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**POSTOPERATIVE VOLUME THERAPY IN CARDIAC SURGERY:
EFFECTS ON HEMOSTATIC AND CIRCULATORY VARIABLES**

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Academic Dissertation

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Yliopistopaino

***To my wife, Diana,
and children, Emilia and Marcus***

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ORIGINAL PUBLICATIONS

- I. Schramko A, Suojaranta-Ylinen R, Kuitunen A, Kukkonen S, Niemi T. Rapidly degradable hydroxyethyl starch solutions impair blood coagulation after cardiac surgery: a prospective randomized trial. *Anesth Analg* 2009;108:30-6.
- II. Schramko A, Suojaranta-Ylinen R, Kuitunen A, Raivio P, Kukkonen S, Niemi T. Hydroxyethyl starch and gelatin solutions impair blood coagulation after cardiac surgery: a prospective randomized trial. *Br J Anaesth* 2010;104:691-7.
- III. Schramko A, Kuitunen A, Suojaranta-Ylinen R, Niemi T. Role of fibrinogen-, factor VIII- and XIII-mediated clot propagation in gelatin haemodilution. *Acta Anaesthesiol Scand* 2009;53:731-5.
- IV. Niemi T, Schramko A, Kuitunen A, Kukkonen S, Suojaranta-Ylinen R. Haemodynamics and acid-base equilibrium after cardiac surgery: comparison of rapidly degradable hydroxyethyl starch solutions and albumin. *Scand J Surg* 2008;97:259-65.
- V. Schramko A, Suojaranta-Ylinen R, Kuitunen A, Raivio P, Kukkonen S, Niemi T. Comparison of the effect of 6% hydroxyethyl starch and gelatin on cardiac and stroke volume index: a randomized, controlled trial after cardiac surgery. *Perfusion*, *in press*.

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ABBREVIATIONS AND ACRONYMS

ACT – activated clotting time

BE – base excess

CABG – coronary artery bypass grafting

CI – cardiac index

CFT – clot formation time

CO – cardiac output

CPB – cardiopulmonary bypass

CT – clotting time

CVP – central venous pressure

DO₂ – oxygen delivery

FVIII+vWF – coagulation factor VIII+von Willebrand factor

FXIII – coagulation factor XIII

FFP – fresh frozen plasma

G – shear elastic modulus

HES – hydroxyethyl starch

ICU – intensive care unit

kDa – kiloDalton

LY30 – clot lysis after 30 minutes

LY60 – clot lysis after 60 minutes

MAP – mean arterial pressure

MCF – maximum clot firmness

MW – molecular weight

P_aCO₂ – partial pressure of carbon dioxide in arterial blood

P_aO_2 – partial pressure of oxygen in arterial blood

PCWP – pulmonary capillary wedge pressure

PVRI – pulmonary vascular resistance index

TEM – thromboelastometry

SD – standard deviation

SID – strong ion difference

SVI – stroke volume index

SvO_2 – mixed venous saturation

SVRI – systemic vascular resistance index

VO_2 – oxygen consumption

ABSTRACT

Background: Patients may need massive volume-replacement therapy after cardiac surgery because of large fluid transfer perioperatively. Cardiopulmonary bypass (CPB) also elevates the risk for bleeding. After cardiac surgery, hemodynamic stability is better maintained with colloids than with crystalloids when a similar volume of fluid is given. However, colloids have more adverse effects such as coagulation disturbances and impairment of renal function than do crystalloids.

The present study examined the effects of modern hydroxyethyl starch (HES) and gelatin solutions on blood coagulation and hemodynamics. The mechanism by which colloids disturb blood coagulation was investigated by modified thromboelastometry (TEM) after cardiac surgery, and *in vitro* by use of experimental hemodilution.

Materials and methods: Ninety patients scheduled for elective primary cardiac surgery (Studies I, II, IV, V), and twelve healthy volunteers (Study III) were included in this study. The patients had preserved left ventricle function preoperatively and had neither coagulation disturbances nor renal nor liver failure. During surgery, Ringer's acetate solution was given for volume replacement. After admission to the cardiac surgical intensive care unit (ICU), patients were randomized to receive either 15 mL kg⁻¹ of HES 130/0.4, HES 200/0.5, or 4% albumin solution (Studies I, IV). The effect of cumulative doses of 7 mL kg⁻¹, 14 mL kg⁻¹, 21 mL kg⁻¹, and 28 mL kg⁻¹ of HES 130/0.4 or 4% gelatin solution was compared to that of Ringer's acetate (Studies II, V).

To explore in more detail the mechanisms by which colloids disturb coagulation, venous blood collected from twelve healthy volunteers was diluted by gelatin *in vitro*. Thereafter, single coagulation factor concentrates (coagulation factors VIII+von Willebrand factor (FVIII+vW), coagulation factor XIII (FXIII), and fibrinogen, as well as fresh frozen plasma (FFP) were added to diluted samples.

Coagulation was assessed by TEM, and hemodynamic measurements were based on pulmonary artery pressures and thermodilutionally measured cardiac index (CI).

Results: HES 130/0.4 and HES 200/0.5, as well as gelatin solutions impaired whole blood coagulation similarly as measured by TEM (Studies I, II) even at a small dose of 7 mL kg^{-1} . These solutions reduced clot strength and prolonged clot formation time (CFT), and these effects were more pronounced with increasing doses of colloids (Study II). The albumin solution did not disturb whole blood coagulation after cardiac surgery. Ringer's acetate enhanced coagulation by increasing clot strength slightly after a relatively large cumulative dose (14 mL kg^{-1} or more). Fibrinogen concentrate, but neither FVIII+vW nor FXIII improved clot strength after gelatin hemodilution *in vitro* (Study III).

Coagulation disturbance after infusion of HES 130/0.4, HES 200/0.5, or gelatin solutions was clinically slight, and postoperative blood loss was comparable with that of Ringer's acetate or albumin solutions.

Both single dose (Study IV) and multiple dose (Study V) of all the colloids increased CI and stroke volume index (SVI) postoperatively, and this effect was dose-dependent in Study V. Ringer's acetate had no effect on CI or SVI (Study V). On the first postoperative morning, CI and SVI were similar in all the study groups, but higher than before the infusion of study solution. The hemodynamic effect of HES 130/0.4 was similar to that of the HES 200/0.5 solution, but more pronounced than that of albumin (Study IV). At a small dose (7 mL kg^{-1}), the effect of gelatin on CI and SVI was comparable with that of Ringer's acetate solution and significantly less than that of HES 130/0.4 (Study V). However, when the dose was increased to 14 and 21 mL kg^{-1} , the hemodynamic effect of gelatin rose and became comparable to that of HES 130/0.4.

