being classified as strong recommendations. They recommended treatment with CRYO or fibrinogen concentrate if significant bleeding is accompanied by plasma fibrinogen levels of less than 1 g/L. An initial CRYO dose of approximately 15 to 20 units or 50 mg/kg or 3 to 4 g of fibrinogen concentrate was suggested.



Repeat doses should be guided by laboratory values of fibrinogen. The document stated that although no optimal initial dose has been defined, their suggested dose raises plasma fibrinogen level above 1 g/L, which provides sufficient hemostasis. Similar to the majority of published guidelines, type of fibrinogen assay to be used and timing of fibrinogen measurement were not addressed.

### **The British Committee for Standards in Haematology – Guidelines for the Diagnosis and Management of Disseminated Intravascular Coagulation - 2009**

These guidelines were developed by the British Committee for Standards in Haematology Taskforce in Hemostasis and Thrombosis and European experts to help hematologists in diagnosing and managing disseminated intravascular coagulation (DIC) (Levi *et al.*, 2009). This document recommended the use of CRYO or fibrinogen concentrate for severe hypofibrinogenemia in the context of DIC that persists despite administration of FFP. A dose containing 3 g of fibrinogen should be expected to increase plasma fibrinogen levels by approximately 1 g/L, which can be provided by two pools of CRYO (10 donor units) or as 3 g of fibrinogen concentrate, according to these guidelines. They also advised that plasma fibrinogen levels should be interpreted with caution in the setting of DIC. Because fibrinogen is an acute-phase reactant protein, plasma levels can be within normal range for long period of time. These guidelines suggested that serial measurements of fibrinogen might be more helpful and that the Clauss method should be used. However, it should be kept in mind that the high levels of FDP might interfere with the measured fibrinogen by the Clauss assay.

## **Management of Bleeding Following Major Trauma: An Updated European Guideline - 2010**

In 2010, the Multidisciplinary European Task Force for Advanced Bleeding Care published an updated version of their guidelines for the management of bleeding following major trauma (Rossaint *et al.*, 2010). Two major changes were made to the original version with respect to CRYO use in trauma. First, they suggested fibrinogen concentrate and CRYO for fibrinogen replacement if plasma fibrinogen levels of less than 1.5 – 2.0 g/L are found, a more liberal trigger for replacement than the 1.0 g/L previously suggested. Also, it was incorporated a recommendation on the use of thrombelastometric monitoring, as a way to detect functional fibrinogen deficits. Those new recommendations were originated from a growing body of evidence on the pivotal role of fibrinogen in the trauma associated coagulopathy and observations of improved outcomes with a more aggressive approach in replacing fibrinogen in trauma (Rugeri *et al.*, 2007; Hess *et al.*, 2008; Stinger *et al.*, 2008; Fries & Martini, 2010; Tisherman, 2010) . A fibrinogen concentrate dose of 3 – 4 g or 50 mg/Kg of CRYO (15 – 20 units) for an average weight adult (70 kg) were recommended for fibrinogen < 1.5 – 2.0 g/L.

The document included the following statements supporting their new recommendations: (i) based on the thrombelastometry data on trauma patients, maximum clot firmness was associated with plasma fibrinogen levels of approximately 2 g/L (Rugeri *et al.*, 2007); (ii) during massive transfusion, fibrinogen may be the first clotting factor to drop to critical levels (Hippala *et al.*, 1995); (iii) plasma fibrinogen concentration less than 2.0 g/L was the only coagulation variable independently associated with the progression to severe bleeding in postpartum hemorrhage, with a 100% positive predictive value (PPV) (Charbit *et al.*, 2007); (iv) in coronary artery bypass graft surgery, preoperative plasma fibrinogen levels were

inversely correlated to blood loss and transfusion requirements (Karlsson *et al.*, 2008); (v) in major perioperative bleeding, replacement with a median of 2 g (range 1 to 5 g) of fibrinogen increased plasma levels from 1.4 g/L (IQR 1.0, 1.8) to 2.4 (IQR 2.1, 2.6), which was associated with decreased need for allogeneic blood product transfusion (Fenger-Eriksen *et al.*, 2008); (vi) a retrospective observational review involving combat-related trauma suggested that high ratios of fibrinogen to red blood cells (RBC) improves survival rates (Stinger *et al.*, 2008); (vii) postoperative blood transfusions was reduced by increasing fibrinogen from 1.7 ( $\pm 0.3$ ) g/L to 2.4 ( $\pm 0.1$ ) g/L in a randomized controlled trial involving patients undergoing radical cystectomy (Fenger-Eriksen *et al.*, 2009); (viii) the Clauss method for plasma fibrinogen measurement overestimates the actual concentration of fibrinogen in the plasma (Hippala, 1995); (ix) fibrinogen levels are expected to rise regardless of its replacement after major surgery and trauma (Thompson *et al.*, 2007; Wei *et al.*, 1995) , and the effect of fibrinogen replacement during surgery on postoperative levels remains unclear (Fenger-Eriksen *et al.*, 2009; Karlsson *et al.*, 2009); and (x) no thrombotic risk was found in patients with acquired hypofibrinogenemia ( $< 1.5$  g/L) treated with fibrinogen concentrate (Weinkove & Rangarajan, 2008)

**Association of Anaesthetists of Great Britain and Ireland (AAGBI) –  
Guidelines – Blood Transfusion and the Anaesthetist: Management of  
Massive Haemorrhage - 2010**

A Working Party established by the AAGBI, with the contribution from the British Committee for Standards in Haematology Transfusion Task Force and the Appropriate Use of Blood Group, produced a consensus document containing guidelines on the management of massive haemorrhage (Thomas *et al.*, 2010). This document stated that the replacement of fibrinogen can be achieved faster by the use of fibrinogen concentrate, as oppose to CRYO use

that requires thawing and transportation and should be reserved if fibrinogen is not available. They suggested a dose of 30 – 60 mg/kg for fibrinogen concentration and no dose regime for CRYO was recommended. According to these guidelines, emerging evidence suggests that fibrinogen levels higher than 1.5 g/L should be the target. A plasma fibrinogen level of less than 1.0 g/L in the context massive bleeding is indicative of haemostatic failure with impending microvascular bleeding. They also supported the use of the Clauss method for fibrinogen measurement and cautioned against total reliance on coagulation tests, which due to activation of anticoagulant pathways in massive trauma, may be within normal ranges despite hemostatic compromise (Wolberg *et al.*, 2004).

## 7.9 Fibrinogen Measurement

There are currently several laboratory methods available for the measurement of plasma fibrinogen (Nieuwenhuizen *et al.*, 1995; Mackie *et al.*, 2003; Lowe *et al.*, 2004; Verhovsek *et al.*, 2008). The assays differ with respect to their precision of fibrinogen levels determination in different clinical settings; ability to detect total fibrinogen concentrations versus functional fibrinogen levels; degrees of expertise; and time and equipment required. Four main types of assay are used to determine plasma fibrinogen: clotting rate (von Clauss method), total clottable fibrinogen, prothrombin –time derived and immunological (radial immunodiffusion, enzyme-linked immunoabsorbent or nephelometric) assays (Nieuwenhuizen *et al.*, 1995; Mackie *et al.*, 2003; Lowe *et al.*, 2004; Verhovsek *et al.*, 2008).

In general, clotting rate assays, which use clotting time of plasma dilutions for the determination of functional plasma fibrinogen levels, remain the routine assay of choice for the diagnosis of hypofibrinogenemia in the context of bleeding (Mackie *et al.*, 2003). Total clottable protein assays have high accuracy and are used as reference method for plasma

fibrinogen measurement (Mackie *et al.*, 2003). However, these assays are technically difficult and time-consuming to perform; thus rarely required in clinical practice (Lowe *et al.*, 2004). Prothrombin time-derived assays, which estimate fibrinogen concentrations based on optical density or light scatter in automated coagulometers, are easy and cheap to perform (Lowe *et al.*, 2004). Nevertheless, their results show wide variability depending on the reagents and analysers used and give higher values than von Clauss assays (Palateri *et al.*, 1991; Chitolie *et al.*, 1994; Chantarangkul *et al.*, 1994; Kitchen *et al.*, 1995; Chitolie *et al.*, 1998; De Cristofaro *et al.*, 1998; Lawrie *et al.*, 1998; Medical Devices Agency, 2000). Immunological assays, which measure total fibrinogen (clottable or not), are useful to differentiate afibrinogenemias or hypofibrinogenemias from dysfibrinogenemia (Lowe *et al.*, 2004; Verhovsek *et al.*, 2008).

Traditionally, plasma fibrinogen assays have been performed for the investigation, monitoring and management of hemorrhagic risks associated with low congenital or acquired fibrinogen levels. In acute bleeding situations, a simple and fast method to determine plasma concentration of clottable fibrinogen is desirable. In addition, the management of acute bleeding disorders (such as in trauma, in the perioperative period, in obstetric complications and in disseminated intravascular coagulation), requires multiple and frequent measurements. Due to its pivotal role in primary and secondary hemostasis, circulating clottable fibrinogen is considered clinically the most relevant measure. Of note, circulating plasma fibrinogen can present significant heterogeneity in structure and function due to genetic individual differences and degradation by enzymes such as thrombin, plasmin and neutrophil elastase (Nieuwenhuizen *et al.*, 1995). This heterogeneity might render some plasma fibrinogen non-clottable, explaining differences in results between clottable assays and other methods.

Currently, automated hemostasis analysers use mainly two different technologies to assess fibrin clot end-points. The use of mechanical end-point technology is based on the tensile strength of the clot in a magnetic field. Despite being sensitive at low fibrinogen

concentrations, this technique is specially affected by heparin therapy, which can be overcome with in vitro heparin neutralization and re-running of the sample (Mackie *et al.*, 2003). Photo-optical systems are also used and measure the changes in light scatter through 90° or light transmission as a result of fibrin formation. Therefore, this derived fibrinogen assay is based on the changes in optical density, which are proportional to the concentration of fibrinogen. The von Clauss method is the standard assay used at SHSC for fibrinogen measurement.

### **Clotting Rate Assay (von Clauss Method)**

A standardized high concentration of thrombin (varying from 35 to 200 U/ml, but usually about 100 U/ml) is added to diluted test plasma and the clotting time is recorded (Clauss, 1957). The test result is derived by comparison with calibration curves, which are generated by clotting a series of dilutions of a reference plasma sample of known clottable fibrinogen concentration, and is expressed in g/L (SI units). Different analysers can use mechanical end-point or photo-optical systems and a multiplicity of commercial reagents are now available (Medical Devices Agency, 2000; Mackie *et al.*, 2002). Significant differences (approximately 0.1g/L) in fibrinogen estimations were found when different analysers are utilized, but they are unlike to be of any clinical relevance (Medical Devices Agency, 2000). It is estimated that reasonable degree of precision is documented in coagulometers with normal plasma: the coefficient of variation (CV) is characteristically 3 – 7% by optical methods and 6 – 9% by mechanical methods (Marbet & Duckert, 1992; Mackie *et al.*, 2002). Manual techniques are less precise than other methods (CV 10% or above) (Lowe *et al.*, 2004).

### **Total Clottable Fibrinogen Assay**

These assays are performed in the absence of  $\text{Ca}^{2+}$  in citrate plasma by adding thrombin, which converts fibrinogen to fibrin clot during prolonged incubation. Epsilon aminocaproic acid is added to prevent degradation of the clot by plasmin. Soluble proteins in the clot are then washed and dissolved in alkaline urea or other reagents (Ratnoff & Menzie, 1951; Jacobsson, 1955; Blomback & Blomback, 1956; Swain & Feders, 1967; Gaffney & Wong, 1992). Most of the protein in the clot will be fibrin, which will be the equivalent to the fibrinogen concentration. Different from the Clauss assay (Clauss, 1957), where fibrin degradation products (FDPs) inhibits the conversion rate of fibrinogen to fibrin, in clottable assays FDPs may form the clot and increase the assay value. The fibrinogen concentration can be measured by measuring light absorbance at 282nm (Blomback & Blomback, 1956) or by determination of tyrosine in the washed clot (Lowe *et al.*, 2004). Despite being very accurate and used as reference assays for fibrinogen, clottable protein assays are time consuming and technically difficult to perform, limiting their use as routine method in clinical coagulation laboratories (Mackie *et al.*, 2003).

### **Prothrombin –time Derived Test**

This technique is not a direct determination of plasma fibrinogen but an estimation of the concentration of fibrinogen during the clotting process of the prothrombin time (PT) on automated photo-optical coagulometers. In this method, absorbance changes at 450 nm (delta OD) are used to determine the concentration of fibrinogen by analysers which are calibrated by performing PT on a plasma (or a series of plasma dilutions) of known fibrinogen concentration and then by plotting optical changes against fibrinogen concentration (Chitolie *et al.*, 1994; Sobas *et al.*, 2002).

Due to its ease to perform at “no additional costs”, the method rapidly gained popularity among hemostasis laboratories (Chantarangkul *et al.*, 1987; De Metz & van Wersch, 1987;

Hoffmann & Verhappen, 1988; Rossi *et al.*, 1988). However, there is controversy regarding the suitability of PT-derived assays for clinical use (Sobas *et al.*, 2002; Cunningham *et al.*, 2002). It has been showed to yield higher fibrinogen levels than the Clauss method, particularly in anticoagulated patients (Chitolie *et al.*, 1994; Sobas *et al.*, 2002).

### **Immunological Fibrinogen Assays**

Several immunological assays are available for the determination of plasma fibrinogen: radial immunodifusion (RID), enzyme-linked immunoabsorbent (ELISA) or nephelometric. In immunonephelometric and radial immunodifusion techniques, polyclonal antibodies to fibrinogen are incubated with diluted plasma and immune complexes formed. The detection of theses immune complexes are performed by diffusion and precipitation in agarose gel in RID assays and by light-scattering in immunonephelometry. In ELISA techniques, specific antibodies on polystyrene surfaces capture the fibrinogen molecules from diluted plasma. The detection method uses a second enzyme-conjugated antibody to bind fibrinogen on microplates or tubes. All immunological assays measure protein concentration rather than functional activity. Degraded forms of fibrinogen are present and may produce “falsely elevated concentrations of functional fibrinogen”. This effect explains discrepancies between immunological and functional assays such as Clauss and Prothrombin-derived. Due to this limitation, immunological assays are indicated for conditions where normal or elevated fibrinogen levels are expected, such as studies assessing fibrinogen as a risk factor for cardiovascular disease (Cremer *et al.*, 1994; Sweetman *et al.*, 1996, 1998).

Recently, the issue of long turn-around time for lab tests was addressed in a study by Chandler *et al.* (Chandler *et al.*, 2010). The authors implemented a series of modifications to stat coagulation testing such that: i) the emergency hemorrhage panel was prioritized; and ii) speed in processing samples was improved at the expense of precision, but to still produce clinically



relevant results (shortened centrifugation time [from 8 at 2000g to 2min at 4440g instead]; elimination of checks for clots and hemolysis; and extension of fibrinogen calibration curve down to 0.53mg/dL with repeated test, but results as < 0.53mg/dL released). They were able to reduce their turn-around time from 34 to 13 minutes (Chandler *et al.*, 2010).

## 7.10 Dosage Recommendations for Cryoprecipitate

### Transfusion

Currently, due to its high content of fibrinogen, CRYO is used as a way to raise fibrinogen levels in the context of bleeding. One unit of CRYO contains 0.25 g of fibrinogen, thus an average dose of 8 units contains 2 g of fibrinogen. The content of a standard dose of CRYO is equivalent to the amount of fibrinogen found in an average adult dose of FFP (4 units), where each unit of FFP contains 0.5 g of fibrinogen (Callum *et al.*, 2011; Nascimento *et al.*, 2010). Of note, the total volume of a standard dose of CRYO is approximately 150 ml, while the total volume of a FFP dose is equivalent to 1000 ml.

As described previously, typically, an adult dose of 8 – 12 units (one unit per 5 - 10 kg of body weight) is recommended for plasma fibrinogen concentration < 1.0 g/L in the presence of bleeding. However, there is preliminary evidence that challenges this target and recommends a higher target for fibrinogen levels (above 1.5 g/L) and increased doses of CRYO or fibrinogen according to the most recent guidelines (Rossaint *et al.*, 2010; Thomas *et al.*, 2010). A fibrinogen concentrate dose of 3 – 4 g or 50 mg/Kg of CRYO (15 – 20 units) for an average sized adult (70 kg) have recently been recommended for fibrinogen < 1.5 – 2.0 g/L (Rossaint *et al.*, 2010).

A summary of dosage recommendations from major guidelines and their supporting evidence is displayed on table 1.

**Table 1. Dosage Recommendations for Cryoprecipitate Transfusion**

<b>Guidelines</b>	<b>CRYO Dosage</b>	<b>Plasma Fibrinogen Response</b>	<b>Reference</b>
College of American Pathologists - 1994	1 unit/5-10kg	50% increase	No
American Association of Anesthesiologists - 1996	1 unit/10kg	50mg/dL increase	No
British Task Force – 2004	No recommendation	Not stated	No
British Task Force - 2006	2 packs of pooled CRYO for an adult	Not stated	No
American Association of Anesthesiologists - 2006	1 unit/10kg	50mg/dL increase	No
Trauma Guidelines - 2006	10 units	Not stated	No
British Columbia - 2007	No recommendation	Not stated	No
European Guidelines -2007	15 – 20 units or 50mg/kg for 70kg	Raised to 1.0g/L	No
British Committee -2009	10 units	Raised to 1.0g/L	No
European Guidelines - 2010	15 – 20 units or 50mg/kg for 70kg	Not stated	No
Great Britain and Ireland Guidelines - 2010	No recommendation	Not stated	No

The American Association of Blood Banks physician handbook states that 1 unit will increase the fibrinogen by 5 to 10 mg/dl (0.05-0.1 g/L) in an average sized adult (American Association of Blood Banks, 2002) . Also, the American Association of Blood banks technical manual (Brecher, 2005), recommends the use of the following calculation:

- Blood volume = weight (kg) x 70 mL/kg
- Plasma volume = Blood volume x (1-hematocrit)
- mg of fibrinogen required = (desired fibrinogen-current fibrinogen in mg/dl) x plasma volume divided by 100 ml/dL
- Bags of cryoprecipitate required = mg of fibrinogen divided by 250 mg

This calculation assumes that 100% of the infused fibrinogen is measured as plasma fibrinogen concentration by standard fibrinogen assays. However, the content of fibrinogen in CRYO is variable (Callum *et al.*, 2009), wide variation among different fibrinogen measurement methods exist (Nieuwenhuizen *et al.*,1995; Mackie *et al*, 2003; Lowe *et al.*,2004; Verhovsek *et al.*, 2008), and the plasma fibrinogen response to CRYO administration is unclear but likely significant lower than 100%. These recommendations are likely based on expert opinion only and their clinical utility are limited.

## 7.11 The Trauma-associated Coagulopathy

Approximately 40% of all in hospital trauma-related deaths are directly caused by hemorrhage (Sauaia *et al.*, 1995; Kauvar *et al.*, 2006) and many are regarded as being potentially preventable (Tien *et al.*, 2007; Teixeira *et al.*, 2007; Saltzheer *et al.*, 2010; Tien *et al.*, 2010; Sanddal *et al.*, 2011). In massively hemorrhaging patients, current resuscitation protocols regularly fail to prevent the development of coagulopathy in a proportion of patients. It has been estimated that mortality rates can vary from 20 to 50% in trauma patients requiring

massive transfusion, usually defined as the transfusion of 10 or more units of RBC within 24 hours of hospitalization (Malone *et al.*, 2006; Huger-Wagner *et al.*, 2007; Sauaia *et al.*, 1995).

Large-volume crystalloid infusion, early aggressive transfusion of RBC and late replacement of clotting elements may cause dilution, which, associated with clotting factor consumption, hypothermia and acidosis are the multiple factors implicated in the development of the complex coagulopathy following major injury and bleeding (Cotton *et al.*, 2006; Tieu *et al.*, 2007). In 2003, Brohi and MacLeod separately described that trauma coagulopathy occurs early following injury and is present on hospital admission in 25% of all severely traumatized patients (Brohi *et al.*, 2003; MacLeod *et al.*, 2003). Of note, the studies used different cut-off values for PT and partial prothrombin time (PTT) (Brohi *et al.* used PT > 18 and PTT > 60 seconds and Macleod *et al.* defined as PT > 14 seconds and PTT > 34 seconds) Further studies suggest that early coagulopathy is associated with the presence of shock and the amount of tissue destruction, independent of clotting factors consumption or dilution, and is associated with a 3-fold increase in mortality increase (Brohi *et al.*, 2003; MacLeod *et al.*, 2003; Brohi *et al.*, 2007; Hess *et al.*, 2008). Furthermore, a unique coagulopathy in traumatic brain injury (TBI) has long been described, where the release of brain tissue factor causes systemic activation of coagulation (disseminated intravascular coagulation), exhaustion of clotting elements and hyperfibrinolysis (Gando, 2006; Gando *et al.*, 1999). While coagulopathy is common and critically important in TBI, the controversial existing evidence suggests it may not differ from trauma coagulopathy in general (Gando, 2009; Gando *et al.*, 211). The evidence of this early trauma-associated coagulopathy has led to a paradigm shift in the way massively bleeding trauma patients are currently resuscitated, prompting the majority of trauma hospitals to adopt a more aggressive and earlier use of plasma and clotting factors (Holcomb, 2010).

The early trauma-associated coagulopathy concept has challenged the current crystalloid-based resuscitation where coagulopathy is not addressed until late during trauma

resuscitation. Recently, a more aggressive use of plasma and platelets with restriction of crystalloids has been suggested. This hemostatic resuscitation, commonly named “Damage Control Resuscitation” (DCR) or “reconstituted whole blood resuscitation”, proposes early and aggressive FFP and platelets transfusion to treat or prevent early trauma-associated coagulopathy while providing volume replacement (Holcomb *et al.*, 2007; Beekley, 2008).

## 7.12 The Role of Fibrinogen in Trauma-associated Coagulopathy

Fibrinogen is essential in the coagulation process and clot stabilization in order to form a strong clot. It is cleaved by thrombin to form fibrin polymers, which are capable of binding factor XIII, with subsequent cross-linkage to produce a robust fibrin network (Velik-Salchner *et al.*, 2007). Furthermore, fibrinogen plays an important role in platelet activation and aggregation by its binding to  $\alpha_2\beta_3$  integrin glycoprotein (GP) IIb/IIIa fibrinogen receptors present on the surfaces of platelets, which are the main substrate for primary hemostasis. However, in trauma-associated coagulopathy multiple causal factors may directly affect fibrinogen polymeration and metabolism (Fries & Martini, 2010).

### **Dilutional Coagulopathy**

Traditional resuscitation strategies utilize crystalloid to correct shock in order to maintain euvolemia, blood pressure, cardiac output and thus adequate oxygen delivery to tissues. Aggressive initial fluid replacement causes dilution, which compromises coagulation and has been associated with worse outcomes in trauma. Despite being affected by direct dilution, fibrinogen metabolism is not directly disturbed by crystalloid use (Martini *et al.*, 2006).

Hydroxyethyl starch (HES) is also a solution used as fluid replacement in acute resuscitation in trauma. HES may have several deleterious effects on coagulation and has been associated with increased bleeding tendency. It induces a blockage of the fibrinogen receptor (GIIb/IIIa); interferes with fibrin polymerization; and causes platelet coating, hypocalcemia, and a vWB-like syndrome (Mittermayr *et al.*, 2007). HES solutions are routinely used in resuscitation protocols in European countries, but not commonly employed in Canada.

Gelatin products may also cause dilution and impair fibrinogen polymerization (Mardel *et al.*, 1998).

### **Hyperfibrinolysis**

Fibrinolysis is a physiologic response to various insults such as trauma, surgery, infection and ischemia to maintain hemostasis (Levi *et al.*, 1993). Normally, this initial fibrinolytic phase is followed by a suppression phase when anti-fibrinolytic systems are activated (Levi *et al.*, 1993). Tissue and endothelial damage result in the release of tissue plasminogen activator (t-TPA), which causes fibrinolysis through the conversion of plasminogen to plasmin; and its antagonist, plasminogen activator inhibitor type 1 (PAI – 1), which regulates fibrinolysis by inhibiting the formation of plasmin (Cesarman-Maus & Hajjar, 2005; Chandler, 1996; Medcalf, 2007). In trauma, hyperfibrinolysis (increased fibrinolysis) occurs due to the combined effects of both extensive tissue injury and shock (Hess *et al.*, 2008). It is believed that this hyperfibrinolysis develops to limit the coagulation to the site of bleeding, but in the context of extensive tissue damage, this regulatory process may be imbalanced (Hess *et al.*, 2008). This exacerbated fibrinolysis is considered to be an important contributor of hemorrhage and coagulopathy (Tanaka *et al.*, 2009). It seems to be associated with the severity of trauma and specific tissue traumas, where the increased release of thromboplastins are present, such as traumatic brain injury and long-bone fractures (Zehtabchi *et al.*, 2008; Hulka *et*

al., 1996; Stein & Smith, 2004; Gando *et al.*, 1999; Kushimoto *et al.*, 2003; Kupe *et al.*, 1971; Mellor & Soni, 2001).

The bleeding tendency in hyperfibrinolysis can be treated by the use of antifibrinolytic drugs such as tranexamic acid and aprotinin. In fact, a recent randomized controlled trial evaluated an impressive 20,110 number of trauma patients at risk of significant hemorrhage who were treated with 2g tranexamic acid or placebo, and showed reduced mortality rates in favor of the intervention for all-cause in-hospital mortality within 4 weeks (Shakur *et al.*, 2010). Subsequent subgroup analysis of this trial demonstrated the importance of early (first 3 hours following injury) administration of tranexamic acid for its survival benefit (Roberts *et al.*, 2011). Despite its antifibrinolytic action, no corresponding increase in thromboembolic events was observed. The efficacy of antifibrinolytic therapy has also been documented in liver transplant, cardiac and orthopedic surgery (Henry *et al.*, 2007).

### **Hypothermia**

In trauma, hypothermia is defined as core temperature of 34°C or lower and is commonly observed in trauma patients (Tsuei & Kearney, 2004). The deleterious effect on coagulation are well described and include decrease in fibrinogen synthesis and in activity of the complex tissue factor-FVIIa, and inhibition of platelet function (Fries & Martini, 2010; Reed *et al.*, 1990; Wolberg *et al.*, 2004; Meng *et al.*, 2003). Core temperatures below 32°C are associated with excessive mortality rates in trauma (Gentilello *et al.*, 1997; Shafi *et al.*, 2005).

### **Acidosis**

Acidosis is frequently found in trauma patients usually due to the shock state and excessive administration of normal saline (ionic chloride) during acute resuscitation (Siegel *et al.*, 1990; Rutherford *et al.*, 1992). It is an independent predictor of coagulopathy in trauma patient and its severity is directly correlated with mortality rates (Siegel *et al.*, 1990; Rutherford

*et al.*, 1992). Acidosis affects adversely coagulation by reducing protease activity and depleting fibrinogen and platelet counts. As oppose to hypothermia, acidosis did not affect fibrinogen synthesis but significantly increased fibrinogen degradation by an order of almost two in a recent animal study (Martini & Holcomb, 2007).

## **Evidence and Risks of Fibrinogen Replacement in Trauma-associated Coagulopathy**

Fibrinogen was the first clotting factor to decrease to critical levels in coagulopathic patients who underwent major abdominal surgery with estimated blood loss of more than 200 % of the patients' calculated total volemia (Hiippala *et al.*, 1995). Furthermore, compensatory mechanism of synthesis could not increase fibrinogen levels to overcome the increased breakdown in a porcine model of moderate dilutional coagulopathy, suggesting that hypofibrinogenemia can not be entirely explained by blood loss and dilution (Martini *et al.*, 2005). It has been shown that low preoperative levels of fibrinogen are associated with increased postoperative bleeding (Blome *et al.*, 2005; Ucar *et al.*, 2007b; Karlsson *et al.*, 2008). In postpartum patients, low levels of fibrinogen had a PPV of 100% for severe bleeding (Charbit *et al.*, 2007). Finally, besides the importance of fibrinogen in fibrin formation and platelet function, the replacement of exogenous fibrinogen could theoretically bypass other missing components of the coagulation cascade.

Currently, fibrinogen can be provided through plasma, CRYO and fibrinogen concentrate. FFP and CRYO are human blood products that contain high concentrations of fibrinogen. One unit of CRYO contains 0.25 g of fibrinogen, thus an average dose of 8 units contains 2 g of fibrinogen. The content of a standard dose of CRYO is equivalent to the amount of fibrinogen found in an average adult dose of FFP (4 units), where each unit of FFP contains 0.5 g of fibrinogen (Callum *et al.*, 2011; Nascimento *et al.*, 2010). Of note, the total volume of



a standard dose of CRYO is approximately 150 ml, while the total volume of a FFP dose is equivalent to 1000 ml.

However, the use of these two human blood products as a source of fibrinogen has important and practical limitations, such as extended turnaround time for thawing and pooling, transfusion-related risks and unproven efficacy (Stanworth *et al.*, 2004; Stanworth, 2007). Of note, CRYO is not available in most European countries (Haas *et al.*, 2008), but it is still widely used in North America and the UK. For FFP transfusion, time permitting, blood group matching is required; and both FFP and CRYO are stored frozen, which require thawing before using. These factors contribute to long turnaround times (from the time products are requested to its completion of transfusion). Furthermore, FFP is not submitted to viral inactivation procedures in North America, carrying, although low, a risk of infectious disease transmission. Pathogen inactivation treatments can be used for FFP, but it can reduce the content of fibrinogen present in the product (Cardigan *et al.*, 2009). These procedures cannot be employed for the CRYO product itself. Other potential complications of these products, particularly FFP, include transfusion-associated circulatory overload (TACO) and transfusion-related acute lung injury (TRALI) (Stanworth, 2007; Callum *et al.*, 2009; O'Brien, 2007; Renaudier *et al.*, 2009). TRALI is the commonest cause of transfusion-related mortality (Popovsky, 2006; Renaudier *et al.*, 2009). Finally, the evidence on the clinical efficacy of FFP and CRYO for the replacement of fibrinogen is limited (Chowghury *et al.*, 2004; Danes *et al.*, 2008). A recent retrospective study by Stinger and colleagues, reviewing 252 massively transfused injured soldiers, found that the transfusion of an increased ratio of fibrinogen (given as combination of all blood and blood product administered) to RBC was independently associated with improved survival, mainly by reducing hemorrhagic deaths (Stinger *et al.*, 2008). Of note, the vast majority of fibrinogen administered to these patients in this study was in the form of plasma, thus survival improvement could also be attributable to the infusion of other coagulation factors.

As compared to FFP and CRYO, fibrinogen concentrate preparations are routinely subjected to pathogen reduction technologies treatment in their manufacturing process (Groner, 2008) and their content of fibrinogen are standardized (Fenger-Eriksen *et al.*, 2009a). With fibrinogen concentrate, there is no thawing time and due to its small volume, the turnaround time and administration time is short. The infusion time for 100ml (approximately 2g of fibrinogen) of reconstituted RiaSTAP<sup>®</sup>, a recently approved and commercially available human fibrinogen concentrate in the United States, is approximately 20 min. It has been shown to be effective and well tolerated in a variety of congenital and acquired fibrinogen deficiency in medical and surgical clinical settings (Fenger-Eriksen *et al.*, 2008; Fenger-Eriksen *et al.*, 2009a,b; Rodriguez *et al.*, 1988; Kreuz *et al.*, 2005; Aygoren-Pursun *et al.*, 2007; Heindl *et al.*, 2005; Danes *et al.*, 2008; Haas *et al.*, 2008; Weinkove & Rangarajan, 2008; Rahe-Meyer *et al.*, 2009a,b). In animal models of dilutional coagulopathy, fibrinogen concentrate substitution prior to injury was associated with reduction in blood loss, improvement in coagulation parameters and increase in survival rates (Fries *et al.*, 2005; Fries *et al.*, 2006; Grottke *et al.*, 2010). As a consequence of this, in Europe, a marked increase in the use of fibrinogen concentrates for the management of bleeding patients has been documented in the last few years (Meyer *et al.*, 2011).

Despite the intense interest in this concentrate and growing use, there is a lack of studies demonstrating its efficacy in trauma. A comprehensive review on fibrinogen concentrates for bleeding trauma patients published early this year (Meyer *et al.*, 2011), identified only four relevant studies. None of these studies were randomized controlled trials. One observational study, including 128 trauma patients (Schochl *et al.*, 2010b) and three cases reports comprised this review (Schochl *et al.*, 2010a,c; Brenni *et al.*, 2010). The main finding of these studies was that the administration of fibrinogen concentrate sometimes associated with prothrombin complex concentrate, might be an alternative hemostatic strategy in trauma

patients treated with synthetic colloids during resuscitation, as compared to the traditional plasma strategy. This review highlights the urgent need for higher quality study designs in order to address this question (Meyer *et al.*, 2011). No published studies comparing the efficacy of CRYO with fibrinogen concentration are available to date.

## 7.13 Appropriateness of Use of CRYO in Transfusion

### Medicine

#### **Assessing Appropriateness of Use**

The appropriateness of a medical or surgical procedure is evident when “the expected health benefit exceeds the expected negative consequences by a sufficiently wide margin that the procedure is worth doing, exclusive of cost” (Brook *et al.*, 1986; Park *et al.*, 1986).

Physician and patients are daily faced with the challenge to make important decisions about diagnostics tests, surgical procedures and medical treatments, which require knowledge about the best evidence available and its application in an individual basis to minimize inappropriate risky and expensive medical interventions. Several methods have been developed to assess appropriateness of care that identifies the opinion of experts about an intervention (Fink *et al.*, 1984). In general, they use a structured process for incorporating information from the scientific evidence with the expert judgment to generate explicit criteria in order to establish the appropriateness of particular procedures. It usually involves defined steps that include but is not limited to: (i) literature review; (ii) development of clinical scenarios to be assessed by a panel of experts; (iii) selection of experts; (iv) rating and discussion of scenarios; (v) obtain input from patients and other physicians when applicable; and (vi) update recommendations when necessary (Brook, 2009).

Once a medical or surgical procedure is assessed and appropriateness criteria are agreed upon, basically two conditions are needed to evaluate if its use is appropriate in clinical practice: (i) guidelines for the intervention have to be in place and users (physicians) aware of its existence and (ii) enough information about the indication for the intervention from the responsible physician available for verification.

## **Anticipated Challenges in Assessing Appropriateness of CRO**

### **Transfusion in Trauma**

CRYO transfusion is considered an important strategy in the management of refractory hemorrhage in trauma patients. In the setting of massive hemorrhage in trauma, transfusion decisions are usually made according to physicians' clinical judgment, available laboratory testing results and transfusion protocols where available, to provide some general guidelines, usually customized by the individual patient's needs.

### **Patient's characteristics**

Massively bleeding patients are at great risk of coagulopathy and refractory shock, which occur soon after admission, leading to early in-hospital hemorrhagic deaths (Moore *et al.*, 2008b). In fact, Acosta *et al.* reviewed autopsies and time to death of patients who died from trauma and described that 70% of all deaths occurred in the first 24 hours of hospitalization, with a significant number of these deaths (35%) occurring within 15 min of hospital arrival (Acosta *et al.*, 1998). Blood and blood product transfusion is essential part of the resuscitation of these patients and an estimated 80% of all RBC transfusions occur in the first 6 hours of admission (Moore *et al.*, 2008a; Kashuk *et al.*, 2008). This illustrates the urgent nature of transfusion in this population of massively bleeding trauma patients, which might affect compliance with transfusion guidelines, due to complete lack of laboratory test results in the first 30 – 60 min of arrival.

## **Low Use of Guidelines**

The failure of many institutions to adopt published transfusion guidelines is an important consideration for the assessment of appropriateness of CRYO transfusion. A recent national survey conducted in the UK on the use of transfusion guidelines for major trauma documented that guidelines were infrequently utilized (Westerman *et al.*, 2008). Out of 109 major emergency departments which responded to the questionnaire, only 17 (16%) have transfusion guidelines in place to guide the transfusion management of trauma patients. Also, an international survey of clinical practice for the management of coagulopathy in patients with multiple injuries has recently been published. This survey involved 25 countries and reported that 45 % of the respondents claimed to follow their institution's massive transfusion protocol, 19% reported having a protocol but with inconsistent use and 34% do not have a protocol.