After infusion of gelatin solution, the strong ion difference (SID) was greater than after infusion of HES 130/0.4 or Ringer's acetate solutions. However, there were no differences in acid-base equilibrium between the HES 130/0.4, gelatin, or Ringer's acetate groups.

Conclusions: In cardiac-surgery patients postoperatively, HES and gelatin solutions impaired clot strength in a dose-dependent manner. The potential mechanisms were interaction with fibrinogen and fibrin formation, resulting in decreased clot strength, and hemodilution.

ABSTRACT

Although the use of HES 130/0.4, of HES 200/0.5, or of gelatin inhibited coagulation, postoperative bleeding after elective cardiac surgery was not higher than for albumin and Ringer's acetate. Chest tube drainage on the first postoperative morning in all the study groups was similar.

A single dose of HES 130/0.4 and HES 200/0.5 solutions improved CI and SVI postoperatively more than did gelatin, albumin, or Ringer's acetate. However, when administered in a repeated fashion, (cumulative dose of 14 mL kg⁻¹ or more), no differences were evident between HES 130/0.4 and gelatin. On the first postoperative morning, CI and SVI in all study groups were similar.

The study solutions caused no clinically significant changes in acid-base equilibrium postoperatively.

INTRODUCTION

After major surgery, colloid solutions (HES, gelatin, albumin, dextran) ensure volume resuscitation. Because of good volume expansion capacity, colloids are recommended in partial replacement of the blood loss. On the other hand, colloid solutions have led to impaired blood coagulation, therefore aggravating risk for postoperative bleeding. Both clinical and experimental studies have shown that HES solutions reduce clot strength as assessed by whole blood coagulation analysis (i.e., TEM). These effects depend on the molecular weight (MW), degree of substitution, and the C2:C6 ratio of HES solutions. High-MW HES solutions cause increased bleeding after cardiac surgery (Cope *et al.* 1997). The impact of rapidly degradable HES solutions of low MW (i.e., HES 130/0.4) on postoperative blood loss is minimal or absent (Van der Linden *et al.* 2005), although laboratory analysis has revealed some negative effects (Felfernig *et al.* 2003). The effect of gelatin on blood coagulation is controversial. It has been suggested that the effect of gelatin on hemostasis is less than that of HES, probably due to the low MW of gelatin (30 kDa). After cardiac surgery, a gelatin solution has impaired blood coagulation to a similar extent as that of HES (Niemi *et al.* 2006), and reduced platelet aggregation (Boldt *et al.* 1992).

Crystalloid solutions do not impair blood coagulation more than hemodilution could explain, but their volume expansion effect is less than that of colloids. After major surgery, maintenance of an adequate preload requires a volume of crystalloids double or more than that of colloids (Lang *et al.* 2001). Thus, risk for tissue edema and risk for an increased amount of extravascular lung water can rise, as has occurred in critically ill patients (Vrancken *et al.* 2005).

After CPB, patients are predisposed to bleeding complications because CPB induces platelet dysfunction, reduces the amount of coagulation factors, and promotes fibrinolysis (Parolari *et al.* 2003). Hemodilution and hypothermia as well as the use of heparin also have detrimental effects on blood coagulation.

The main indication for colloids after cardiac surgery is to stabilize intravascular volume and maintain hemodynamics. The study of Kuitunen and co-workers (2007) showed that after cardiac surgery, the ef-

fect of gelatin on cardiac output (CO) and oxygen delivery (DO₂) is inferior to that of HES 200/0.5.

No evidence exists of benefit to patients' outcome with regard to different types of colloid or crystalloid solutions (Perel and Roberts 2007). However, the clinical effects of various colloids are dissimilar: blood coagulation, hemodynamics, and kidney dysfunction are related to the type of colloid given. Especially after cardiac surgery, the characteristics of the colloid solution are of clinical importance. The ideal colloid must improve hemodynamics rapidly, without disturbing blood coagulation or leading to increased postoperative risk for bleeding.

The objective of the present study was to compare several modern colloid solutions (i.e., HES 130/0.4, HES 200/0.5, and gelatin) after elective cardiac surgery and assess their effects on coagulation and hemodynamics.

REVIEW OF THE LITERATURE

1. Principles of volume replacement therapy after major surgery

During cardiac surgery with CPB, homeostasis is affected because of fluid therapy, CPB-related coagulation disturbances, perioperative bleeding, hypothermia, and mechanical ventilation. Administration of fluids aims not only to stabilize macrohemodynamics, but also to induce beneficial effects on microcirculation and tissue oxygenation (Lang *et al.* 2001).

According to the current concept, any deficit in circulating plasma should be replaced with a colloid, and this infusion of colloid should be guided by goal-directed methods (Chappell *et al.* 2008, Hildebrand *et al.* 2009).

Many controversies continue in regard to the quantity and quality of fluids appropriate for postoperative plasma volume expansion. Recent findings have changed the principles of volume replacement therapy in patients undergoing surgery. Research work in physiology has shown the important role of the endothelial glycocalyx (Levick and Michel 2010) in inhibiting fluid shift from intravascular into the interstitial space. The glycocalyx is a physiological barrier between the microcirculatory vessel (arteriole, capillary) wall and the interstitial space (Rehm *et al.* 2004). Preventing damage to the endothelial glycocalyx should therefore minimize fluid shifting and tissue edema.

Some studies have shown that the fluid overload during and after surgery may prove harmful (Brandstrup *et al.* 2003), even fatal. Lowell and co-workers (1990) demonstrated that after major surgery those patients with a body weight increase of over 20% died, but a moderate fluid overload postoperatively could improve the DO_2 (Arkilic *et al.* 2003) as well as the microcirculation in critically ill patients (Lang *et al.* 2003). Nevertheless, many studies report a better physiological outcome using the optimal model of volume replacement (Tølløfsrud *et al.* 1995, Lobo *et al.* 2002, Brandstrup *et al.* 2003, Nisanevich *et al.* 2005).

The Cochrane library meta-analysis could not show that outcome after surgery or in critically ill patients can be modified by administra-

tion of different types of fluids (Perel and Roberts 2007). However, many investigators have shown that colloid solutions improve hemodynamics better than do crystalloids in animal experiments (Su *et al.* 2007), in septic patients (Huang *et al.* 2009), and after cardiac or major vascular surgery (Karanko *et al.* 1987, Verheij *et al.* 2006a). Some studies show body weight gain and extravascular lung water to be increased through the use of crystalloid solutions (Huang *et al.* 2009), and after major surgery, this fluid overload can prolong the ICU stay (Gan *et al.* 2002). One study in septic patients showed also that the fluid overload which occurs within a few days requires some weeks to resolve (Cheng *et al.* 1998).