## **Variability in Practice**

The lack of consensus on major aspects of massive transfusion is evident. A recent review on massive transfusion protocols, including 14 large medical academic centers, documented the presence of important variations in transfusion practice (Young *et al.*, 2011). Of 14 massive transfusion protocols, 5 (36%) indicate CRYO use based on ratios of blood with blood products, 5 (36%) use CRYO upon request and 4 (28%) do not recommend its use. The lack of agreement on major aspects of prevention and treatment of coagulopathy in patients receiving massive transfusions was also evident in a recent international forum (Levi *et al.*, 2011). This forum received 12 contributions from experts in the area, who had to compile their answers to five questions addressing 5 major aspects of massive transfusion, representing 8 different countries (Austria, Belgium, Canada, Denmark, France, Germany, Netherlands, Switzerland and USA). Interestingly, all 12 contributions used a different criteria or language to define massive transfusion. Other significant variations could be appreciated with respect to:

(i) the utilization of a preset ratio of blood products and/or which ratio should be used if adopted; (ii) the use of standard coagulation tests (platelet count, International Normalized Ratio [INR], partial thromboplastin time [PTT], fibrinogen levels), viscoelastic coagulation testing (thromboelastography [TEG] or rotational thromboelastometry [ROTEM]), and no testing at all during massive transfusion; (iii) the use of adjuncts to hemostasis (antifibrinolytic drugs; fibrinogen concentrate; prothrombin complex concentrate, clotting factor XIII concentrate, etc.)

### **Adequacy of Documentation**

Another potential challenge in assessing appropriateness in major trauma, particularly through retrospective audits of physician's orders, is the adequacy of physician and nursing documentation of transfusion. Due to urgent nature of CRYO transfusion in trauma, documentation is frequently regarded as secondary in face of other clinical priorities. Friedman & Edrahim conducted a retrospective review of the adequacy of documentation for RBC transfusion in two New York acute care hospital facilities in the US (Friedman & Edrahim, 2006). Transfusions of RBC were deemed justified or not by comparing with hospital transfusion guidelines. Events with suboptimal (intermediate and inadequate) documentation accounted for 49% of all medical record reviewed transfusion events and 62% could not be justified. The authors noted that there was a significant correlation between suboptimal documentation and failure to justify transfusion (Friedman & Edrahim, 2006).

All of these might produce obstacles to assessing compliance with published guidelines in this patient population.

### **Evidence on Appropriateness of CRO Transfusion in Trauma**

To date, no studies were performed assessing the appropriateness of CRYO transfusion exclusively in massively transfused trauma patients. Three studies evaluated the

appropriateness of CRYO transfusion in a variety of clinical setting, including a small subpopulation of trauma patients (Schofield *et al.*, 2003; Pantanowitz *et al.*, 2003; Alport *et al.*, 2008). These studies, one a prospective audit and two retrospective reviews of transfusion records, considered appropriate CRYO use according to published guidelines on plasma fibrinogen triggers and type of clinical settings. They did not explore physician's clinical judgment and perceptions, neither specific patient's clinical characteristics, which might affect clinical decisions on laboratory monitoring and/or CRYO transfusion.

Alport *et al.* conducted a prospective audit involving 25 Canadian hospitals, involving 603 transfusion events, and reported an overall 24% of appropriateness for CRYO use in different clinical settings (Alport *et al.*, 2008). The authors categorized CRYO transfusions as likely appropriate if a fibrinogen level, taken within 6 hr before or after transfusion was not more than 1.0g/L. However, the authors did not report on individual appropriateness rates for the different clinical settings.

In 2003, an Australian study reviewed 64 CRYO transfusions and reported that only 38% of CRYO transfusions were considered appropriate when the same fibrinogen level criteria were applied (Schofield *et al.*, 2003). When a more relaxed fibrinogen level (< 1.5g/L) was used, 57% of CRYO transfusions were considered appropriate. However, the time to fibrinogen measurement in this report was not stated.

An audit from the United States also published in 2003, applying different criteria for appropriateness, reported that 76% of CRYO transfusions were appropriate in 51 patients (Pantanowitz *et al.*, 2003). In that report, CRYO transfusion was considered appropriate for a fibrinogen level less than 1.0g/L when bleeding, and in addition, for tissue plasminogen activator-related hemorrhage, transfusion of 10 or more PRBC units irrespective of fibrinogen level, uremic bleeding and von Willebrand's disease. The two latter indications are considered inappropriate by others due to the availability of desmopressin, virally inactivated human-

derived factor VIII/von Willebrand factor concentrates and fibrin (Janson *et al.*, 1980; Maierhofer *et al.*, 1981; Triulzi *et al.*, 1990; Weigert *et al.*, 1998; Mannucci, 2004).

### **Appropriateness of Red Blood Cell and Plasma Transfusion**

Although guidelines for RBC and FFP have been published, adherence to these guidelines is frequently low. An international multidisciplinary panel of 15 expert reviewed all pertinent literature on RBC transfusion, and applying establish criteria for assessing appropriateness of a procedure (RAND/UCLA Appropriateness Method), determined the appropriateness of RBC transfusion (based on its expected impact on outcomes) in 450 typical inpatient medical, surgical, or trauma scenarios (Shander *et al.*, 2011). Panelists rated RBC transfusion as appropriate in 53 of the scenarios (11.8%), inappropriate in 267 (59.3%), and uncertain in 130 (28.9%). This observation indicates that the available evidence on RBC transfusion is limited and many areas of disagreement exist. It also supports a more judicious use of this product. In another UK-wide survey on FFP transfusion practices, including 4969 transfusions in 190 different hospitals, 43% of all FFP transfusion in adults was not support by published recommendations (Stanworth *et al.*, 2011). The appropriateness of FFP transfusion was recently evaluated in a Canadian intensive care unit (Lauzier *et al.*, 2007). In this review, 47.6% of the FFP were considered inappropriate according to national guidelines. An academic hospital prospective audit documented that 45% of FFP orders were inconsistent with national guidelines (Luke *et al.*, 2002). Others have also found important inconsistencies between FFP use and accepted recommendations (Rao *et al.*, 2002; Dara *et al.*, 2005; Wallis & Dzik, 2004).

## **8. Aims**

This study reviews the practice of CRYO transfusion during a period of 10 years at a large tertiary care trauma hospital. It analyzes a cohort of massively transfused trauma patients where CRYO was used for the management of bleeding/coagulopathy following injury. It aims



to assess the appropriateness and fibrinogen response to CRYO transfusion. The study is divided in two major parts:

### **Part A (Appropriateness of CRYO transfusion)**

The study aims to assess appropriateness of CRYO transfusion in trauma by applying two different definitions:

(i) Strict definition: CRYO transfusion was considered appropriate if fibrinogen levels were  $< 1.0\text{g/L}$  2 hour before and/or  $\leq 1.5\text{g/L}$  within 2 hour after CRYO transfusion. If no fibrinogen levels were measured within 2 hour of CRYO use the event was classified as outside guidelines. This definition was thought to be more appropriate by the authors in the setting of major trauma with high rates of bleeding and aggressive resuscitation.

(ii) Lenient definition: CRYO transfusion was considered appropriate if fibrinogen levels were  $< 1.0\text{ g/L}$  6 hour before and/or  $\leq 1.5\text{ g/L}$  within 6 hour after CRYO transfusion. If no fibrinogen levels were measured within 6 hr of CRYO use the event was classified as outside guidelines. This definition has been used previously by other studies to characterize appropriateness in a variety of clinical settings including trauma (Schofield *et al.*, 2003; Pantanowitz *et al.*, 2003; Alport *et al.*, 2008).

### **Part B (Fibrinogen Response to CRYO Transfusion)**

It analyzes a subgroup of trauma patients where fibrinogen levels were obtained within 1hr before and after CRYO transfusion, and with no transfusion of plasma and/or platelets within 2hr prior to the start of CRYO. This analysis aims to assess the plasma fibrinogen response to CRYO transfusion alone.

## **8.1 Specific Aims – Part A**

A.1 - To determine the frequency of appropriate CRYO transfusion events in the setting of trauma. Appropriateness was defined based on published and our hospital guidelines.

A.2 - To assess the rate of appropriateness of CRYO use in trauma over time at a single institution;

A.3 - To assess patients factors associated with appropriateness; to compare demographics and baseline characteristics of patients in the group defined as appropriate to patients in the group outside guidelines; to compare transfusion practice for RBC, plasma and platelets between appropriate and outside guideline groups;

A.4 – To describe our hospital massive transfusion protocol;

A.5 – To describe our transfusion medicine program;

A.6 – To describe our hospital emergency laboratory protocol;

A.7 - To discuss system-related factors associated with appropriateness of CRYO transfusion at a single institution;

A.8 – To describe CRYO utilization according to the clinical setting at a large teaching hospital;

A.9 – To describe CRYO dosage in different clinical settings in a large teaching hospital.

## 8.2. Specific Aims – Part B

B.1 - To determine the incremental changes in plasma fibrinogen levels within 1hr of CRYO transfusion in trauma patients;

B.2 - To compare plasma fibrinogen response to CRYO transfusion between trauma patients with documented low and high fibrinogen levels before the administration of CRYO;

B.3 - To explore patient factors associated with differences in fibrinogen response to CRYO transfusion; to compare demographics, baseline characteristics and transfusion data between patients with low and high fibrinogen levels before CRYO transfusion;

## 9. Hypotheses

### 9.1 Hypothesis – Part A

We hypothesize that our rates of appropriateness for CRYO precipitate transfusion in the context of trauma are higher than previous reported rates in mixed trauma, surgical and medical patients due to the presence of institutional guidelines, active involvement of transfusion medicine physicians in transfusion management, and adequate turnaround time for fibrinogen levels.

Previous audits documented rates of appropriateness in a variety of clinical indications ranging from 24 to 76% (Schofield *et al.*, 2003; Pantanowitz *et al.*, 2003; Alport *et al.*, 2008). This wide variability is mostly due to different definitions for appropriateness being used in different settings. In 2003, an audit from the United States (Pantanowitz *et al.*) reported high rates of appropriate use of CRYO utilizing a very inclusive definition. In this audit, in addition to the commonly accepted indication (fibrinogen level < 1.0 g/L in the context of bleeding) for CRYO transfusion, its use was also considered appropriate for transfusion of 10 or more RBC units irrespective of fibrinogen level, for tissue plasminogen activator-related hemorrhage, and for uremic bleeding and vWD. The two latter indications are considered inappropriate by others due to the availability of desmopressin, virally inactivated human-derived factor VIII/von Willebrand factor concentrates and fibrin (Janson *et al.*, 1980; Maierhofer *et al.*, 1981; Triulzi *et al.*, 1990; Weigert *et al.*, 1998; Mannucci, 2004). Furthermore, the indication for CRYO based solely on the number of units of RBC transfused is not supported by most published

guidelines (American Society of Anesthesiologists, 1996; O'Shaughnessy *et al.*, 2004; Droubatchevskaia *et al.*, 2007; Levi *et al.*, 2009). Therefore, the rate of 76% appropriate CRYO transfusion reported by Pantanowitz *et al.* is inappropriately high.

We believe with an organized system where a transfusion protocol is in place and monitored by transfusion medicine specialist and blood bank technologists, despite the challenges and limitations of trauma resuscitation, over 50% of the transfusion events in trauma will be considered appropriate (transfusion of CRYO will be accompanied by fibrinogen levels below 1.0g/L preceding the transfusion and/or < 1.5g/L after transfusion).

## 9.2 Hypothesis – Part B

The American Society of Anesthesiology, in their guidelines for blood component therapy, states that an average dose of 1 unit of CRYO per 10kg of body weight raises plasma fibrinogen by approximately 0.5 g/L in the absence of continued consumption or massive bleeding (American Society of Anesthesiologists, 1996). In trauma, CRYO is commonly transfused after high volumes of crystalloids, RBC, plasma and platelets had been infused, and when dilution and continued blood loss are present. Furthermore, acidosis, hypothermia and consumption of coagulation contribute to coagulopathy in trauma. We hypothesize that current recommended dose of CRYO transfusion is inadequate in the context of dilution and ongoing blood loss, and would result in a plasma fibrinogen increment below 0.5 – 1.0g/L per standard CRYO dose (8 – 10 units of CRYO per dose) in trauma patients.

## 10. Method

### 10.1 Setting

SHSC is a large academic hospital fully affiliated with the University of Toronto where the vast majority of CRYO is used for major hemorrhage in cardiac surgery and trauma. SHSC is an urban level 1 trauma centre in Toronto, Canada. Approximately 1100 injured patients (80% blunt traumas; 70 – 80% males) are treated by the trauma team at SHSC annually, and these patients have an average Injury Severity Score (ISS) of 20. ISS is an anatomic trauma scoring system which takes into account the squared values of the three mostly injured body areas based on the Abbreviated Injury Scale (AIS) and correlates strongly with mortality (Baker *et al.*, 1976; Baker *et al.*, 1974; Linn, 1995; Copes, 1988; Senkowski, 1999). It varies from minor traumas with practically no significant injuries (score point=1) to unsalvageable injuries (score point=75). Mortality increases significantly for scores of 15 or higher. Score values of 25 or above are representative of severe trauma with high mortality rates (Baker *et al.*, 1976; Baker *et al.*, 1974; Linn, 1995; Copes, 1988; Senkowski, 1999).

Of 1100 patients seen at SHSC annually, approximately 35 to 40 patients receive massive transfusions ( $\geq 10$  units of RBC) within the first 24h of admission. In our institution, similar to recent published data, 80% of all blood transfusions to severely injured patients occur within the first 6 hours of hospitalization. This group of massively transfused patients has a mean ISS of 42 ( $\pm 15$ ) and has an in-hospital mortality rate of 45%.

In the trauma room at our institution, patients are assessed and treated by the trauma team, led by a trauma team leader who can be a trauma surgeon, emergency physician or anesthesiologist. Acutely, care may also be provided by attending staff from Critical Care Medicine and/or Anesthesia. For massively bleeding trauma patients, transfusion medicine specialists are also automatically contacted, and they are available for assistance with transfusion decisions. Our emergency blood stock consists of 16 units of O+ RBCs, and 8 units of O- RBCs (reserved for women less than 45 years of age). Our emergency stock is immediately available, with half stored in the trauma room and half in the blood bank.

Currently, thawed plasma is not available due to our low utilization of this product (1500 units per year or approximately 4 units per day) and potential for wastage. FP is thawed by water bath method which usually takes about 30 minutes for its completion. Based on our current transfusion policy, our blood bank technologists proactively thaw 4 – 8 units of FP when they anticipate a patient will use a large amount of blood. In emergency situations, when the patient's blood type is unknown, AB plasma is used until type-specific plasma can be provided.

The study proposal was reviewed and approved by SHSC Research Ethics Board.

## 10.2 Study Design

This is a retrospective cohort review of all consecutive cases of CRYO use in various specialties during a period of 10 years (from January 1998 until June 2008) at SHSC.

## 10.3 Data Acquisition

The Hospital Blood Bank generated a report on all cases of CRYO transfusions during the study period and transfusion data were obtained from the blood bank databases (Hemocare, Medware Information Systems, Inc. Revision 6.1.1, USA and Sunquest, Information Systems, Inc. version 6.2, USA). Clinical and laboratory data were retrieved from the hospital chart and electronic patient records.

## 10.4 Study Variables

Data collected include age, gender, reasons for hemorrhage (type of trauma in injured patients). For trauma patients, ISS and presence of head injury (Head Abbreviated Injury Severity Score – Head AIS) were also determined. Transfusion variables analyzed include indications for CRYO transfusion, time of blood product issue, number of blood products transfused before CRYO transfusion (RBC, platelets and FP) and dosing. Laboratory results

included initial pH, INR, PTT, platelet count and fibrinogen. For the analysis of fibrinogen response to CRYO transfusion, plasma fibrinogen levels recorded within 2 hours before and after transfusion were utilized.

## 10.5 Definitions

### **Appropriateness of CRYO Transfusion**

CRYO transfusion was considered appropriate if the fibrinogen levels were  $< 1.0$  g/L 2 hour before and/or  $\leq 1.5$  g/L within 2 hour after CRYO transfusion. It was classified as outside guidelines if fibrinogen levels were above these thresholds or no fibrinogen levels performed before and after CRYO transfusion.

A more lenient definition of appropriateness, using 6 hour before and after CRYO transfusion, for the same levels of fibrinogen ( $< 1.0$  g/L 6 hour before and/or  $\leq 1.5$  g/L within 6 hour after CRYO) was also applied. Using the 2 hour cut-off definition (strict definition), patients were categorized according to the appropriateness of their transfusion events into appropriate and outside guideline groups. Demographics, baseline characteristics and transfusion data were analyzed for both groups.

### **Severe Head Injury**

The Head AIS was used to classify patients with severe head injury. In this injury scoring system, the severity of injury is rated from minor (AIS=1) to unsalvageable (AIS=6) (Table 2).

### **Table 2. The Abbreviated Injury Scale (AIS) Rating System**

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<b>Score</b>	<b>Severity of Injury</b>
AIS = 1	Minor
AIS = 2	Moderate
AIS = 3	Serious but not life threatening
AIS = 4	Severe, life threatening, survival probable
AIS = 5	Critical, survival uncertain
AIS = 6	Unsurvivable

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Based on this rating system, severe head injury is considered to be Head AIS >3 (Baker *et al.*, 1976; Baker *et al.*, 1974; Linn, 1995; Copes, 1988; Senkowski, 1999).

The AIS is the most widely used anatomic injury severity rating system in the world (Garthe *et al.*, 1999). It was developed in 1971 by the American Medical Association Committee on Medical Aspects of Automotive Safety to provide researchers with an accurate method for rating and comparing injuries from automotive crashes, as well as to establish a



common language when describing injuries (Committee on Medical Aspects of Automotive Safety, 1971, 1972). A subsequent number of revisions followed its original version: (1) 1980, several nonanatomic components were included (Committee on Injury Scaling, 1980); (2) 1985, the scale body regions were expanded from five to nine and expanded sections on thoracic, abdominal and pelvic regions were added. New entries for penetrating, vascular and skin injuries were incorporated (Committee on Injury Scaling, 1985); (3) 1990, more descriptors of penetrating injuries, specific codes for pediatric trauma and a different classification for the body regions were included (Association for the Advancement of Automotive Medicine, 1990).