After cardiac surgery, intravenous fluid administration has received wide attention (Karanko *et al.* 1987, Kuitunen *et al.* 2007, Boldt *et al.* 2007, 2008). After open heart surgery, postoperative volume replacement is challenging and should be guided individually by each patient's requirements. Patients need adequate fluid therapy to create an optimal preload and maximize tissue perfusion. Decreased intravascular volume after open cardiac surgery can result in low-output syndrome and hypoperfusion of the tissues. On the other hand, fluid overload causes tissue edema, which may have negative effects on outcome (Brandstrup *et al.* 2003). The goal-directed use of colloids, crystalloids, and blood products, as needed, is the basis of modern fluid therapy (Gan *et al.* 2002, Boldt 2007).

2. Colloid solutions

2.1 Hydroxyethyl starches

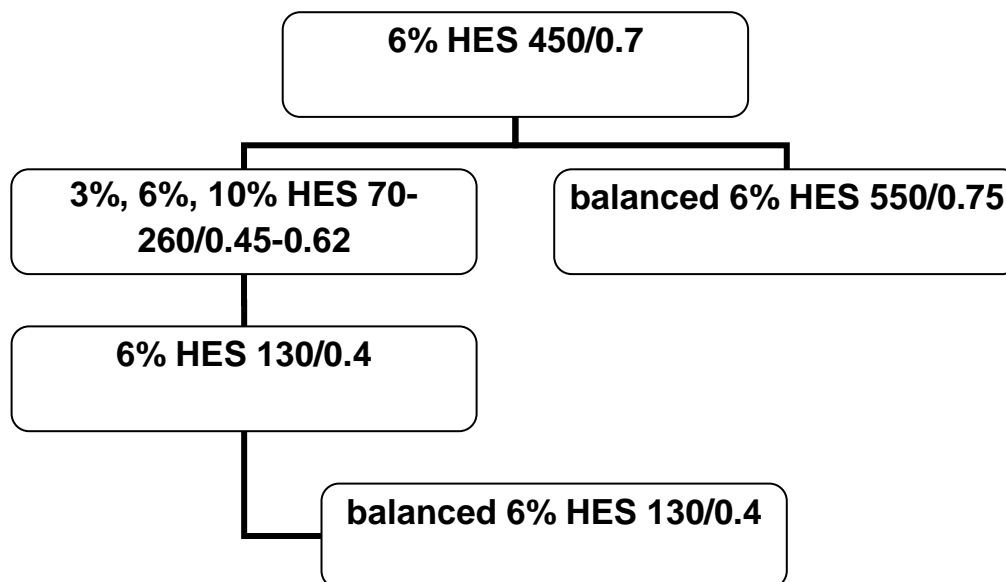
Natural starches cannot serve as plasma substitutes because they are rapidly degradable by circulating amylase, and they are insoluble at a neutral pH. HES solutions are widely used colloids in cardiac surgery patients, with new HES solutions being developed rapidly. HESs are polymers of glucose units derived from amylopectin with substitution of hydroxyl by hydroxyethyl groups on glucose molecules. HES solutions are polydisperse, and their *in vitro* MW ranges from a few kilodaltons (kDa) to several hundred. After administration of HES, the low-MW fraction (< 70 kDa) is removed rapidly by renal elimination, and the large molecules are progressively hydrolyzed, resulting in an

average *in vivo* MW which is lower than the MW of the infused fluid. The degradation of HES molecules depends on the degree of substitution, which is the proportion of glucose units having a hydroxyethyl group instead of a hydroxyl group. A high degree of substitution results in slower degradation and increased solubility (Treib *et al.* 1996).

The currently available HES solutions have a degree of substitution between 0.4 and 0.7. According to the degree of substitution, HES solutions are divided into tetra- (degree of substitution = 0.4), penta- (degree of substitution = 0.5), hexa- (degree of substitution = 0.6), and hetastarches (degree of substitution = 0.7). Because substitution is possible at positions 2, 3, or 6 of the glucose unit, different patterns of substitution are possible. The substitution pattern is characterized by the C2:C6 hydroxyethylation ratio. Clearance of HES is slowest with high C2:C6 ratios (Westphal *et al.* 2009). HES solutions are therefore classified by their initial MW, degree of substitution, and C2:C6 ratio (de Jonge *et al.* 2001). Usually, HES molecules are mixed with normal saline, but recently HES solutions have also been available in their balanced form (electrolyte concentrations corresponding to those of plasma).

The further development of HES started from the high MW (400-600 kDa) solutions with a high degree of substitution 0.6-0.7 (slowly degradable HES solutions) (Fig. 1), which has been demonstrated to impair blood coagulation (Felfernig *et al.* 2003) and renal function (Schortgen *et al.* 2001). In 1999, the rapidly degradable tetrastarch with a MW of 130 kDa was synthesized (“third generation” HES).

Figure 1. Development of HES solutions.



2.1.1. Coagulation effects of hydroxyethyl starches

Discussion is continuous as to the safety of HES solutions regarding blood coagulation. The first reports of negative effects of high-MW HES solutions on blood coagulation were published in 1965 (Thompson and Gadsden). The possible mechanisms have been widely studied in experimental (Hiippala 1995, Nielsen 2005) and clinical studies (Langeron *et al.* 2001, Boldt *et al.* 2002). Large HES molecules interfere with fibrinogen, coagulation factor VIII, and von Willebrand factor, and their negligible effect on coagulation is more than can be expected as a result of hemodilution (Omar *et al.* 1999). HES also promotes platelet dysfunction by disturbing platelet aggregation *in vitro* (Boldt *et al.* 1993). In studies assessing viscoelastic properties of whole blood, HES has been consistently demonstrated to disturb fibrin formation and to reduce clot firmness (Niemi and Kuitunen 1998, Boldt *et al.* 2007). The resulting thrombus is therefore less stable and more susceptible to lysis (Nielsen 2006). After these findings it was not surprising that even slight coagulation disorders were considered as a contraindication for the use of hetastarches.

Rapidly degradable HES solutions such as tetrastarch HES 130/0.4 have good plasma-expanding capacity and good hemodynamic effects without clinically significant impairment of blood coagulation after

cardiac surgery (Gallandat Huet *et al.* 2000, Kuitunen *et al.* 2004, Boldt *et al.* 2008), or in critically ill patients (Palumbo *et al.* 2006).

In 2001, Wilkes and co-workers published a meta-analysis of randomized studies in cardiac surgery: the average 24-hour postoperative blood loss after cardiac surgery was 96 mL greater after use of HES solutions than after albumin, which was significantly higher. That particular meta-analysis included HES solutions with a MW of 200 to 400 kDa and a degree of substitution of 0.5 to 0.7. They had been administered both intra- and postoperatively. The recent pooled analysis of randomized trials (Kozek-Langenecker *et al.* 2008) showed that blood loss in patients undergoing major surgery and receiving HES 130/0.4 solution was less than in patients who received HES 200/0.5.