## 10.6 Sunnybrook Hospital Transfusion Protocol

### (Appendix 1)

During the study period, the hospital transfusion guidelines recommended 1 unit of CRYO/10 kg of body weight (i.e. 8 to 12 units per dose) if plasma fibrinogen level was < 1.0 g/L in the context of massive or microvascular bleeding and regardless of the number of RBC, FP or PLT transfused. During our massive transfusion protocol, coagulation testing, including fibrinogen levels are performed in an expedited fashion and results are usually delivered within 30 to 60 minutes of sample arrival. Our transfusion guidelines recommend hourly fibrinogen levels during massive transfusion, which is overseen by a transfusion medicine specialist who is automatically contacted for any request of large amounts of blood. In our hospital transfusion guidelines, RBC transfusions are administered to maintain euvolemia, and to maintain hemoglobin levels between 70 g/L and 100 g/L. FP is transfused in 3-4 unit aliquots, for INR>1.8. Platelets are transfused one pool at a time (4 units/ pool of Buffy coat platelets or

one single donor apheresis; before the buffy coat platelets, each pool of platelets corresponded to 5 units) to maintain the platelet count above  $50 \times 10^9/L$  ( $> 100 \times 10^9/L$  with co-existing brain injury). FP is thawed in the standard warm water bath fashion. If blood group is unknown at the activation of our massive transfusion protocol, AB plasma is thawed in anticipation and issued in an expedited fashion in order to assure constant availability of plasma. Anti-fibrinolytic drugs were not routinely recommended during the study period.

## 10.7 Sunnybrook Blood Bank

The blood bank is staffed by at least 2 technologists during nights and 10 – 12 during days. The blood bank technologist routinely checks the fibrinogen levels at any request for CRYO transfusion and often prompts testing when not requested. CRYO is also prepared in anticipation based on testing independently of medical request as per our massive transfusion guidelines. Blood bank is notified by an automatic alarm of a potential massively transfused trauma patient, if the blood fridge in the trauma room is accessed for emergency stock RBC.

## 10.8 Fibrinogen Measurement

### **Sample Collection**

Venous or arterial punctures in a clean and fast (in order to minimize blood stasis) fashion were performed for whole blood acquisition for fibrinogen testing. Blood samples for fibrinogen measurement were collected using tubes containing 3.2% trisodium citrate. During

sample collection, tubes were filled up to achieve the 9 vol of blood to 1 vol of 0.105 mol/L (3.2%) trisodium citrate standard recommendation (World Health Organization, 1999). After collection, samples were transferred to the Coagulation Laboratory area for processing.

### **Handling and Processing of Coagulation Specimens**

Our coagulation laboratory has a flowchart for handling coagulation samples that takes into account the acuity of the area the samples are originated from for processing (Appendix 2). Samples coming from areas treating patients with high acuity such as emergency room, operating rooms and intensive care units require STAT handling and processing. Samples are initially checked for possible clots by removing the tube caps. If the presence of clotted sample is detected, laboratory personnel notify the clinical staff and the sample is rejected. Samples with no evidence of clotting are then centrifuged. At our institution, STAT coagulation specimens are prioritized over routine samples; and rapid turnaround times are achieved mainly by shortening labeling and spinning times (barcode pre-labeled tubes are used and placed in STAT spinner with tops off at 7200 rpm for only 2 minutes, instead of at 3500 rpm for 10 minutes used for routine samples) (Appendix 1). After centrifugation, the sample plasma is assessed for evidence of hemolysis. If the presence of hemolysis is confirmed, clinical staff is informed and sample is rejected.

### **Fibrinogen Assay**

The assay was performed at 37<sup>0</sup>C on ACL TOP analyzer (Instrumentation Laboratory, IL), which has its endpoint based on the optical density of formed clot.

Plasma fibrinogen levels were determined using the Clauss method for the entire duration of the study. A standardized high concentration of thrombin is added to diluted test

plasma and the clotting time is recorded (Clauss, 1957). The test result is derived by comparison with calibration curves generated by clotting a series of dilutions of a reference plasma sample of known clottable fibrinogen concentration and expressed in g/L (SI units).

In our laboratory, 100U/mL of thrombin solution (Fibrinogen-C, HemosII, Instrumentation Laboratory) is added to the citrated diluted plasma. The recommended (1:10) dilution is initially used. Test samples are re-diluted if they are outside the linear range of the assay. Dilutions of 1:5 and 1:20 are used for very low and high levels of fibrinogen, respectively. Our laboratory has four different reference range calibration curves. The range of detectable fibrinogen is from 0.08 g/L to 10.0 g/L. Therefore, fibrinogen levels as low as 0.08g/L can be detected.

Quality control samples for normal and low fibrinogen levels are performed every time the Hemostasis analyzer is utilized for fibrinogen determination.

The Clauss assay uses a high concentration of thrombin, which has the tendency of sticking to foreign surfaces and may contaminate subsequent samples. In order to avoid carry-over, appropriate cleaning is employed on the reagent delivery system of the analyzer between tests.

### **Reporting of Results**

In order to minimize turnaround time for laboratory results in emergency situations, our hospital policy requires the coagulation technologist to initially report fibrinogen results direct to the clinical area by telephone and subsequently to enter results into the patient's electronic laboratory record system. Fibrinogen levels are reported in g/L (SI units).

## **11. Study Outcomes**

### **Main Study Outcome – Part A**

It analyzes the frequency of appropriate and outside guideline CRYO transfusion in the setting of trauma using both definitions discussed above (2 and 6 hour cut-off criteria).

### **Main Study Outcome – Part B**

To determine the fibrinogen response to CRYO transfusion in trauma patients (the increase in plasma fibrinogen level following CRYO transfusion in g/L and percentage increase will be determined). For this second outcome, CRYO transfusion events with fibrinogen levels measured 1hr before and after; and with no transfusions of plasma and/or platelets within 2 hr prior to the start of CRYO, were included in this analysis so as to avoid confounding by plasma or platelet transfusion as a source of fibrinogen.

### **Secondary Study Outcomes – Part A**

SOA1 – Total CRYO use according to clinical setting during study period (in percentage, units of CRYO, transfusion events, number of patients and units/patients);

SOA2 – Dosage of CRYO in different clinical settings (in units of CRYO and number of doses);

SOA3 – Percentage of single dose, two doses or more than three doses per clinical setting;

SOA4 – Total CRYO use (in number of units & percentages) by year in different clinical settings;

SOA5 – Number of CRYO transfusion events and patients in cardio-vascular surgery over study period

SOA6 – Number of CRYO transfusion events and patients in trauma over study period;

SOA7 – Hourly rates of fibrinogen measurement before and after CRYO transfusion in trauma;

SOA8 – Hourly fibrinogen levels  $< 1.0$  g/L within 6 hours before CRYO transfusion in trauma;

SOA9 – Hourly fibrinogen levels  $\leq 1.5$  g/L within 6 hours after CRYO transfusion in trauma;

SOA10 – Rates of appropriateness of CRYO transfusion in trauma by year;

SOA11 – Comparison of demographics, baseline characteristics and transfusion of RBC, FP and platelets before CRYO transfusion in appropriate and outside guideline groups;

SOA12 - Comparison of demographics, baseline characteristics and transfusion of RBC, FP and platelets before CRYO transfusion in appropriate and outside guideline (without fibrinogen levels) groups;

SOA13 - Comparison of demographics, baseline characteristics and transfusion of RBC, FP and platelets before CRYO transfusion between events with and without fibrinogen measured within 1 hour.

### **Secondary Study Outcomes – Part B**

SOB1 – Plasma fibrinogen response (in g/L and percentage increase) in events with fibrinogen level  $< 1.0$  g/L before CRYO;

SOB2 - Plasma fibrinogen response (in g/L and percentage increase) in events with fibrinogen level  $\geq 1.0$  g/L before CRYO;

SOB3 - Comparison of demographics, baseline characteristics and transfusion of RBC, FP and Platelets before CRYO transfusion between patients with fibrinogen  $<1.0$  g/L and fibrinogen  $\geq 1.0$ g/L within 1 hour before CRYO.

## **12. Statistical Analysis**

Summary data for continuous variables were presented as means and standard deviations or medians and interquartile ranges depending on the distribution. The discrete variables were summarized as frequency and percentages.

Comparisons between appropriate and outside guideline groups were performed using Student's *t* Test for continuous variables and  $\chi^2$  analysis for categorical data. The analysis of fibrinogen response was conducted using Student's *t* Test. Two-tailed tests were applied and statistical significance was defined at  $p < 0.05$ . All analyses were performed with SPSS 15.0 (Chicago, IL).

## 13. Results

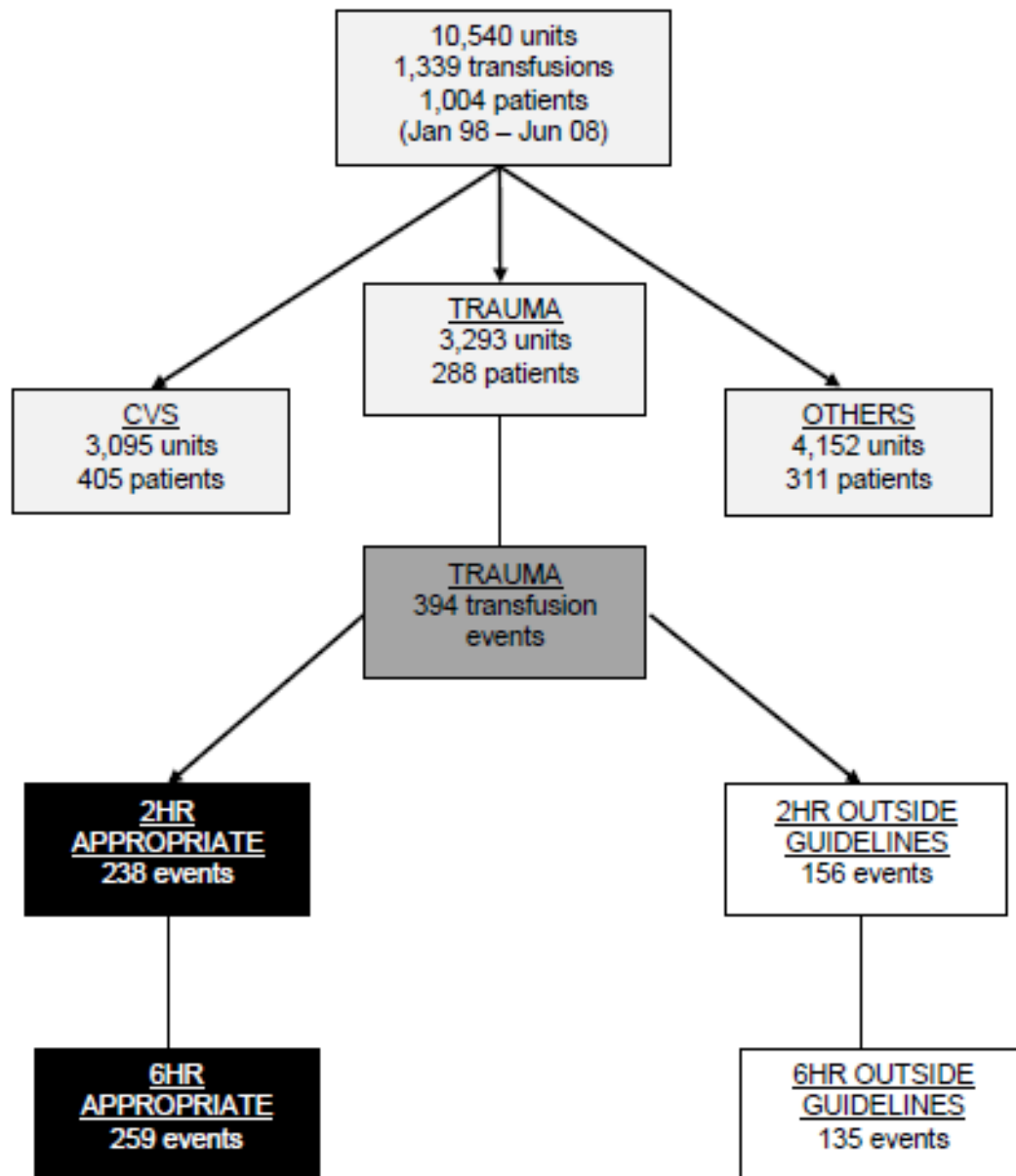
### **Results – Part A**

During the 10-year period between January 1998 and June 2008, 10,540 units of CRYO were transfused in 1,339 transfusion events in a total of 1,004 patients at SHSC (Figure 2). Hemorrhage in patients with low plasma fibrinogen levels due to cardiac surgery and trauma were the most common indications for CRYO use, corresponding to 405 (40%) and 288 (29%) of all patients, respectively (Table 3). Other less frequent indications included: cancer surgery, 59 (6%); cancer-related hemorrhage, 48 (5%); vascular surgery, 48 (5%); general surgery, 34(3%); orthopedics, 27 (2.7%); gastro-intestinal bleeding, 26 (2.6%); sepsis, 15 (1.5%); neurosurgery, 15 (1.5%); urology, 12 (1.2%); burns, 8 (0.8%); cardiology, 8 (0.8%), obstetric, 5 (0.5%); neurology, 4 (0.4%) and von Willebrand's Disease, 2 (0.2%) (Table 3). Overall, a mean CRYO dose of 7.9 ( $\pm 1.8$ ) units was given after transfusion of 10.5 ( $\pm 9.4$ ) units of RBC, 6.5 ( $\pm 5.4$ ) units of plasma and 9.4 ( $\pm 8$ ) units of platelets. Units of CRYO per patient, dose administered and number of patients receiving single dose by diagnostic category are displayed in Table 4. The highest individual dose was on average 8.4 units, and similarly seen

in trauma, general surgery and sepsis. The lowest individual dose, 6.7 ( $\pm 2.3$ ) units, was used for vWD. The percentage of single dose by clinical setting ranged from 50% in vWD to 100% in Cardiology, which was the only setting requiring a single dose. Relatively, sepsis and obstetrics had the highest proportions of three or more doses, 26% and 20%, respectively (Table 4). Despite accounting for the largest proportion of CRYO transfusion for the entire study period, cardiac surgery utilization decreased over the study period and was surpassed by trauma between 2003 and 2004 (Figure 3). In cardio-vascular surgery, both the absolute numbers of transfusion events and patients requiring CRYO declined continuously throughout the study period (Figure 4). In contrast, the number of CRYO transfusion events and patients receiving transfusions continued to rise gradually from 1998 until 2005, dropped in 2006, increased again in 2007, and finally dropped again in 2008 (Figure 5).



Fig 2. Study Flow Diagram - Part A



CVS, cardio-vascular surgery

**Table 3. Cryoprecipitate Transfusion at Sunnybrook (1998-2008)**

<b>Clinical Settings</b>	<b>Units of CRYO (%)</b>	<b>Transfusion Events</b>	<b>Patients</b>	<b>Units/Patients</b>
All settings	10,540 (100%)	1339	1004	10.5
Cardio-vascular surgery	3095 (37%)	523	405	9.6
Trauma	3293 (31%)	396	288	11.4
Oncologic surgery	634 (6%)	77	59	10.7
Cancer related-bleeding	607 (5.8%)	78	48	12.6
Vascular surgery	533 (5%)	66	48	11
General surgery	363 (3.4%)	43	34	10.7
Orthopedic surgery	209 (2%)	31	27	7.7
GI bleeding	240 (2.3%)	31	26	9.2
Sepsis	202 (1.9%)	24	15	13.5
Neurosurgery	141 (1.3%)	18	15	9.4
Urology	106 ( $\pm$ 1)	13	12	8.8
Burn	99 (0.9%)	12	8	12.4
Cardiology	66 (0.6%)	8	8	8.2
Obstetrics	78 (0.7%)	10	5	15.6
Neurology	44 (0.4%)	6	4	11
von Willebrand's Disease	20 (0.2%)	3	2	10

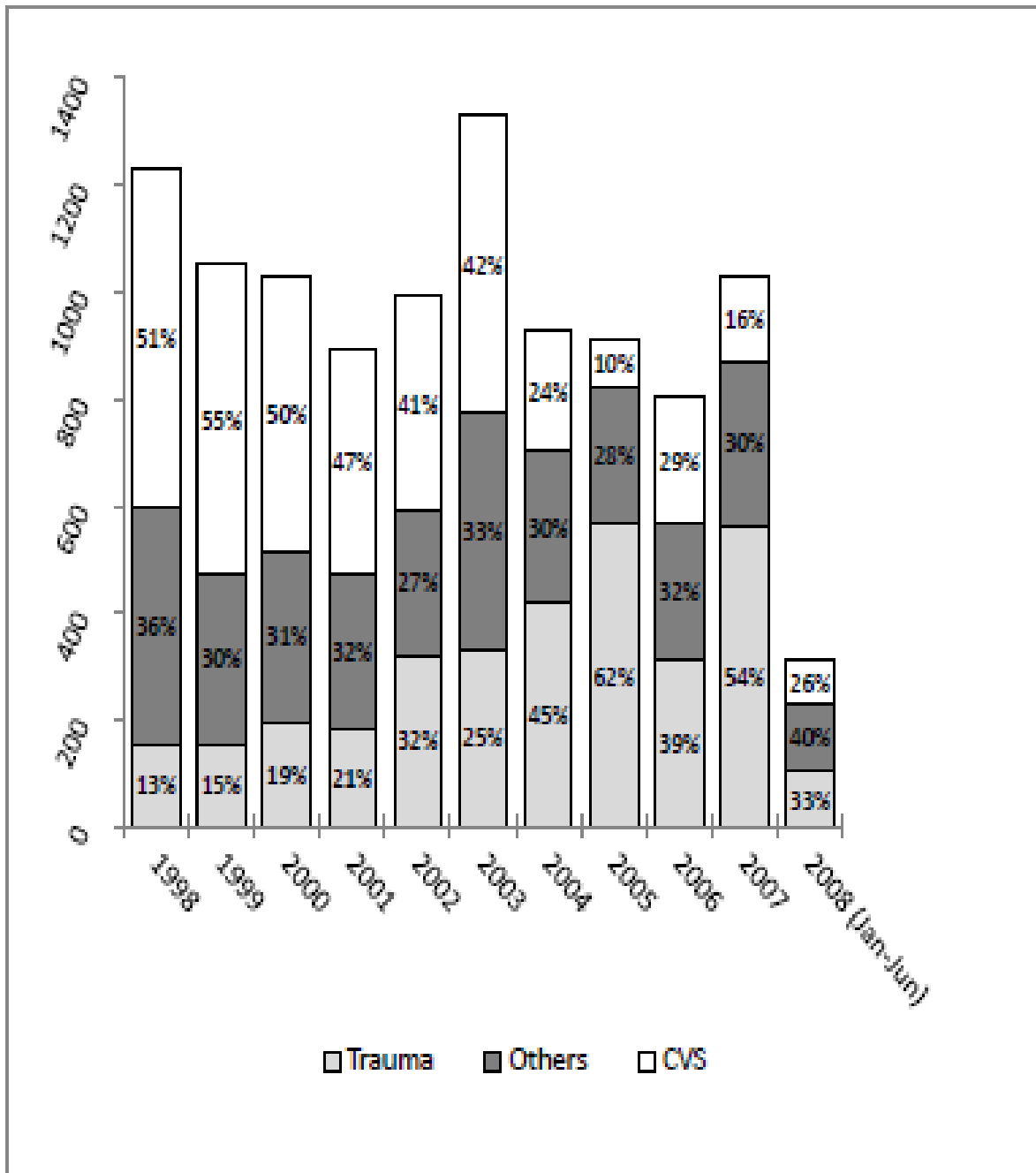
Data presented as number, percentage or mean when appropriate. CRYO, cryoprecipitate.

**Table 4. Dose of Cryoprecipitate at Sunnybrook (1998-2008)**

<b>Clinical Settings</b>	<b>Dose per patient (units)</b>	<b>Single dose (%)</b>	<b>Two doses (%)</b>	<b>Three or more doses (%)</b>
All settings	7.9 ( $\pm$ 1.8)	780 (78%)	150 (15%)	74 (7%)
Cardio-vascular surgery	7.5 ( $\pm$ 1.6)	319 (78%)	61 (15%)	25 (7%)
Trauma	8.4 ( $\pm$ 1.8)	218 (75%)	50 (17%)	20 (8%)
Oncologic surgery	8.2 ( $\pm$ 1.9)	51 (86%)	6 (10%)	2 (4%)
Cancer related-bleeding	7.8 ( $\pm$ 2.1)	35 (73%)	6 (12%)	7 (15%)
Vascular surgery	7.8 ( $\pm$ 1.3)	35 (73%)	7 (15%)	6 (12%)
General surgery	8.4 ( $\pm$ 2.6)	26 (76%)	4 (12%)	4 (12%)
Orthopedic surgery	6.7 ( $\pm$ 1.8)	23 (85%)	4 (15%)	0
GI bleeding	7.7 ( $\pm$ 2.2)	23 (88%)	1 (4%)	2 (8%)
Sepsis	8.4 ( $\pm$ 2.6)	10 (67%)	1 (7%)	4 (26%)
Neurosurgery	7.8 ( $\pm$ 1.1)	12 (80%)	3 (20%)	0
Urology	8.1 ( $\pm$ 0.5)	11 (92%)	1 (8%)	0
Burn	8.2 ( $\pm$ 2.2)	5 (62%)	2 (25%)	1 (13%)
Cardiology	8.2 ( $\pm$ 0.7)	8 (100%)	0	0
Obstetrics	7.8 ( $\pm$ 1.5)	3 (60%)	1 (20%)	1 (20%)
Neurology	7.3 ( $\pm$ 1.6)	2 (50%)	2 (50%)	0
von Willebrand's Disease	6.7 ( $\pm$ 2.3)	1 (50%)	1 (50%)	0

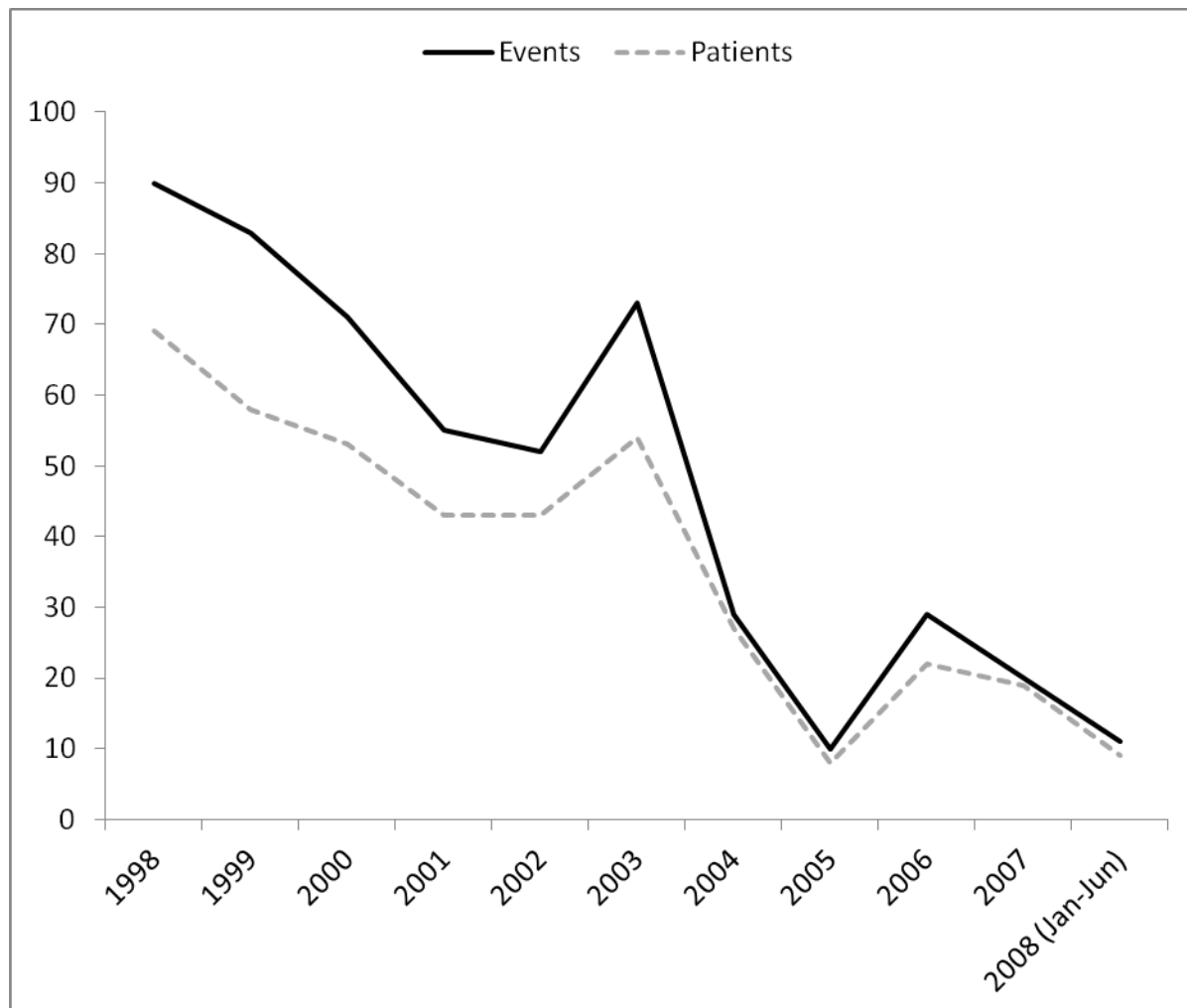
Data presented as number, percentage or mean ( $\pm$  standard deviation) when appropriate.  
CRYO, cryoprecipitate

**Fig 3. Cryoprecipitate Use according to Clinical Settings over Study Period**

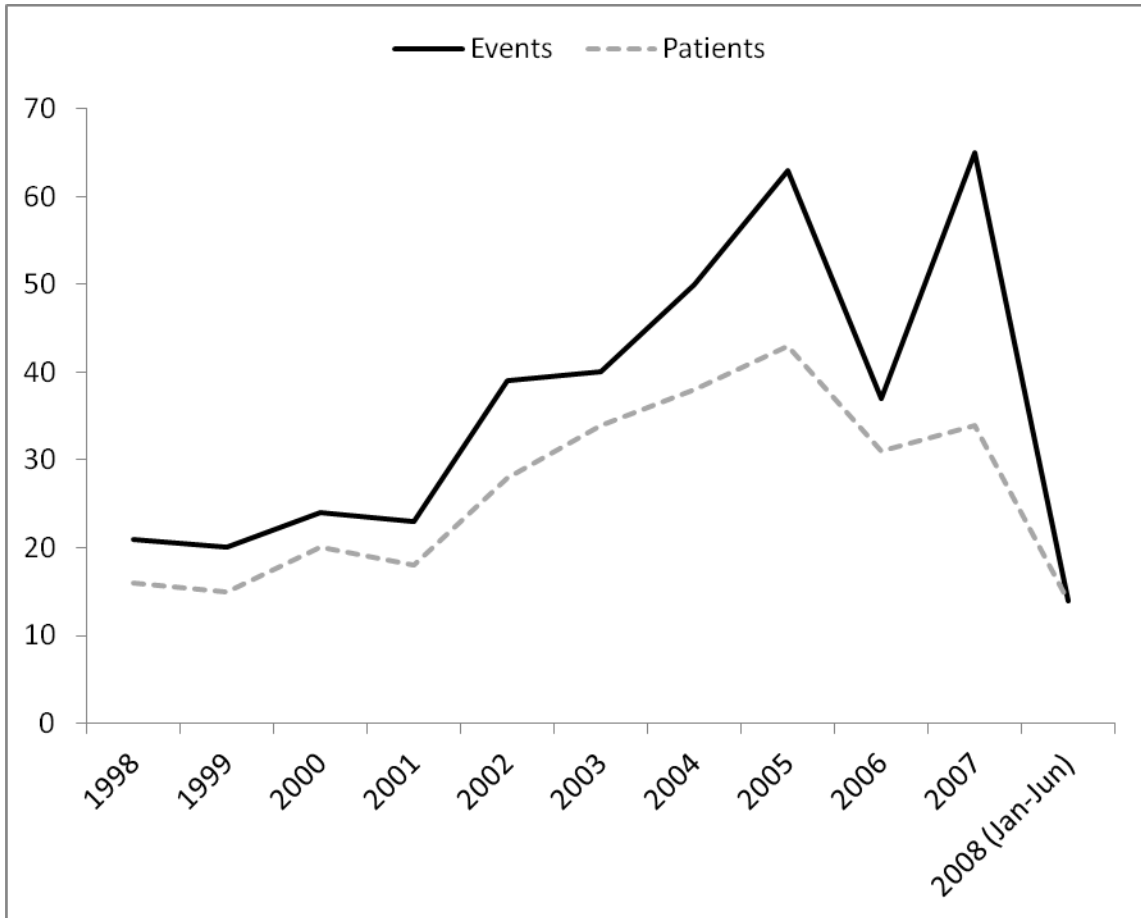


Data presented as absolute numbers and percentage by year of study. CRYO, cryoprecipitate

**Fig 4. Number of Cryoprecipitate Transfusion Events and Patients Receiving Cryoprecipitate in Cardio-vascular Surgery over Study Period**



**Fig 5. Number of Cryoprecipitate Transfusion Events and Patients Receiving Cryoprecipitate in Trauma over Study Period**

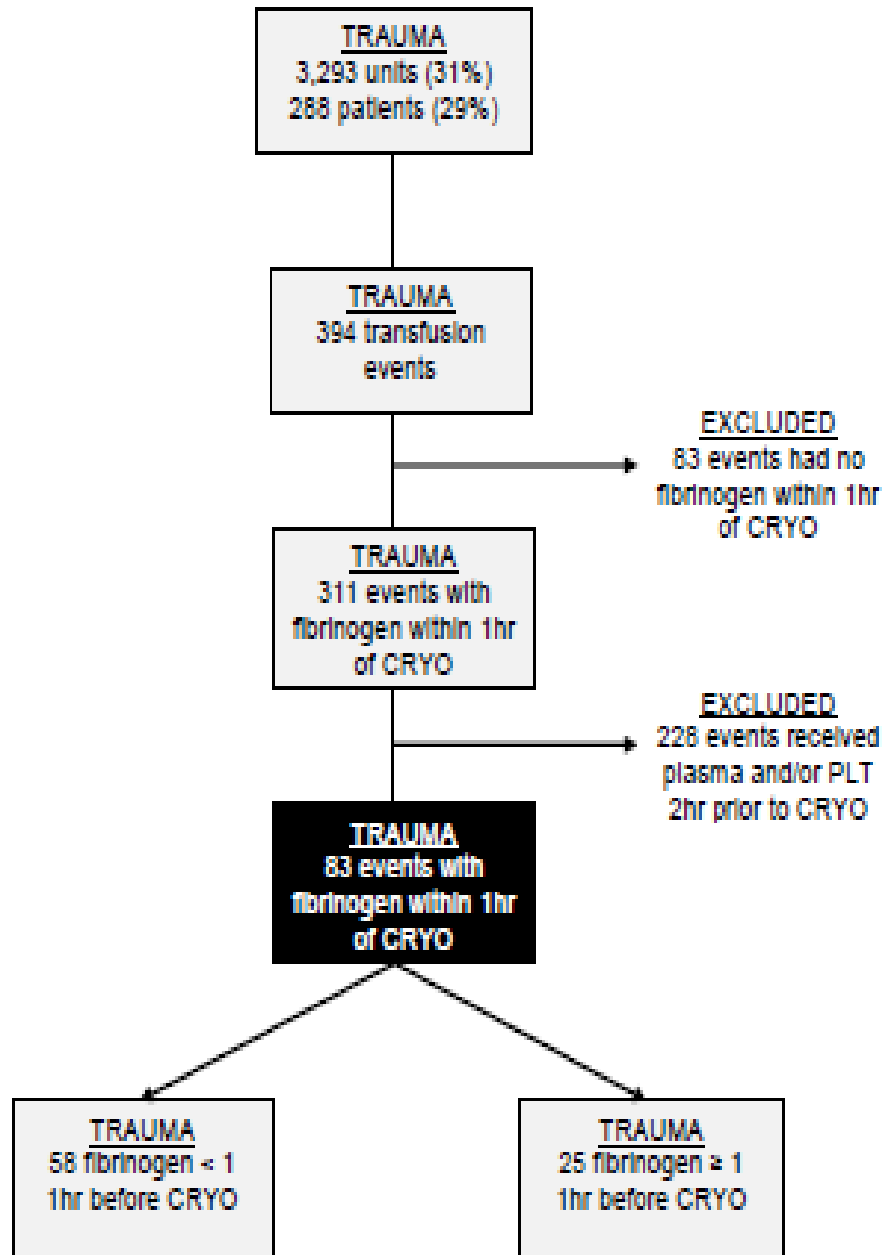


In trauma patients, 3293 units of CRYO were given in 394 transfusion events, involving 288 patients (Figure 6). The mean number of units per dose of CRYO given was 8.4 ( $\pm 1.8$ ), at a median of 4.5 hour (2.9, 7.5) from hospital admission. Out of 394 transfusion events, 82 (21%) had no fibrinogen measurement within 2 hour before and/or after CRYO transfusion and 49 (12%) had no fibrinogen levels measured at any time. The appropriateness rates by both 2 hour and 6 hour cut-off criteria for 394 CRYO transfusion events were 60% and 66%, respectively (Figure 7). The rates for fibrinogen measurement before and after increased gradually to the highest levels within 1 hour of the transfusion event (Figure 8). The proportions of events with  $< 1.0$  g/L were higher within 2 hour prior to the CRYO transfusion (Figure 9). Hourly fibrinogen measurements after CRYO transfusion showed that rates of failure to raise fibrinogen levels to  $> 1.5$  g/L ranged from 35% at 3 hour to 59% at 1 hour within 6 hours following the event (Figure 10). Apart from an isolated drop in 2001, rates of appropriateness of CRYO transfusion in trauma increased steadily over the study period, improving from 38.1% in the beginning to 78.6% by the end of the study period (Figure 11).

The comparison between patients who had appropriate and outside guideline CRYO transfusions revealed they were similar with respect to age, gender, type of trauma, ISS, presence of severe TBI, standard coagulation tests and degree of acidosis (Table 5). However, the appropriate group had less RBC and plasma transfusions before CRYO than the outside guideline group (Table 6).

Subgroup analysis comparing patients with appropriate events to patients with no fibrinogen levels revealed no significant differences in demographics, severity of injury, coagulation parameters, acidosis and transfusion of blood and blood products between groups (Tables 7 & 8).