2.1.2 Hemodynamic effects of hydroxyethyl starches

Many studies have demonstrated the successful use of HES solutions in maintaining hemodynamic goals after different types of surgery, and in critically ill patients (Boldt *et al.* 1996, Lang *et al.* 2003, Kuitunen *et al.* 2007). HES solutions have a good plasma-expansive property, which lasts several hours. The volume-expansion effect is dependent on the degree of the volume deficit before the administration of the HES solution, as well as the concentration and MW of the HES. Usually, in hypovolemic patients the plasma-expansion effect of HES is about 100% (Zaar *et al.* 2009), but actually it is individual and can range from 70 to 220% (Kröll *et al.* 1993). The MW of the HES molecule plays a big role in the volume replacement. Because of their relatively high MW (70 to 670 kDa), HES molecules do not shift through the endothelium as do colloids with low MW (<70 kDa), or as do crystalloids, and they can remain in the intravascular space. Degree of tissue edema is therefore less than with the use of crystalloids (Lang *et al.* 2001).

Actually, it is unclear whether various HES solutions have equal hemodynamic effects. A few studies have compared different HES solutions in some specific situations (trauma, major orthopedics), but the results are still discordant (Langeron *et al.* 2001, Persson and Grande 2006).

The hemodynamic effects of HES solutions have also been compared with those of other types of fluids. Some trials have reported

that HES is superior to albumin or gelatin solutions (Palumbo *et al.* 2006, Kuitunen *et al.* 2007). In an experimental model of hemorrhagic shock, the hemodynamic effect was better with HES than with crystalloid solutions (Persson and Grande 2005). The amount of crystalloid needed to maintain similar hemodynamics was greater than that of HES (Lang *et al.* 2001). On the other hand, some studies found no difference in volume efficacy with different colloid solutions (Moggio *et al.* 1983, Beyer *et al.* 1997).

2.1.3 Other effects of hydroxyethyl starches

One of the clinically important side-effects of HES solutions is their possible adverse effect on renal function. In 2008, the multicenter randomized study showed that use of HES 200/0.5 in septic patients was worse for renal function than was Ringer's solution (Brunkhorst *et al.* 2008). However, in sub-group analysis, mortality was lowest in the group of patients administered the recommended dose of HES 200/0.5 ($<22 \text{ mL kg}^{-1}$) (31%) in comparison with patients administered Ringer's (41%) or a high dose of HES (58%) solutions. That study was criticized for using high doses of pentastarch HES 200/0.5 in the treatment of patients with already evident renal dysfunction (exclusion criteria: serum creatinine over $320 \mu\text{mol L}^{-1}$).

The renal effects of HES 130/0.4 have also been studied. Those relatively small studies which include 20 to 25 patients per group demonstrated that this solution did not impair renal function after major vascular surgery (Mahmood *et al.* 2007). Even in patients with previous renal dysfunction, serum creatinine, glomerular filtration rate, and cystatin C plasma levels as well as urine output were comparable between HES and albumin groups after cardiac surgery (Boldt *et al.* 2007). Boldt and co-workers (2004) demonstrated also that after cardiac surgery the effect of HES 130/0.4 on endothelial inflammatory response was better than that of gelatin.

The use of the "first-" and "second generation" HES solutions was associated with accumulation of high-MW HES molecules in different tissues. Because of this, the repeated administration of HES was regarded as contraindicated in patients with renal or liver failure. The prolonged persistence of HES in plasma and tissues can be avoided by use of rapidly degradable HES solutions with degree of substitution

<0.5 (tetrastarches) (Jungheinrich and Neff 2005). Waitzinger and co-workers (2003) showed that even the use of repeated doses of HES 130/0.4 caused no accumulation in the plasma of human volunteers. Accumulation of tetrastarches in the tissues has not been studied.

Although allergic reactions are possible in patients receiving HES solutions, such reactions after administration of tetrastarches occurred in only 0.05%, less than after a gelatin solution (Dieterich *et al.* 1998).

2.2 Gelatin

Gelatins are polydisperse polypeptides produced by degradation of bovine collagen. Two separate forms are available: succinylated (modified) gelatins, or polygelatins. Modified gelatins have their ammonium groups replaced by acetate groups due to reaction of the basic peptide with succinic acid anhydride; polygelatins consist of polypeptides cross-linked by urea bonds. Gelatins are widely used in some countries for plasma volume replacement because they are considered to have no adverse effects on renal function or blood coagulation (Haisch *et al.* 2001, Schortgen *et al.* 2001). Gelatin maintains intravascular volume better and causes less tissue edema than crystalloids do (Van der Heijden *et al.* 2009).

In Scandinavia the use in ICUs of HES 130/0.4 is greatest in comparison with other colloids (60%). The use of gelatin has decreased to less than 5%, and in 2008 it was comparable to that of dextrans or albumin (Perner *et al.* 2008).

2.2.1 Coagulation effects of gelatin

Gelatin solutions disturb the reticular fibrin mesh and reduce functional clot quality *in vitro* (Mardel *et al.* 1998), making the clot less stable and more sensitive to lysis in comparison to an intact clot. Gelatin impairs blood coagulation in experimental studies (de Jonge *et al.* 1998, Niemi and Kuitunen 2005), and the effect of gelatin on blood coagulation is comparable to that of tetrastarches, according to modified TEM (Fries *et al.* 2006). In *in vitro* conditions, gelatin impairs blood coagulation less than hetastarches do (Petroianu *et al.* 2000).

Several clinical studies have reported similar blood loss and use of blood products after infusion of gelatin or HES 130/0.4 solutions after cardiac surgery (Van der Linden *et al.* 2005, Niemi *et al.* 2006). The effect of gelatin is slight, without any evidence of increased blood loss perioperatively. However, Niemi and co-workers (2006) demonstrated that the clot quality in TEM correlates with postoperative bleeding.

2.2.2 Hemodynamic effects of gelatin

The plasma volume expansion effect of gelatin, lasting 2 to 4 hours, is relatively short. Because of its rapid metabolism without tissue accumulation (MW of 30 kDa), gelatin has served perioperatively for volume replacement.

The capacity of gelatin to maintain hemodynamics has been studied in experimental and clinical investigations (Marx *et al.* 2002, Verheij *et al.* 2006b). Kröll and co-workers had already shown in 1993 that the volume expansion effect of 500 mL of gelatin in healthy volunteers is significantly inferior to that of HES and even shorter than that of crystalloid solution (Ringer).