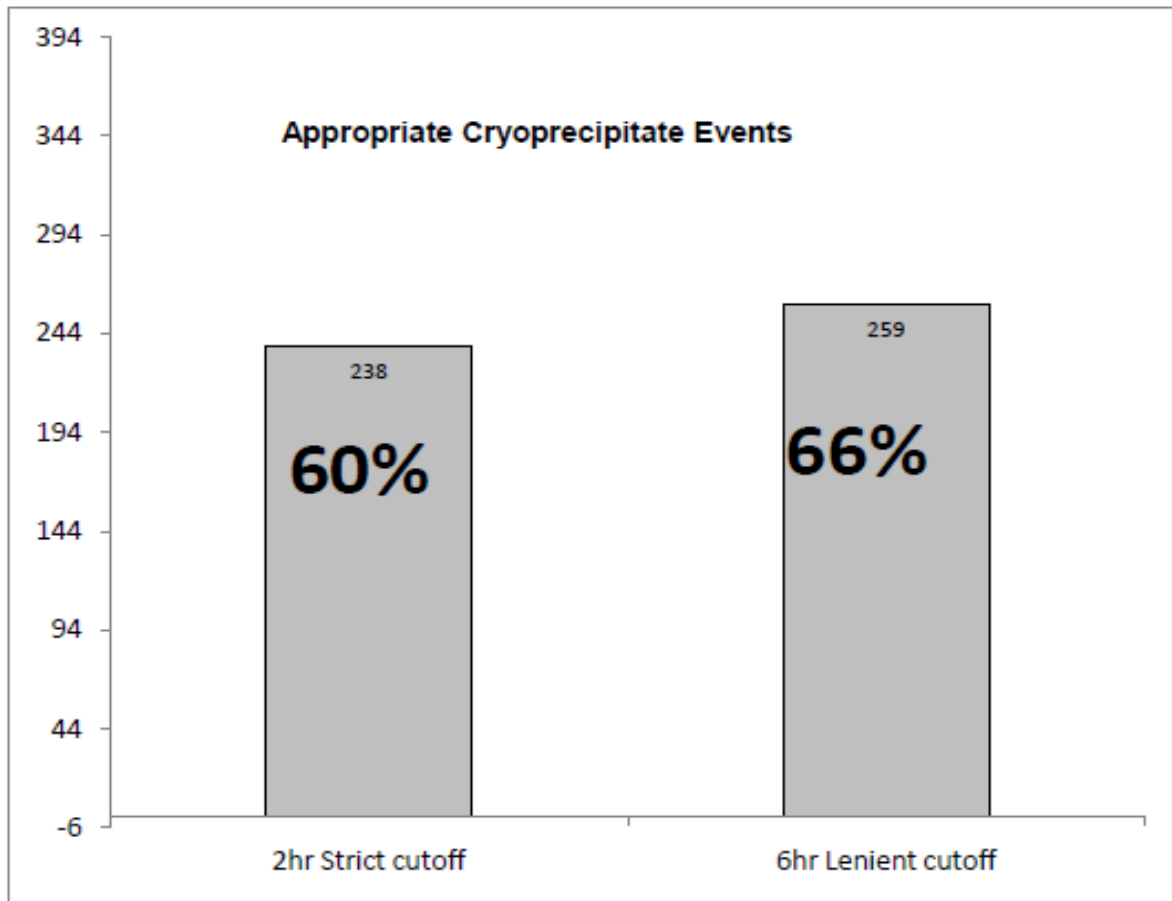
Fig 6. Study Flow Diagram - Part B



CRYO, cryoprecipitate; PLT, platelets

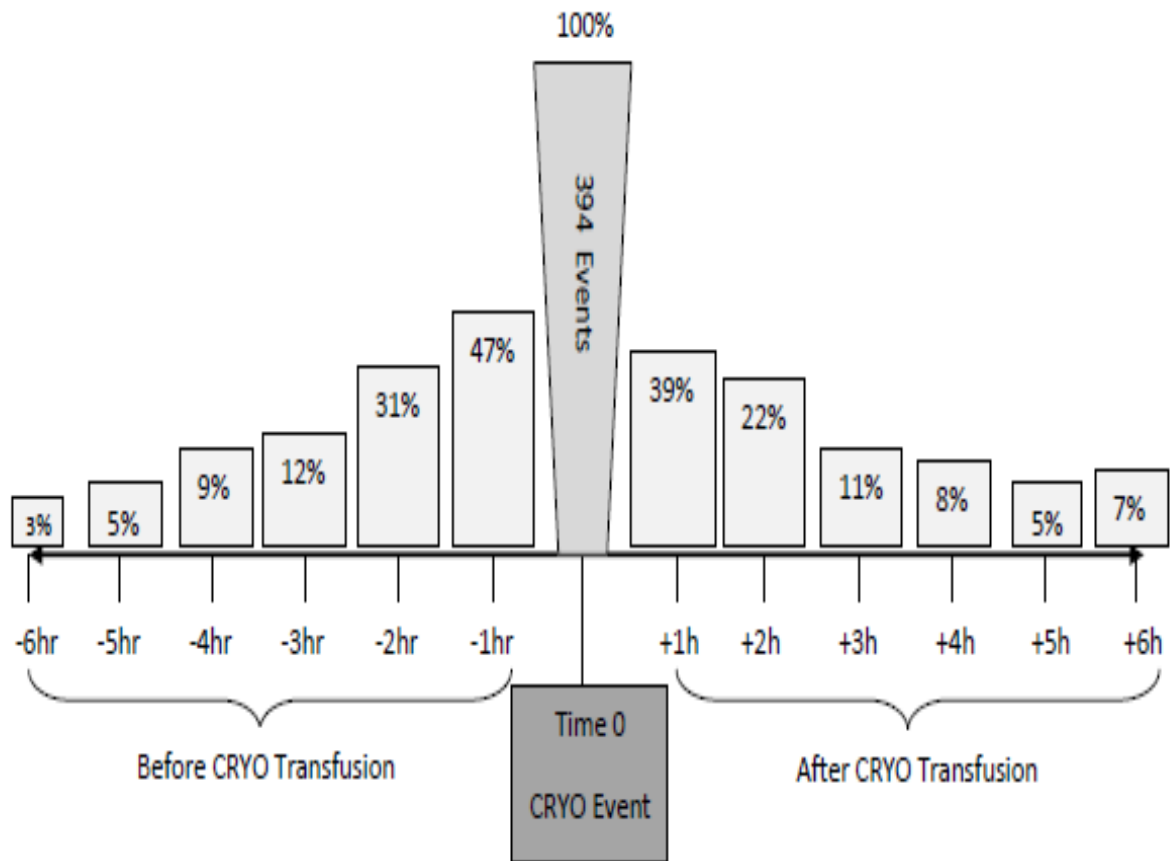


**Fig 7. Sunnybrook Rates of Appropriateness for Cryoprecipitate Transfusions in Trauma Using Strict (2hr cutoff) and Lenient (6hr cutoff) Criteria**



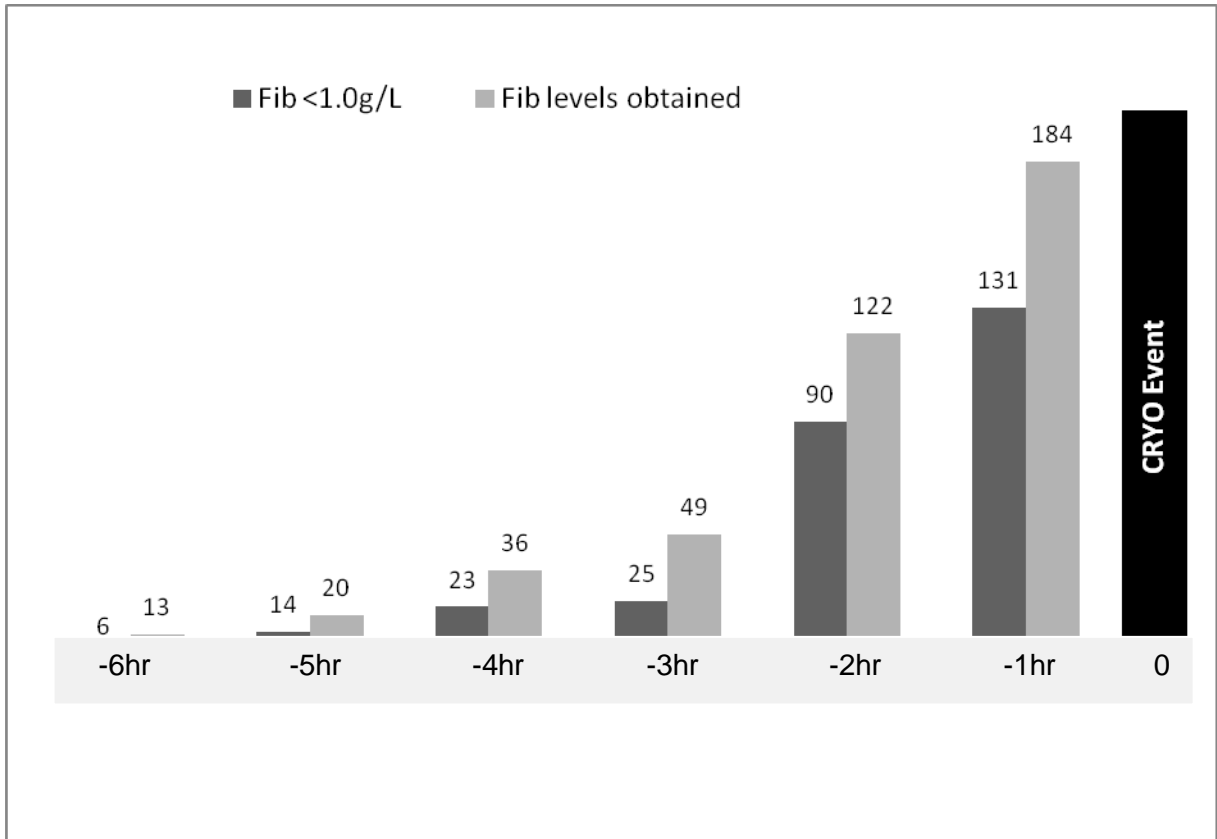
Cryoprecipitate transfusion was considered appropriate if fibrinogen levels were < 1.0g/L before and/or 1.5g/L within 2 hours (strict criteria) or 6 hours (lenient criteria). Data presented as percentage.

**Fig 8. Hourly Rates of Fibrinogen Measurement before and after Cryoprecipitate Transfusion**



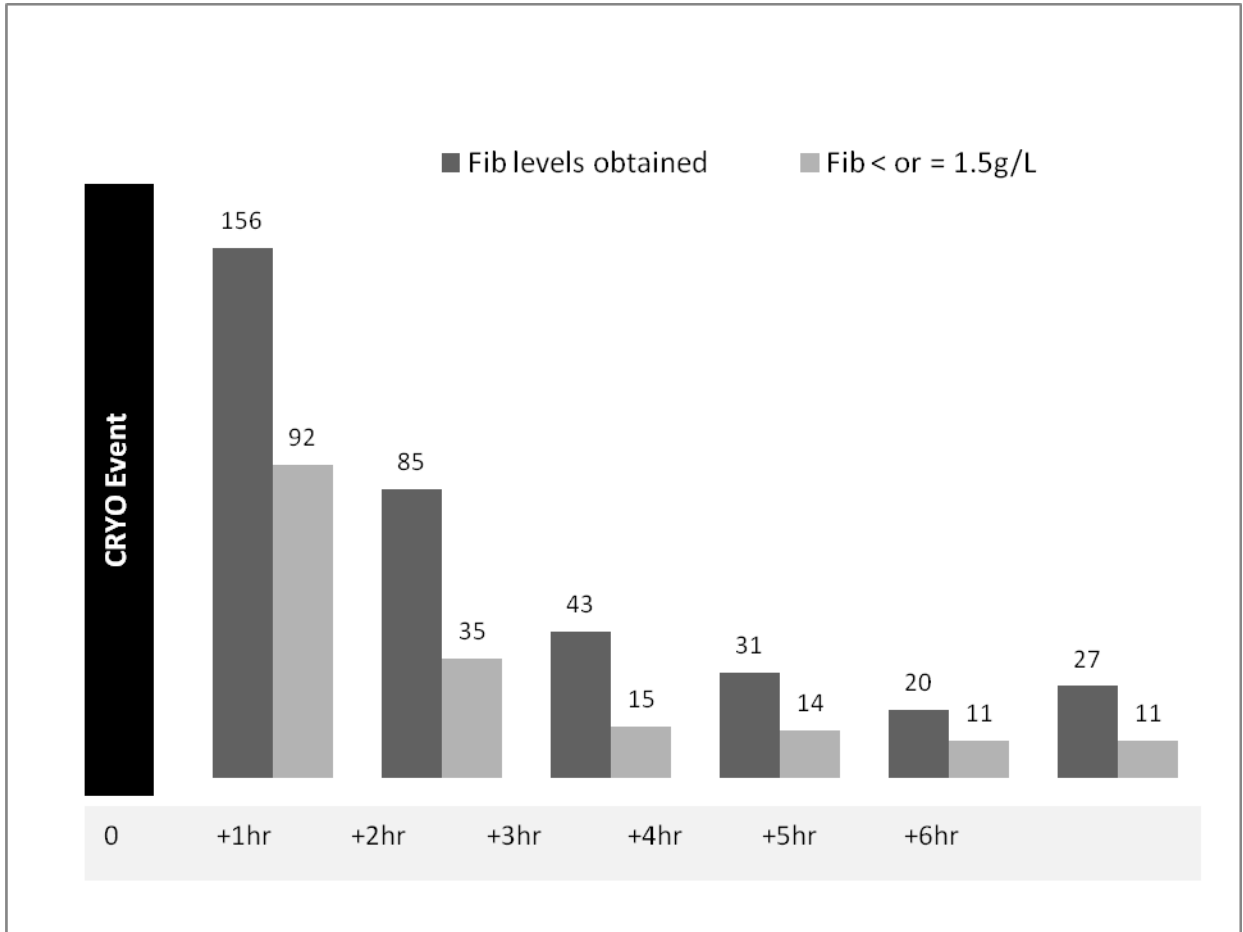
Data presented as percentages. CRYO, cryoprecipitate.

**Fig 9. Hourly Fibrinogen Measurements and Fibrinogen Levels <1.0 g/L within 6 hours before Cryoprecipitate Transfusion for 394 Transfusion Events in Trauma**



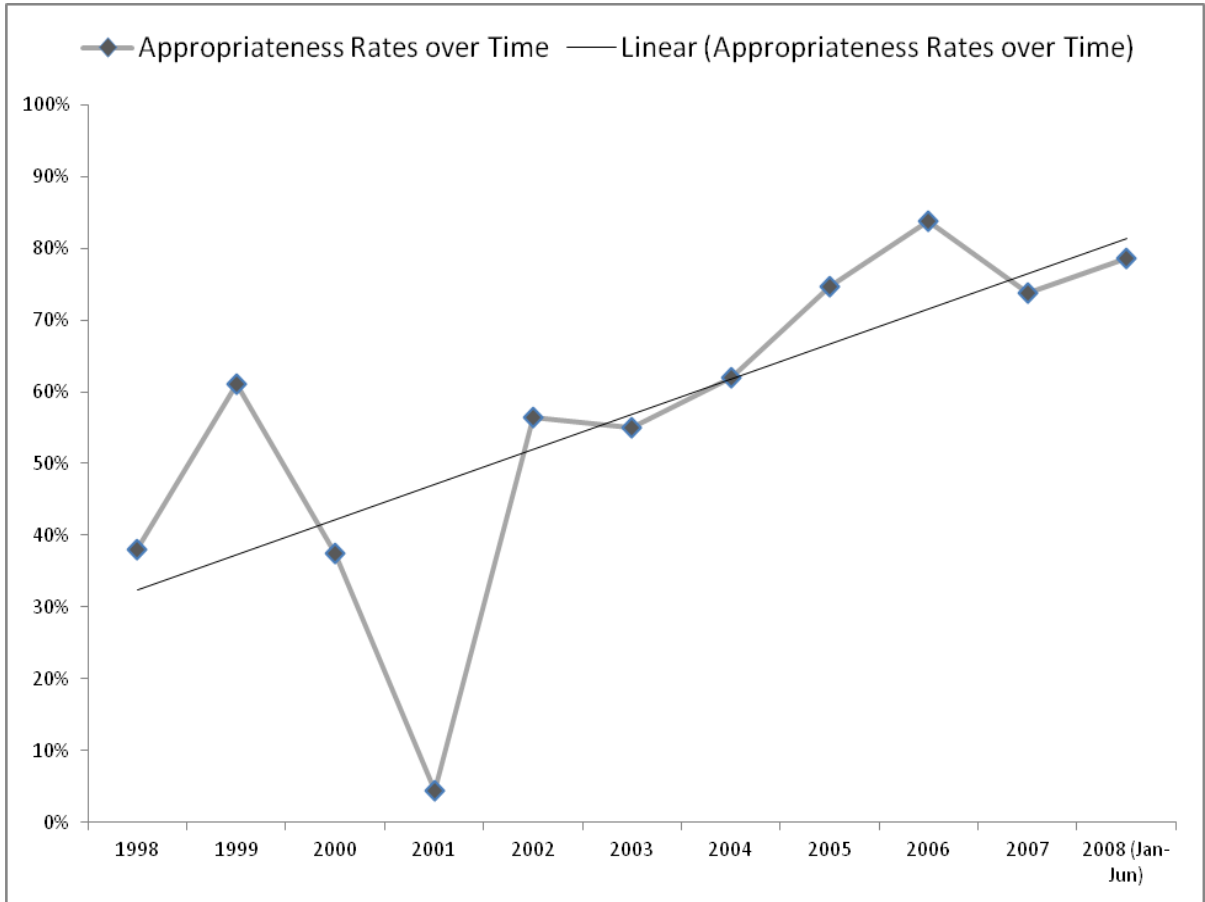
Fib, fibrinogen; CRYO, cryoprecipitate.

**Fig 10. Hourly Fibrinogen Measurements and Fibrinogen Levels  $\leq 1.5$  g/L within 6 hours after Cryoprecipitate Transfusion for 394 Transfusion Events in Trauma**



Fib, fibrinogen; CRYO, cryoprecipitate.

**Fig 11. Appropriate Cryoprecipitate Transfusions in Trauma over Study Period**



Data presented as percentages by year of study.

**Table 5. Comparison of Baseline Characteristics for Patients with Appropriate and Outside Guidelines Cryoprecipitate Transfusion Events in Trauma Patients**

<b>Variables</b>	<b>Total Cohort (n=288)</b>	<b>Appropriate (n=156)</b>	<b>Outside guidelines (n=132)</b>	<b>P</b>
Age (years)	39 ( $\pm$ 19)	39 ( $\pm$ 20)	38 ( $\pm$ 18)	0.86
Male	210 (73%)	96 (73%)	114 (74%)	0.9
Penetrating	61 (21%)	36 (23%)	25 (19%)	0.47
ISS	40 ( $\pm$ 15)	41 ( $\pm$ 15)	40 ( $\pm$ 14)	0.48
Severe TBI	109 (38%)	54 (41%)	55 (35%)	0.47
INR	1.82 (1.5, 2.4)	1.8 (1.5, 2.7)	1.8 (1.5, 2.3)	0.19
PTT (s)	60 (45, 109)	64 (45, 113)	62 (46, 101)	0.69
Platelets ( $\times 10^9/L$ )	66 (40, 106)	62 (36, 96)	69 (45, 113)	0.77
pH	7.19 ( $\pm$ 0.17)	7.19 ( $\pm$ 0.16)	7.18 ( $\pm$ 0.17)	0.5

Data presented as number, percentage and median (interquartile ranges) or mean ( $\pm$ SD) when appropriate; p values generated by Student's t and Wilcoxon Tests or  $\chi^2$  Analysis, as appropriate. Two-sided test performed. CRYO, cryoprecipitate; ISS, injury severity score; Severe TBI (traumatic brain injury) = Head AIS > 3; INR, international normalized ratio; PTT, partial thromboplastin time. Laboratory values at admission.

**Table 6. Comparison of Transfusion Data for Patients with Appropriate and Outside Guidelines Cryoprecipitate Transfusion Events**

<b>Variables</b>	<b>Total Cohort (n=288)</b>	<b>Appropriate (n=156)</b>	<b>Outside guidelines (n=132)</b>	<b>P</b>
RBC before CRYO (units)	13 ( $\pm$ 8)	14.7 ( $\pm$ 9)	12.2 ( $\pm$ 7.8)	0.01
FP before CRYO (units)	7 ( $\pm$ 4)	7.8 ( $\pm$ 5)	6.3 ( $\pm$ 3.6)	< 0.01
PLT before CRYO (units)	5 (5, 10)	5 (5, 11)	5 (5, 10)	0.12

Data presented as median (interquartile ranges) or mean ( $\pm$ SD); p values generated by Student's t and Wilcoxon Tests or  $\chi^2$  Analysis, as appropriate. Two-sided test performed and p < 0.05 considered significant. CRYO, cryoprecipitate; RBC, red blood cells; FP, frozen plasma; PLT, platelets.

**Table 7. Subgroup Comparison of Demographics and Baseline Characteristics for Patients with Appropriate and Outside guidelines (without Fibrinogen Levels) Cryoprecipitate Transfusion Events**

<b>Variables</b>	<b>Appropriate (n=156)</b>	<b>No Fibrinogen Levels (n=38)</b>	<b>P</b>
Age (years)	39 ( $\pm$ 20)	35 ( $\pm$ 17)	0.36
Male	96 (73%)	30 (79%)	0.53
Penetrating	36 (23%)	7 (18%)	0.66
ISS	41 ( $\pm$ 15)	39 ( $\pm$ 15)	0.7
Severe TBI	54 (41%)	12 (32%)	0.7
INR	1.8 (1.5, 2.7)	1.75 (1.4, 2.2)	0.08
PTT (s)	64 (45, 113)	53 (43, 101)	0.56
Platelets ( $\times 10^9/L$ )	62 (36, 96)	74 (51, 181)	0.004
pH	7.19 ( $\pm$ 0.16)	7.2 ( $\pm$ 0.15)	0.5

Data presented as number, percentage and median (interquartile ranges) or mean ( $\pm$ SD) when appropriate; p values generated by Student's t and Wilcoxon Tests or  $\chi^2$  Analysis, as appropriate. Two-sided test performed and  $p < 0.05$  considered significant. ISS, injury severity score; Severe TBI (traumatic brain injury) = Head AIS > 3; INR, international normalized ratio; PTT, partial thromboplastin time. Laboratory values at admission.



**Table 8. Subgroup Comparison of Transfusion Data for Patients with Appropriate and Outside guidelines (without Fibrinogen Levels) Cryoprecipitate Transfusion Events**

<b>Variables</b>	<b>Appropriate (n=156)</b>	<b>No Fibrinogen Levels (n=38)</b>	<b>P</b>
RBC before CRYO (units)	14.7 ( $\pm$ 9)	15.4 ( $\pm$ 9.8)	0.06
FP before CRYO (units)	7.8 ( $\pm$ 5)	7.6 ( $\pm$ 4)	0.07
PLT before CRYO (units)	5 (5, 11)	8.5 (5, 14)	1.0

Data presented as median (interquartile ranges) or mean ( $\pm$ SD); p values generated by Student's t and Wilcoxon Tests or  $\chi^2$  Analysis, as appropriate. Two-sided test performed and p < 0.05 considered significant. CRYO, cryoprecipitate; RBC, red blood cells; FP, frozen plasma; PLT, platelets.

In 2001, 22 patients received 23 CRYO transfusions, of which, 22 were classified as outside guidelines (95.6%). Comparing 2001 patients versus non-2001 patients, no differences in baseline characteristics were found except for lower platelet count in the outside guideline group (Tables 9 & 10).

Interestingly, a subgroup analysis evaluating only events which fibrinogen levels were obtained 1 hour before CRYO against events without fibrinogen, demonstrated more severe TBI, worse coagulation status and acidosis related to events where fibrinogen measurements were performed (Table 11). No transfusion differences were observed between groups (Table 12).

There was no difference in hospital mortality rates when appropriate and outside guideline groups were compared (48% versus 48%,  $p=0.9$ ).

## **Results – Part B**

For the fibrinogen response analysis, we first selected all transfusion events that had fibrinogen levels measured 1 hour before and after CRYO transfusion (311 events) (Figure 6). In order to avoid confounding (transfusion of plasma and platelets as a source of fibrinogen), we then excluded 228 events of 311 events which were associated with any FP and/or platelet transfusion within 2 hour prior to the start of CRYO transfusion. Overall, 83 of the total 394 CRYO transfusions in trauma were included in the final analysis to determine the plasma fibrinogen response. Out of 83 CRYO events, 58 had fibrinogen  $< 1.0\text{g/L}$  and 25 had fibrinogen  $\geq 1.0\text{g/L}$  before the CRYO transfusion occurred.

The overall increase in plasma fibrinogen levels after transfusion of an average of 8.7 ( $\pm 1.7$ ) units of CRYO in trauma was  $0.55\text{ g/L}$  ( $\pm 0.24$ ), or  $0.06\text{g/L}$  per unit of CRYO (Table 13). Out of 83 CRYO transfusions, 59 (71%), 38 (46%) and 13 (16%) resulted in plasma fibrinogen levels  $\geq 1.0\text{ g/L}$ ,  $\geq 1.5\text{ g/L}$  and  $\geq 2.0\text{ g/L}$ , respectively. In 58 events where fibrinogen was  $<$

1.0g/L before CRYO, 8.4 units ( $\pm 1.5$ ) of CRYO caused an increase in plasma fibrinogen of 0.69 ( $\pm 0.4$ ) (Table 14). However, when fibrinogen levels were  $\geq 1.0$  g/L before CRYO, despite higher doses (9.2 units), a rise of only 0.21 g/L ( $\pm 0.2$  g/L) was observed (Table 15).

Comparison between patients associated with either events where fibrinogen were  $< 1.0$  g/L or fibrinogen were  $\geq 1.0$  before CRYO, revealed the groups were similar with respect to age, gender, type of trauma, severity of injury, coagulation tests, degree of acidosis and number of units of RBC, FP and platelet transfused prior to the CRYO event (Table 16 & 17).

**Table 9. Subgroup Comparison of Demographics and Baseline Characteristics for 2001 (year) patients and non-2001 patients with Outside Guidelines Cryoprecipitate Transfusion Events**

<b>Variables</b>	<b>Non-2001 outside guidelines (n=131)</b>	<b>2001 outside guidelines (n=22)</b>	<b>P</b>
Age (years)	38 ( $\pm$ 18)	40 ( $\pm$ 23)	0.6
Male	93 (71%)	19 (86%)	0.19
Penetrating	25 (19%)	6 (27%)	0.39
ISS	42 ( $\pm$ 15)	39 ( $\pm$ 17)	0.37
Severe TBI	50 (38%)	8 (36%)	1.0
INR	1.78 (1.5, 2.7)	1.96 (1.8, 2.5)	0.24
PTT (s)	59 (43, 109)	66 (52, 150)	0.15
Platelets ( $\times 10^9/L$ )	69 (33, 124)	42 (28, 62)	0.01
pH	7.19 ( $\pm$ 0.16)	7.2 ( $\pm$ 0.1)	0.7

Data presented as percentage and median (interquartile ranges) or mean ( $\pm$ SD) when appropriate; p values generated by Student's t and Wilcoxon Tests or  $\chi^2$  Analysis, as appropriate. Two-sided test performed and  $p < 0.05$  considered significant. ISS, injury severity score; Severe TBI (traumatic brain injury) = Head AIS > 3; INR, international normalized ratio; PTT, partial thromboplastin time. Laboratory values at admission.

**Table 10. Subgroup Comparison of Transfusion Data for 2001 (year) patients and non-2001 patients with Outside Guidelines Cryoprecipitate Transfusion Events**

<b>Variables</b>	<b>Non-2001 outside guidelines (n=131)</b>	<b>2001 outside guidelines (n=22)</b>	<b>P</b>
RBC before CRYO (units)	15.9 ( $\pm$ 10)	20.4 ( $\pm$ 13)	0.08
FP before CRYO (units)	8.6 ( $\pm$ 5.9)	9.8 ( $\pm$ 5.9)	0.39
PLT before CRYO (units)	9.7 ( $\pm$ 8.9)	12.8 ( $\pm$ 8.4)	0.13

Data presented as median (interquartile ranges) or mean ( $\pm$ SD); p values generated by Student's t and Wilcoxon Tests or  $\chi^2$  Analysis, as appropriate. Two-sided test performed and p < 0.05 considered significant. CRYO, cryoprecipitate; RBC, red blood cells; FP, frozen plasma; PLT, platelets.

**Table 11. Subgroup Comparison of Demographics and Baseline Characteristics for CRYO events with and without fibrinogen measured within 1hr before Cryoprecipitate Transfusion**

<b>Variables</b>	<b>Events with fibrinogen 1hr before CRYO (n=83)</b>	<b>Events without fibrinogen 1hr before CRYO (n=311)</b>	<b>P</b>
Age (years)	39 ( $\pm$ 18)	38 ( $\pm$ 23)	0.79
Male	59 (72%)	232 (74%)	0.57
Penetrating	22 (26%)	69 (22%)	0.46
ISS	38 ( $\pm$ 14)	41 ( $\pm$ 15)	0.05
Severe TBI	28 (34%)	152 (49%)	0.02
INR	2.06 (1.6, 4.4)	1.9 (1.5, 2.8)	0.06
PTT (s)	88 (53, 150)	66 (45, 124)	0.02
Platelets ( $\times 10^9/L$ )	58 (30, 93)	64 (32, 100)	0.03
pH	7.14 ( $\pm$ 0.18)	7.19 ( $\pm$ 0.16)	0.03

Data presented as number, percentage and median (interquartile ranges) or mean ( $\pm$ SD) when appropriate; p values generated by Student's t and Wilcoxon Tests or  $\chi^2$  Analysis, as appropriate. Two-sided test performed and  $p < 0.05$  considered significant. ISS, injury severity score; Severe TBI (traumatic brain injury) = Head AIS > 3; INR, international normalized ratio; PTT, partial thromboplastin time. Laboratory values at admission.

**Table 12. Subgroup Comparison of Transfusion Data for CRYO events with and without fibrinogen measured within 1hr before Cryoprecipitate transfusion.**

<b>Variables</b>	<b>Events with fibrinogen 1hr before CRYO (n=83)</b>	<b>Events without fibrinogen 1hr before CRYO (n=311)</b>	<b>P</b>
RBC before CRYO (units)	15 ( $\pm$ 10)	16.7 ( $\pm$ 11)	0.25
FP before CRYO (units)	8.2 ( $\pm$ 6)	8.9 ( $\pm$ 6.2)	0.34
PLT before CRYO (units)	10 (5, 15)	10 (5, 15)	0.52

Data presented as median (interquartile ranges) or mean ( $\pm$ SD); p values generated by Student's t and Wilcoxon Tests or  $\chi^2$  Analysis, as appropriate. Two-sided test performed and  $p < 0.05$  considered significant. CRYO, cryoprecipitate; RBC, red blood cells; FP, frozen plasma; PLT, platelets.

**Table 13. Plasma Fibrinogen Response to Cryoprecipitate Transfusion during Massive Transfusion in Trauma**

<b>N</b>	<b>Pre-CRYO fibrinogen (g/L)</b>	<b>Post-CRYO fibrinogen (g/L)</b>	<b>Δ (Post – Pre)</b>	<b>Increase (%)</b>
83	0.83 (±0.35)	1.38 (±0.59)	0.55 (±0.24)	66%

Data presented as mean (±SD) and percentages.

**Table 14. Plasma Fibrinogen Response to Cryoprecipitate Transfusion for Events with Fibrinogen <1.0 g/L before Administration of Cryoprecipitate during Massive Transfusion in Trauma**

<b>N</b>	<b>Pre-CRYO fibrinogen (g/L)</b>	<b>Post-CRYO fibrinogen (g/L)</b>	<b>Δ (Post – Pre)</b>	<b>Increase (%)</b>
58	0.65 (±0.2)	1.35 (±0.6)	0.69 (±0.4)	106%

Data presented as mean (±SD) and percentages.



**Table 15. Plasma Fibrinogen Response to Cryoprecipitate Transfusion for Events with Fibrinogen  $\geq 1.0$  g/L before Administration of Cryoprecipitate during Massive Transfusion in Trauma**

<b>N</b>	<b>Pre-CRYO fibrinogen (g/L)</b>	<b>Post-CRYO fibrinogen (g/L)</b>	<b><math>\Delta</math> (Post – Pre)</b>	<b>Increase (%)</b>
25	1.2 ( $\pm 0.3$ )	1.45 ( $\pm 0.5$ )	0.21 ( $\pm 0.2$ )	17%

Data presented as mean ( $\pm$ SD) and percentages.

**Table 16. Subgroup Comparison of Demographics and Baseline Characteristics for patients with fibrinogen < 1.0g/L and with fibrinogen ≥ 1.0g/L within 1hr before Cryoprecipitate transfusion.**

<b>Variables</b>	<b>Fibrinogen &lt; 1 (n=58)</b>	<b>Fibrinogen ≥ 1 (n=25)</b>	<b>P</b>
Age (years)	39.8 (±18)	37 (±21)	0.62
Male	34 (65%)	19 (79%)	0.29
Penetrating	13 (25%)	5 (21%)	0.78
ISS	39 (±14)	39 (±15)	0.98
Severe TBI	15 (29%)	8 (33%)	0.79
INR	2 (1.6, 3.3)	1.9 (1.7, 4.1)	0.69
PTT (s)	83 (48, 150)	78 (60, 115)	0.8
Platelets (x10 <sup>9</sup> /L)	58 (34, 100)	61 (32, 82)	0.55
pH	7.12 (±0.2)	7.18 (±0.15)	0.18

Data presented as percentage and median (interquartile ranges) or mean (±SD) when appropriate; p values generated by Student's t and Wilcoxon Tests or  $\chi^2$  Analysis, as appropriate. Two-sided test performed and  $p < 0.05$  considered significant. ISS, injury severity score; Severe TBI (traumatic brain injury) = Head AIS > 3; INR, international normalized ratio; PTT, partial thromboplastin time. Laboratory values at admission.

**Table 17. Subgroup Comparison of Transfusion Data for patients with fibrinogen < 1.0g/L and with fibrinogen ≥ 1.0g/L within 1hr before Cryoprecipitate transfusion.**

<b>Variables</b>	<b>Fibrinogen &lt; 1 (n=58)</b>	<b>Fibrinogen ≥ 1 (n=25)</b>	<b>P</b>
RBC before CRYO (units)	15 (±10)	16 (±11)	0.5
FP before CRYO (units)	7.5 (±4)	8.9 (±8.8)	0.33
PLT before CRYO (units)	10 (5, 15)	5 (4.7, 15)	0.79

Data presented as median (interquartile ranges) or mean (±SD); p values generated by Student's t and Wilcoxon Tests or  $\chi^2$  Analysis, as appropriate. Two-sided test performed and  $p < 0.05$  considered significant. CRYO, cryoprecipitate; RBC, red blood cells; FP, frozen plasma; PLT, platelets.

## 13. Discussion

### **Discussion – Part A**

Compared to previous audits, our review found high rates of appropriateness for CRYO use (60% and 66% based on fibrinogen levels of  $<1.0\text{g/L}$  before and/or  $\leq 1.5\text{g/L}$  after CRYO transfusion, measured at 2 or 6 hour cut-off points, respectively) in the context of massively transfused trauma patients, with notable improvement during the study period. Of note, in the final year of the study 78.6% were appropriate.

During massive transfusion, the definition of appropriateness can be challenging. CRYO preparation involves thawing, reconstitution and pooling which requires about 30 min. The turnaround time for laboratory results of fibrinogen levels usually requires up to 30 – 60 minutes. In addition, the emergent nature of massive blood loss might preclude performing and/or waiting for laboratory results before transfusing blood and/or blood products. In our cohort of trauma patients, CRYO was administered at a median of 4.5 hr from hospital admission. Also, the subgroup analysis comparing patients who had appropriate and outside guideline events, showed that both groups had the same severity of injury, degree of acidosis and coagulopathy, but had different amounts of RBC and plasma transfused prior to CRYO. Patients in the outside guideline group received more RBC and plasma than the appropriate group, implying a possible difference in bleeding rates, possibly more urgency for CRYO transfusion, and more unstable clinical setting. Unlike a previous audit that used fibrinogen levels taken 6 hr before/after transfusion to categorize appropriateness (Alport *et al.*, 2008), we adopted a narrower time window of 2 hr, as we believed this to be more appropriate for the clinical setting of actively bleeding patients.

Alport *et al.* conducted an audit involving 25 Canadian hospitals and reported an overall 24% of appropriateness for CRYO use in different clinical settings (Alport *et al.*, 2008). The

single most common indication for CRYO use was cardiac surgery (45.4% of events), followed by noncardiac surgery (16.9%) and trauma, which corresponded to only 12.3% of the events. The authors categorized CRYO transfusions as likely appropriate if a fibrinogen level, taken within 6 hr before or after transfusion was not more than 1.0g/L. However, the authors did not report on individual appropriateness rates for the different clinical settings. In 2003, an Australian study reported that only 38% of CRYO transfusions were considered appropriate when the same fibrinogen level criteria were applied (Schofield *et al.*, 2003). However, the time to fibrinogen measurement in this report was not stated. Finally, an audit from the United States also published in 2003, applying different criteria for appropriateness, reported that 76% of CRYO transfusions were appropriate in 51 patients (Pantanowitz *et al.*, 2003). In that report, CRYO transfusion was considered appropriate for a fibrinogen level less than 1.0g/L when bleeding, and in addition, for tissue plasminogen activator-related hemorrhage, transfusion of 10 or more PRBC units irrespective of fibrinogen level, uremic bleeding and von Willebrand's disease. The two latter indications are considered inappropriate by others due to the availability of desmopressin, virally inactivated human-derived factor VIII/von Willebrand factor concentrates and fibrin (Janson *et al.*, 1980; Maierhofer *et al.*, 1981; Triulzi *et al.*, 1990; Weigert *et al.*, 1998; Mannucci, 2004).

We believe that there are several local hospital/medical factors associated with the high rates of appropriateness seen in our review as discussed below.

First, there is a high compliance rates among trauma team, surgeons, anesthesiologists and intensivists with the hospital massive transfusion guidelines, which were officially introduced as hospital policy in 2003. However, it was in place as transfusion medicine recommendations since the onset of this review. Our transfusion guidelines recommend hourly fibrinogen levels during massive transfusion, which is overseen by a transfusion medicine specialist and blood bank technologists. During our massive transfusion protocol, coagulation

testing, including fibrinogen levels are performed in an expedited fashion and results are usually delivered within 30 to 60 minutes of sample arrival. Furthermore, in our institution, the blood bank technologist routinely checks the fibrinogen levels at any request for CRYO transfusion and often prompts testing when the test has not been requested on the most recent coagulation sample.