Su and co-workers (2007) showed in an animal model of septic shock that the volume effect of gelatin is superior to that of crystalloids, and this study demonstrated also that the effect of HES is better than that of gelatin solutions.

When gelatin has been compared with HES solutions, clinical studies show conflicting results. In some studies after cardiac surgery, gelatin has improved CI and mixed venous saturation (SvO₂) as effectively as HES solutions did (Van der Linden *et al.* 2004). On the other hand, Kuitunen and co-workers (2007) showed that the hemodynamic effect (as monitored by CI and SVI) of a single dose of pentastarch was better and lasted longer than that of gelatin.

2.2.3 Other effects of gelatin

No recommendation exists for maximal daily dose of gelatin. It has been safely used for plasma volume replacement in critically ill patients with less effect on renal function than with HES (Schortgen *et al.* 2001). In a multicenter randomized study by this group, when gela-

tin or hexastarch with a MW of 200 kDa was administered to septic patients, the gelatin induced significantly lower peak creatinine concentrations and a lower frequency of renal failure than did hexastarch in patients with septic shock. However, other investigators have reported the opposite findings. After cardiac and major vascular surgery, serum creatinine and α -1 microglobulin were higher, and creatinine clearance and glomerular filtration lower after use of gelatin than after tetrastarch (Mahmood *et al.* 2007, Boldt *et al.* 2008).

The frequency of allergic reactions with gelatin solution (0.35%) is more than six-fold that with HES (Laxenaire *et al.* 1994).

2.3 Albumin

Before 1990, the use of albumin as a colloid solution was dominant. In 1990, the Transfusion Practices Committee of the American Association of Blood Banks recommended minimizing the use of albumin for plasma volume replacement. Their reason was that synthetic crystalloid or colloid solutions lack the potential to transmit infection (Goodnough *et al.* 1990). Even in Finland with its low infection risk after transfusions, the use of albumin continues to decrease. In 2004, results of the SAFE (The Saline versus Albumin Fluid Evaluation) study showed that albumin has no effect on mortality in critically ill patients in comparison with normal saline (Finfer *et al.* 2004).

2.3.1 Coagulation effects of albumin

Albumin solutions do not impair clot strength, a fact important for the treatment of patients after cardiac surgery (Kirklin *et al.* 1984). In comparison with HES and gelatin solutions, albumin causes no changes in TEM (Niemi *et al.* 2006). One study showed slight hypo-coagulation after albumin transfusion, probably because of its hemodilution effect (Tobias *et al.* 1998).

2.3.2 Hemodynamic effects of albumin

Several studies have demonstrated stable hemodynamics to be associated with the use of albumin in experimental (Persson and Grande 2005) and clinical studies (Karanko *et al.* 1987, Kuitunen *et al.* 2007). In comparison with synthetic colloids, no differences were observable

in cardiac performance after the postoperative administration of HES or albumin solutions (Moggio *et al.* 1983, Kirklin *et al.* 1984, London *et al.* 1989, Mastroianni *et al.* 1994). However, the rapidly degradable HES solutions are considered to have an effect on hemodynamics more favorable than that of albumin in critically ill patients (Boldt *et al.* 1996, 1998, Palumbo *et al.* 2006). In the short term, however, albumin has superior resuscitation capacity (i.e., correction of CO, blood lactate, and pH) when compared with the capacity of HES solutions in studies on animals (Walcher *et al.* 1996, Cabrales *et al.* 2005).

2.3.3 Other effects of albumin

Albumin has been shown to induce mild metabolic acidosis in patients undergoing normovolemic hemodilution during surgery, a process explained by the changes in SID (Rehm *et al.* 2000). The frequency of allergic reactions with albumin is below 0.1% (Laxenaire *et al.* 1994).

3. Crystalloids

Crystalloid solutions still remain in wide use as resuscitation fluids. This is the oldest group of fluids used for plasma volume replacement. Many clinicians use crystalloids because of their safety and low cost. Crystalloids, *per se*, have no effect on renal function or blood coagulation (Ruttman *et al.* 2002). The number of different ions in Ringer's acetate solution is nearer that of human plasma than is either unbalanced HES or gelatin. Ringer's solution has therefore been used in the ICUs postoperatively.

Some investigators have shown that crystalloids improve hemodynamics and tissue perfusion as well as do albumin or HES solutions (Lang *et al.* 2001, Klein *et al.* 2002, Finfer *et al.* 2004). The large meta-analysis could demonstrate no benefit in critically ill patients' outcome with the use of either crystalloid or colloid solutions (Perel and Roberts 2007). Several clinical studies have demonstrated a higher amount of extravascular lung water during use of crystalloids, which can aggravate tissue edema and worsen lung function (Lang *et al.* 2001, Vrancken *et al.* 2005).

Ringer's solution is successful for priming of the CPB circuit. A study by Tølløfsrud and co-workers (1995) showed that Ringer's solution can raise the amount of extravascular lung water and of tissue edema intraoperatively.

4. Thromboelastometry

4.1 Original thromboelastometry

Thromboelastography was first described in 1948 by Hartert, but this method was not used for the detection of coagulation disturbances clinically until 1980 (Luddington 2005). The TEM method was developed from thromboelastography, and few technical differences between them exist. Clinical use of TEM started after 2000. This method provides a graphic interpretation of viscoelastic changes in whole blood and evaluates the process of clot initiation, formation, and stability as well as lysis. TEM has the capability to give bedside summarized (within 30 minutes) information on platelet function, coagulation factors and inhibitors, and fibrinolysis. The use of native whole blood is not practical in the laboratory or operation theatre, as the unanticoagulated sample must be analyzed within a few minutes. Therefore, the TEM instrument uses citrated blood samples (Zambruni *et al.* 2004). The use of citrated blood in the time range of 0.5 to 4 hours after sampling has been recommended.

For TEM, blood is incubated at 37 °C in a heated cup. A pin connected to an optical detector system is suspended in the cup. The pin is oscillated at an angle of 4°45' relative to the cup, the motion being initiated by the pin. As fibrin forms between the cup and pin, the change in impedance of rotation of the pin is detected by a light beam; a TEM trace is generated (Luddington 2005). A normal TEM tracing is presented in Figure 2.

The parameters of TEM (Fig. 2) are:

1. Clotting time (CT) – the time before the beginning of blood coagulation which depends upon fibrin meshing.
2. Clot formation time (CFT), a measure of the velocity of fibrin meshing. CT and CFT are prolonged by warfarin and by deficits in

coagulation factors, and are shortened in hypercoagulation situations such as hyperfibrinogenemia.