Our transfusion medicine service has a policy of prospectively auditing all transfusion requests outside our transfusion guidelines. The medical laboratory technologist prompts review by the medical director of transfusion medicine who follows-up these cases on a “case-by-case” basis. The medical director or his/her delegate, first, reviews the transfusion request and patient’s clinical data pertinent to transfusion medicine. Then, the reason for the deviation is discussed in a “real time fashion” with the ordering physician who has to justify their order. This model allows the opportunity for education if lack of knowledge is a factor. Finally, agreement is reached and the request might be: (i) disregarded and documentation for the inappropriateness is performed on the request form by the medical director; (ii) withheld until further clinical/laboratorial information is available and case reassessed; medical director documented reason for withholding on request form and follows-up on the case until final decision is made and/or no additional transfusion medicine input is required; (iii) the transfusion request is approved and executed but justification for the guidelines deviation documented on request form by medical director. A recent systematic review of behavioral interventions, including guidelines, prospective audits, retrospective audits, and reminders, or a combination of them, concluded that such interventions appear to be effective in changing physician transfusion practice and reducing inappropriate blood utilization (Tinmouth *et al.*, 2005).

Finally, CRYO is also prepared in anticipation based on laboratory results independent of the medical request as per our massive transfusion guidelines. The medical team is made

aware of the product availability and responsible for the ultimate decision regarding utilization. Of these non-clinician driven CRYO transfusion events, 100% would be appropriate according to our hospital transfusion policies.

### **Discussion – Part B**

This study also suggests that the response to CRYO transfusion (0.55 g/L increase in plasma levels of fibrinogen) in trauma is at the lower limit of the expected range, when standard CRYO dosing is applied, according to expert opinions. The transfusion of 8.7 units of CRYO resulted in plasma fibrinogen levels of 1.5 g/L or greater in only 47% of the cases. The same dose produced fibrinogen levels of 2.0 g/L or greater in only 16% of the events.

CRYO is exclusively used for the replenishment of fibrinogen at our institution. A standard CRYO dose of 8 units contains at least 2g of fibrinogen. The American Society of Anesthesiology, in their guidelines for blood component therapy, states that an average dose of 1 unit of CRYO per 10kg of body weight raises plasma fibrinogen by approximately 0.5g/L in the absence of continued consumption or massive bleeding (American Society of Anesthesiologists, 1996). In 2002, the American Association of Blood Banks guide to blood transfusion therapy stated that 1 unit of CRYO increases the fibrinogen by 0.05 - 0.1g/L in average sized adult (American Association of Blood Banks, 2002). In 2006, Rossaint et al. suggested that 1 unit of CRYO per 7 – 10kg of body weight increases plasma fibrinogen level by 0.5g/L (Rossaint *et al.*, 2006). Recently, the British guidelines on the management of Disseminated Intravascular Coagulation (DIC) stated that a dose of 3g of fibrinogen would be expected to raise plasma fibrinogen by around 1 g/L (Levi *et al.*, 2009). Interestingly, those statements are not supported by clinical evidence and these statements reflect expert opinion only. To the best of our knowledge, our study, which analyzes the fibrinogen response of 83 CRYO transfusions in a population of massively transfused trauma patients, represents the

largest series addressing the issue in this setting to date. Of note, our fibrinogen response is likely similar to other settings where fibrinogen is used, such as in massive surgical or obstetric hemorrhage.

In the context of significant bleeding in elective surgical patients, a drop in fibrinogen level is the first coagulation defect observed (Hiippala *et al.*, 1995). The cleavage of fibrinogen by thrombin results in the formation of fibrin polymers, which by binding FXIII, produces a strong fibrin network (Velik-Salchner *et al.*, 2007). Fibrinogen also plays an important role in inducing platelet activation and aggregation. It is also a positive acute-phase reactant protein (Lowe *et al.*, 2004), which might be also elevated in a wide spectrum of physiological, pathological and lifestyle conditions, such as increasing age and female sex (Lowe *et al.*, 1997); colder season (Van der Bom *et al.*, 1997); pregnancy and oral contraception (Kluft and Lansink, 1997); menopause (Lowe *et al.*, 1997); smoking (Humphries *et al.*, 1999; Woodward *et al.*, 1997); acute exercise (Humphries *et al.*, 1999); genetic conditions (Humphries *et al.*, 1999); and acute-phase reactions (Hemostasis & Thrombosis, 1994; Humphries *et al.*, 1999). Of note, plasma fibrinogen is expected to increase to levels of approximately 7 g/L by post-operative day 5 after trauma and surgery, even without intra-operative administration (Thompson *et al.*, 2007; Wei *et al.*, 1995). Our study performed fibrinogen measurements during resuscitation with a narrow window (1 hour before and after CRYO), which we believe might reflect more exogenous replacement than physiologic response. Of note, our study used the Clauss method for fibrinogen determination during the entire duration of the study. It determines the functional plasma fibrinogen levels, and remains the routine assay of choice for the diagnosis of hypofibrinogenemia in the context of bleeding (Mackie *et al.*, 2003). No colloids are used for trauma resuscitation in our trauma system, which may potentially alter fibrinogen measurements in the Clauss method.



In trauma, a recent retrospective review of 252 combat-related trauma patients requiring massive transfusion, demonstrated an association between increased fibrinogen (mainly from plasma and CRYO transfusion) to RBC ratios and improved survival (Stinger *et al.*, 2008). The role of fibrinogen for the management of coagulopathy in massive transfusion has been increasingly recognized and alternatives to CRYO use, such as fibrinogen concentrate, also intensively investigated (Sorensen *et al.*, 2010; Fries *et al.*, 2010; Tisherman, 2010).

### **Limitations**

Our study has several limitations as follows:

(i) The retrospective nature of our study did not allow us to further explore the reasons for inappropriate CRYO transfusion at the physician level. In the context of massive bleeding, the characterization of inappropriateness can be extremely challenging. Commonly, the decision to transfuse unstable bleeding trauma patients is based solely on clinical grounds. High rates of bleeding coupled with long turnaround times for fibrinogen level testing precludes waiting for blood test results in some patients. However, we tried to address the issue by analyzing the data using both strict and lenient criteria for appropriateness;

(ii) Our retrospective cohort study design included patients who received CRYO transfusion only, thus did not address the issue of under treatment (trauma patients with fibrinogen level  $< 1.0\text{g/L}$  but no CRYO given), which may be regarded as inappropriate practice;

(iii) We were unable to account for the contribution of other blood products in raising fibrinogen levels, the dilutional effects of crystalloid resuscitation between fibrinogen levels and ongoing consumption. In an attempt to avoid this confounding, we analyzed only patients who did not receive any blood product containing fibrinogen 2hr before CRYO transfusion. Furthermore, we utilized a narrow window for fibrinogen measurements (1 hour

before and after CRYO) in the fibrinogen response analysis, likely limiting potential confounding from physiologic elevation of fibrinogen following trauma and ongoing consumption;

(iv) The lack of control group is another important limitation. The ideal control group would be patients with low fibrinogen levels and no active hemorrhage.

(v) Finally, potential variations in fibrinogen response to CRYO transfusion due to differences in patients' weights and in CRYO doses were not accounted for.

### **Future Directions**

This study also enabled us to identify several areas that deserve further investigations:

(i) The impact of behavioral interventions on physician transfusion practices and patient's outcomes.

Our study demonstrated that it is possible to achieve high rates of appropriateness for CRYO transfusion in trauma when behavioral interventions are in place and constantly reinforced in an organized transfusion and trauma programs. However, further improvement might still be possible, which should be investigated in a prospective research design in order to address appropriateness at the physician level. Moreover, it remains unknown whether or not such interventions would improve patient's outcomes. Therefore, the impact of such interventions on patient's outcomes should be investigated;

(ii) The dosage of cryoprecipitate necessary to adequately replete hypofibrinogenemia.

Our review suggested that, in the context of trauma and bleeding, current recommended dosage for CRYO causes only a modest increase in plasma levels of fibrinogen; and a bleeding trauma patient requires double the dose used in our patients to more consistently achieve a

fibrinogen >1.5 g/L. Further investigation on dose response should be conducted to elucidate the appropriate dosage of CRYO in the setting of active bleeding;

- (iii) The role of fibrinogen concentrates.

Alternatives to CRYO for fibrinogen replacement is warranted due to the potential complications of CRYO transfusion, variability in fibrinogen content and time required for thawing, pooling and issuing;

- (iv) The role of other cryoprecipitate contents.

The role of fibronectin and platelet microparticles in infection and hemostasis should be further investigated;

- (v) The role of cryoprecipitate in formula-driven transfusion strategy.

A growing body of literature suggests a potential role of increased ratios of clotting factors, including fibrinogen, to RBC in trauma resuscitation. The role of FFP, platelets and CRYO in transfusion protocol with fixed ratios of products should be investigated in a prospective fashion;

- (vi) The incidence of adverse events associated with cryoprecipitate transfusion.

The consequences of increased exposure of trauma patients to plasma products remain unclear. It would be important to establish a compulsory national reporting system to record adverse events of CRYO.

## 14. Conclusions

In our institution, CRYO is commonly used in cardiac surgery and trauma for low fibrinogen levels in the context of bleeding. Our study demonstrated that, in an organized system, where massive transfusion protocols are in place and overseen by blood bank technologists and transfusion medicine specialists, it is possible to achieve high rates of

appropriateness for CRYO transfusion in trauma. The role of behavioral interventions in appropriateness of CRYO transfusion and their impact on patient's outcomes should be further explored.

The current recommended dose of CRYO in the context of massive transfusion causes a modest increase in plasma fibrinogen levels (0.55 g/L). Further studies are needed to further define when and how much fibrinogen is needed as well as the best way to provide it in the setting of severe traumatic hemorrhage.

## 15. Disclaimer

Bartolomeu Nascimento is the 2010-11 National Blood Foundation Grant Recipient for the conduct of research related to coagulopathy in trauma.

## 16. Conflict of Interest

Sandro Rizoli received honorarium and speaker's fees (as a member of the Scientific Advisory Board) from NovoNordisk S/A, manufacturer of NovoSeven (recombinant factor VII). The other authors have no conflict of interest relevant to the subject matter of this publication.

## 17. Appendices

## Appendix 1. Sunnybrook Massive Transfusion Guidelines - Trauma

**S** – **SURGERY** for surgical bleeding required?

**M** - **MASSIVE TRANSFUSION** risk? (SBP  $\leq$  90mmHg or base deficit  $>$  5 and bleeding)

**A** - **ACTIVATION** of massive transfusion pack needed? (call blood bank # 4051)

**R** – **RESTRICTION** of crystalloids considered? (maximum 3 litres before blood)

**T** – **TEMPERATURE** controlled? (keep  $>$  36°C)

Product	Goal	Dose
RBCs	70-100 g/L	As per rate of blood loss & risk factors
Platelets	$>50/\mu\text{L}$ , or $> 100/\mu\text{L}$ if CNS injury	1 pool 2 pools if count $<30$ + ongoing massive bleeding)
Plasma	INR $\leq$ 1.8	3 - 4 units
Cryoprecipitate	fibrinogen $> 1.0$ g/L	8 -12 units

- Q1h CBC, INR, aPTT, fibrinogen;
- Q4h ABG, lytes, creatinine, ionized Ca, Mg, Lactate
- If expected transfusion  $>$  4 RBCs before lab results + evidence of massive bleeding:
  - Order massive transfusion pack: 4 RBCs; 4 FFP; 1 Platelet pool (infuse based on laboratory values where possible)

Appropriate Interventions
✓ 2 $\geq$ 16-gauge IVs; initial resuscitation – NS or RL; permissive hypotension (SBP no $>$ 90 mmHg) considered in penetrating torso trauma only
✓ Crystalloid, RBCs & FFP via warmer
✓ Rewarming for $<$ 36°C; room at 28°C; forced air blankets; extra-corporeal;
✓ Notify relevant services (Surgery, Interventional Radiology, ICU, Anesthesia); consider cell-saver in OR
✓ Reassess bleeding & lab between transfusions
✓ 1g CaCl IV if ionized calcium $<$ 1.15mmol/L
✓ Tranexamic acid 1g bolus followed by 1g infusion over 8h

Policy
✓ Massive Blood Loss - $>$ 1 blood volume/24h = 5L or 10 RBCs; half of blood vol. within 3h; bleeding rate $>150\text{ml}/\text{min}$ or 4 RBCs within 1h
✓ Initiation – physician informs Blood Bank - One ordering MD communicating with a single BB tech
✓ Unknown ABO $\rightarrow$ O RBCs & AB plasma until ABO confirmed
✓ Rh-neg $\rightarrow$ Rh -neg. RBC & Platelets issued; Blood Bank may switch to Rh + if inventory short
✓ Unknown Rh $\rightarrow$ Rh -neg. only for females $<$ 45y or anti-D antibody history
✓ RBC, Platelets, FFP, Cryoprecipitate infused with filter (170 – 260 micron) & only with 0.9% NS
✓ Change blood tubing q6 h or as per manufacturer; PTL via fresh tubing
✓ Documentation – careful attention to patient identification; If no identifiers $\rightarrow$ request ER unique anonymous ID; document start time for all blood products; end-time not required in massive transfusion
✓ Bleeding in hereditary coagulopathy or on anti-coagulation $\rightarrow$ transfusion medicine consult (ext.:4051)

Anticoagulant	Antidote	Dose
Unfractionated heparin (UFH)	Protamine	1 mg/100 IU of UFH given within past 4 hours; repeat 50 mg dose if aPTT rebounds
Low molecular weight heparin (LMWH) – Dalteparin	Protamine	1 mg per 1 mg LMWH administered within past 4 hours
Vitamin K antagonists (Coumadin)	Vitamin K	10 mg IV
	PCC	1000 IU if INR $<$ 4 or 2000 IU if INR $>$ 4

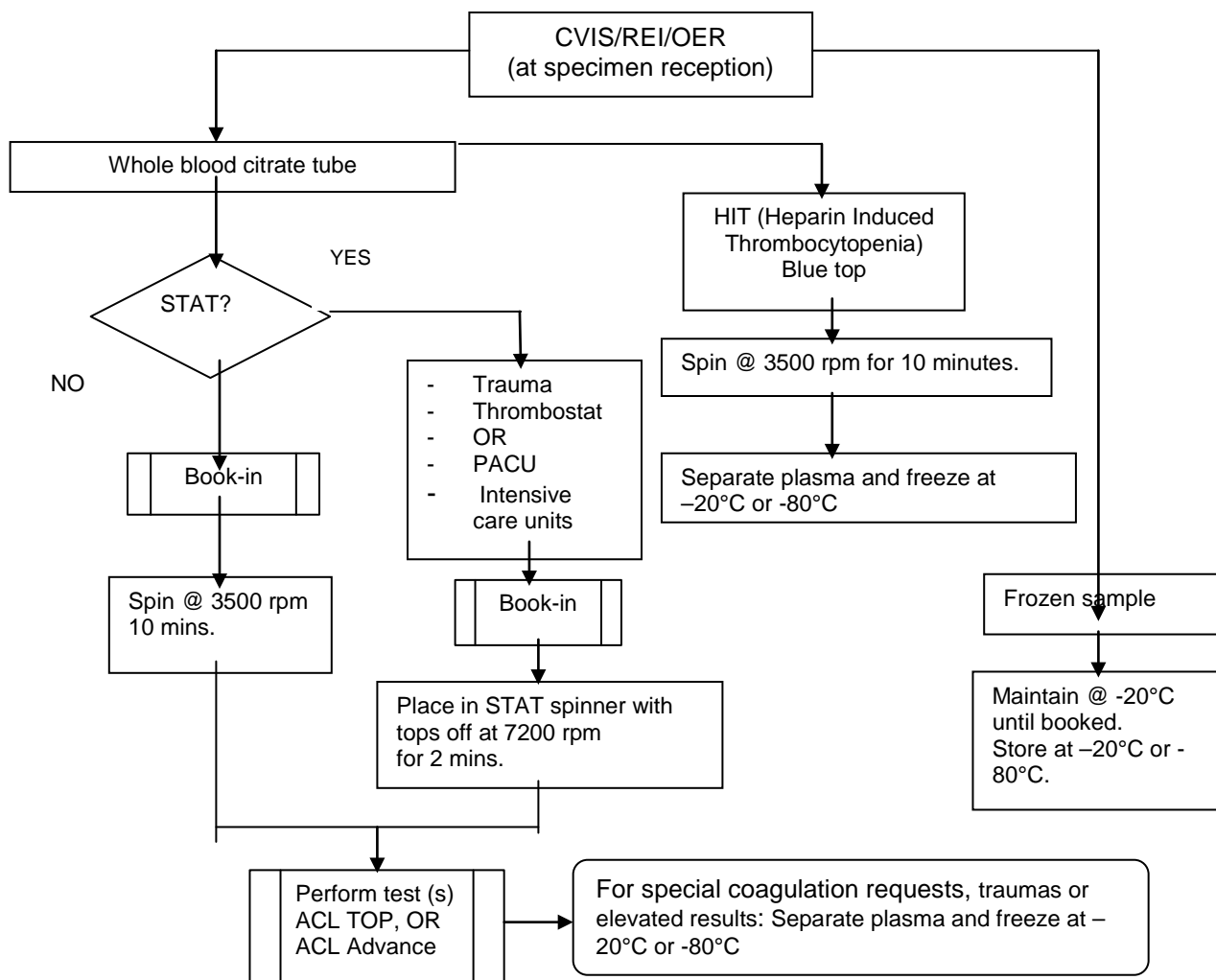
**Transfusion Medicine (TM) consult advised for above cases when bleeding**

**No evidence to support the routine use of rFVIIa in trauma (Consider in consultation with Blood Bank MD)**

Adapted with permission: Sunnybrook Hospital Transfusion Medicine Committee.

## Appendix 2. Handling and Processing of Coagulation Specimens at the

### Sunnybrook Campus Flowchart



Adapted with permission: Integrated and Core Laboratories - Coagulation, Department of Clinical Pathology, Sunnybrook Health Sciences Center. CVIS, indicates samples that had the tests ordered by the floors and clinics and already have a barcode label on the tube; REI, indicates samples that had the tests ordered by the floors and clinics but still need the barcode; OER, indicates sample received with a requisition and the laboratory has to enter all the patient identification information into the lab computer system and order the tests in order to generate the barcode labels for the tubes; OR, operating room; PACU, post-anesthesia care unit; ACL TOP OR ACL Advance, ACL Hemostasis Analyzer, Instrumentation Laboratory, Beckman Coulter.

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