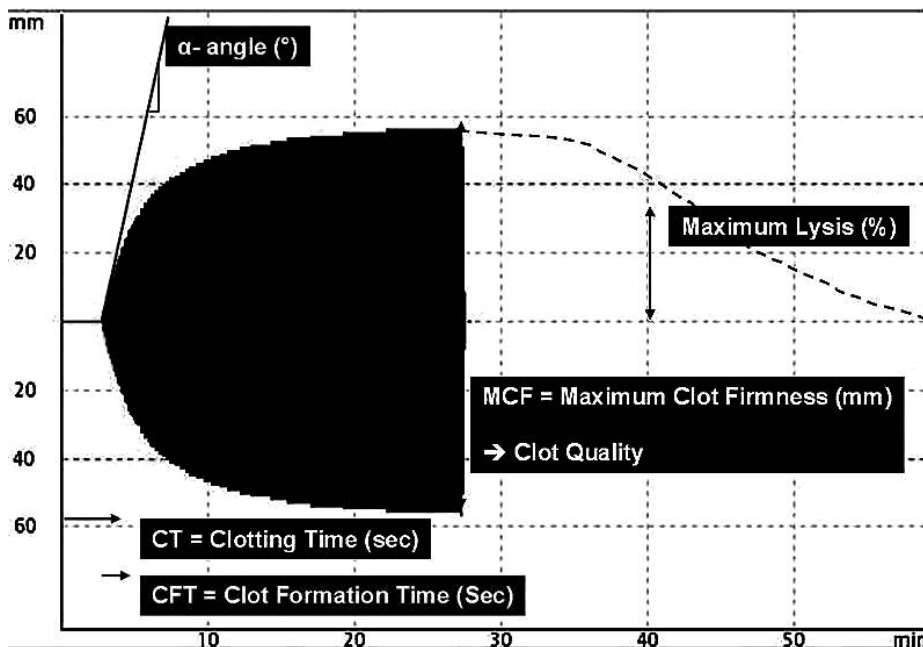
3. Maximum clot firmness (MCF) measures the strength of the developed clot and depends upon platelet function, coagulation factors, and especially fibrinogen concentration. Several studies have shown that MCF correlates with amount of bleeding.

4. An α -angle also reflects clot development.

5. Shear elastic modulus (G) is a parametric measure of clot strength expressed in metric units calculated from MCF (Niemi *et al.* 2006): $G = 5000 \times \text{MCF} [100 - \text{MCF}]^{-1}$.

6. Lysis 30 and 60 (LY30 and LY60) measure the percentage of lysis at 30 minutes and 60 minutes after achievement of MCF.

Figure 2. Normal thromboelastometry tracing and parameters (re-printed with permission from TEM Innovations GmbH, Munich, Germany).



4.2 Modified thromboelastometry

Different possibilities exist to modify TEM. Coagulation can be examined in the presence of different reagents in order to detect changes

at the different levels of the coagulation cascade (Ganter and Hofer 2008).

The InTEM reagent contains contact activator (partial thromboplastin phospholipid) and can detect changes in clot formation and fibrin polymerization via the intrinsic pathway (preceded by coagulation factors VIII-XII).

The ExTEM reagent contains tissue factor (thromboplastin) and has the power to assess parameters via the extrinsic pathway (preceded by coagulation factors VII, X).

To the FibTEM reagent, cytochalasin D is added, which removes the role of platelet function. Therefore, FibTEM shows only the fibrin contribution to clot formation. It is also possible to evaluate the impact of platelets on clotting by calculating the MCF difference between ExTEM and FibTEM.

To the ApTEM reagent, aprotinin is added to inhibit fibrinolysis. The degree of fibrinolysis is determined by evaluating the difference in TEM parameters between ExTEM and ApTEM.

HepTEM contains heparinase, and EcaTEM ecarin. Use of HepTEM allows detection of clot formation in spite of the presence of heparin. EcaTEM has been useful with direct thrombin inhibitors.

The modern TEM instrument has four channels to allow the possibility to use simultaneously four different reagents.

4.3 Clinical implications

TEM has served successfully as a point-of-care coagulation monitor and has been able to describe bedside changes in the coagulation system thoroughly within only 30 minutes. The best impact of TEM has been demonstrated in cardiac surgery, in trauma, in obstetric, and in hepatic-failure patients (Rugeri *et al.* 2007, Tripodi *et al.* 2009).

Several studies have shown that after cardiac surgery, inclusion of TEM in the treatment algorithm reduces blood product transfusion need (Spiess *et al.* 1995) as well as bleeding and reoperation rates (Welsby *et al.* 2006, Rahe-Meyer *et al.* 2009). Anderson and co-workers (2006) reported that inclusion of TEM in the transfusion algorithm after cardiac surgery can reduce red blood cell transfusion from

60% to 53%, FFP transfusion from 17% to 12%, and platelets transfusion from 16% to 11%.

A recent study also demonstrated a strong correlation between MCF and CFT with postoperative bleeding after cardiac surgery by use of different TEM reagents (Reinhöfer *et al.* 2008). TEM during cardiac surgery in cases of bleeding provides the possibility of early diagnosis and treatment of coagulation disturbances; a TEM-guided algorithm for the use of blood products is applied in some cardiac surgical centers.

Using InTEM, ExTEM, FibTEM and ApTEM reagents (Straub *et al.* 2008, Rahe-Meyer *et al.* 2009), it is possible to detect and treat, early, various coagulation disturbances. The Rahe-Mayer group demonstrated a reduction in allogenic blood product transfusion after CPB in patients undergoing thoracoabdominal aorta surgery. Those patients with excessive bleeding received fibrinogen to attain a target MCF by use of FibTEM reagent before standard therapy with platelets and FFP. A few case reports concern the use of ApTEM reagent in the evaluation and treatment of fibrinolysis in major surgery (Vorweg *et al.* 2001).

5. Cardiopulmonary bypass

5.1 Risk for bleeding after cardiopulmonary bypass

CPB predisposes patients to major hemorrhagic complications, and possibly to early bypass-related thrombotic events, as well (Parolari *et al.* 2003, Hekmat *et al.* 2004). The incidence of excessive postoperative bleeding in cardiac surgery exceeds 10%, and approximately 5 to 7% of patients experience blood loss in excess of 2 L within the initial 24 hours postoperatively (Despotis *et al.* 2001).

Among patients undergoing isolated CABG, surgical reexploration for postoperative bleeding is reported in approximately 4%. The specific site of bleeding, however, is identified in less than 50% of patients (Dacey *et al.* 1998, Nuttall *et al.* 2003). An acquired hemostatic defect is often responsible for diffuse mediastinal hemorrhage.

5.2 Mechanisms of activation of coagulation during cardiopulmonary bypass

CPB disturbs blood coagulation by several mechanisms (Yavari and Becker 2009). Coagulation factors are activated following direct contact between circulating blood and the inner surfaces of the extracorporeal circuit. During CPB, blood is pumped continuously across 2 to 2.5 m² of non-biological surfaces (Hyde *et al.* 1998), and the oxygenator, venous reservoir, and arterial line filter represent large blood-contact areas (Hyde *et al.* 1998). During CPB, blood is driven under a propulsive force through conduits of differing internal diameters. Moreover, the patients' blood mixes with priming and cardioplegia solutions.

During CPB, both contact factor and tissue factor activation occur. The contact or intrinsic pathway is triggered when factor XII, prekallikrein, and high-MW kininogen interface with nonbiological surfaces. The tissue factor or extrinsic pathway is triggered when factor VIIa complexes with tissue factor being expressed on monocytes, macrophages, fibroblasts, platelets, exposed atherosclerotic plaques, or exposed subendothelial constituents within the vessel wall (Boisclair *et al.* 1993). CPB therefore creates prothrombotic and proinflammatory conditions.

The contact- and tissue factor-activated coagulation pathways represent an integrated system designed to generate thrombin. Factor Xa with factor Va, calcium, and phospholipid substrate form the prothrombinase complex on activated platelets, cleaving prothrombin to form thrombin (Edmunds and Colman 2006), which activates platelets and coagulation factors V, VIII, and XI (Moor *et al.* 1994).

5.2.1 Cardiopulmonary bypass and effects of heparinization on platelets

Heparin inhibits platelet function (John *et al.* 1993, Kestin *et al.* 1993), reduces platelet count, and influences platelet microaggregation (Muriithi *et al.* 2000b). During CPB, platelets that have aggregated are lost from the circulating platelet pool. Previous investigators have also reported a preferential loss during extracorporeal circulation of activated platelets (Wahba *et al.* 1996). Activated platelets may

contribute to the cytokine production observed during CPB by causing endothelial cells to secrete cytokines and adhesion molecules (Henn *et al.* 1998). Platelets sequestered in the body during extracorporeal circulation later return to the circulation and have a normal life span. In theory, the inhibitory effect of heparin on platelet aggregation (Belcher *et al.* 2000, Muriithi *et al.* 2000a) may preserve platelets.

5.2.2 Fibrinolysis during cardiopulmonary bypass

Fibrinolytic activity increases significantly during and following CPB because of intraoperative contact activation, release of tissue plasminogen activator due to kallikrein production, use of relatively high-dose heparin, and inhibition of α 2-antiplasmin (Kongsgaard *et al.* 1989, Ray *et al.* 1994, Altman *et al.* 1998). Antiplasmin levels do not return to normal for 48 to 72 hours postoperatively, whereas plasmin levels after discontinuation of CPB return to normal immediately (Kongsgaard *et al.* 1989). Therefore, after CPB, the fibrinolysis activation is prolonged, which can lead to increased postoperative bleeding (Gelb *et al.* 1996, Casati *et al.* 2001, Linden 2003).

AIMS OF THE PRESENT STUDY

The main objective of the present thesis was to determine the effects of various colloid solutions (6% HES 130/0.4, 6% HES 200/0.5, and 4% gelatin) on whole blood coagulation and hemodynamics after elective cardiac surgery in patients with preserved cardiac function.

The specific goals were to determine:

1. The effects of a single dose of 15 mL kg^{-1} of HES 130/0.4 and HES 200/0.5 solution (Study I) or repeated doses (between 7 mL kg^{-1} and 28 mL kg^{-1}) of the HES 130/0.4 and gelatin solution (Study II) on whole blood coagulation after cardiac surgery, as assessed by TEM.
2. The mechanisms of colloid-induced coagulopathy by TEM analysis *in vivo* (Studies I, II), and *in vitro*, using gelatin hemodilution of whole blood (Study III).
3. The hemodynamic profile after a single dose of 15 mL kg^{-1} of HES 130/0.4 and HES 200/0.5 solution (Study IV) or repeated doses (between 7 mL kg^{-1} and 28 mL kg^{-1}) of HES 130/0.4 and gelatin solution (Study V) after cardiac surgery by use of a thermodilution method.
4. The acid-base equilibrium after administration of HES 130/0.4 and gelatin (Studies IV, V).

A colloid solution of biological origin (4% albumin) or crystalloid solution (Ringer's acetate) served as controls.

PATIENTS AND METHODS

Study design (I – V)

Four prospective clinical studies (I, II, IV, V) were performed, and one randomized cross-over *in vitro* study (III). In each clinical study, 45 patients scheduled for elective primary cardiac surgery were randomized; 12 apparently healthy volunteers without any medication 7 days prior to the experiment were included in the *in vitro* study. The Ethics Committee for Surgery in the Hospital District of Helsinki and Uusimaa (Studies I – V) and the National Agency of Medicines in Finland (I, II, IV, V) approved the study protocols. All patients and volunteers gave then written informed consent to participate in each study. Study IV included the same patient population as did Study I. Study V included the same patient population as did Study II (Table 1).

Patients included showed no renal or hepatic failure. In the clinical studies, preoperative cardiac medications were administered on the morning of surgery, except for angiotensin-converting enzyme inhibitors and angiotensin II antagonists. Preoperatively, all the patients had a normal left ventricular ejection fraction (> 50%). No antithrombotic medication was allowed within 5 days prior to surgery. Study characteristics are summarized in Table 1.

All the patients received different colloids or Ringer's acetate solutions in the early postoperative period (0-18 hours after surgery). TEM, hemodynamic changes, and the amount of bleeding, blood product use, and acid-base equilibrium were studied.

Table 1. Study characteristics.

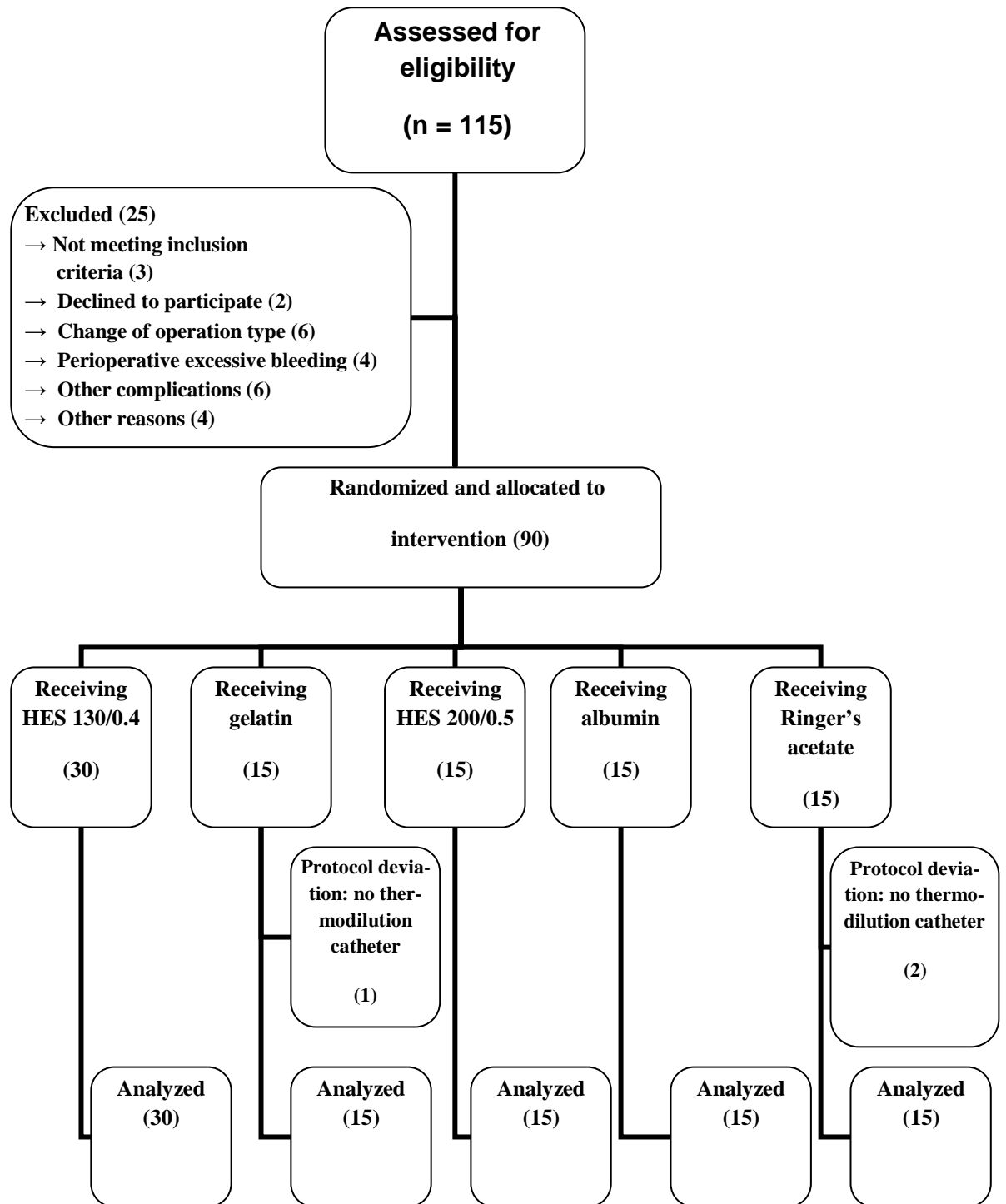
Study	Subjects	n	Design	Intervention	Primary end-point
I	Cardiac surgery patients	45	Prospective, randomized, single-blinded, controlled	15 mL kg ⁻¹ of 6% HES 130/0.4, 6% HES 200/0.5, or 4% albumin	TEM parameters (MCF)
II	Cardiac surgery patients	45	Prospective, randomized, single-blinded, controlled	7, 14, 21, and 28 mL kg ⁻¹ of 6% HES 130/0.4, 4% gelatin, or Ringer's acetate	TEM parameters dose-dependently (MCF)
III	Healthy volunteers	12	Experimental <i>in vitro</i> , randomized, cross-over	40 vol% hemodilution with 4% gelatin	TEM parameters (MCF) with addition of coagulation factors
IV	Cardiac surgery patients	45 (same as in Study I)	Prospective, randomized, open, controlled	15 mL kg ⁻¹ of 6% HES 130/0.4, 6% HES 200/0.5, or 4% albumin	Hemodynamics (CI, SVI) and acid-base equilibrium
V	Cardiac surgery patients	45 (same as in Study II)	Prospective, randomized, open, controlled	7, 14, 21, and 28 mL kg ⁻¹ of 6% HES 130/0.4, 4% gelatin, or Ringer's acetate	Hemodynamics dose-dependently (CI, SVI) and acid-base equilibrium

CI = cardiac index, HES = hydroxyethyl starch, MCF = maximum clot firmness, SVI = stroke volume index, TEM = thromboelastometry

Clinical study protocols (I, II, IV, V)

We recruited 115 patients for the four studies, and 25 were excluded (Figure 3).

Figure 3. Flow chart of patients undergoing elective cardiac surgery.



Anesthesia

Patients were premedicated with lorazepam 0.03 mg kg⁻¹ orally, and received fentanyl 5 µg kg⁻¹ or sufentanil 2 µg kg⁻¹ and propofol

1 to 1.5 mg kg⁻¹ or etomidate 0.2 mg kg⁻¹ for induction of anesthesia. Rocuronium served as the neuromuscular blocking drug. Anesthesia was maintained with a continuous infusion of fentanyl 5 µg kg⁻¹ h⁻¹ or sufentanil 1.5 to 2 µg kg⁻¹ h⁻¹ until the end of surgery. Sevoflurane supplementation (inspiratory concentration 2.5 to 3%) was used to achieve a bispectral index level below 50. After surgery, patients were transported to the cardiac surgical ICU under propofol sedation and on mechanical ventilation.

Cardiopulmonary bypass

CPB was instituted with a nonpulsatile pump and a membrane oxygenator. The bypass circuit was primed with 2000 mL of Ringer's acetate solution and 100 mL of 15% mannitol. During cardiac arrest, the patients were kept in mild hypothermia (nasopharyngeal temperature between 30 and 33°C). For CPB, the patients were anticoagulated with heparin 300 IU kg⁻¹, and 5000 IU of heparin was added to the priming solution. Activated clotting time (ACT using kaolin activator) was measured every 30 minutes and kept above 480 s during CPB with additional doses of 5000 IU of heparin if required. During CPB, hematocrit was kept above 20%. After CPB, heparin was neutralized with 1 mg of protamine for each 100 IU of the initial dose of heparin. Additional doses of 25 mg of protamine were given to achieve the pre-bypass ACT level. Shed mediastinal blood was not retransfused. After termination of CPB, blood from the CPB circuit was collected into nonanticoagulated blood bags and retransfused. During CPB all the patients were rewarmed (nasopharyngeal temperature up to 36°). Intraoperatively, only Ringer's acetate solution was administered.

Surgery

Several types of elective primary cardiac surgery using CPB were included: coronary artery bypass grafting (CABG), valve replacement or valve repair, or combined coronary and valve surgery. The cardiac surgery was not standardized, but all patients underwent full median sternotomy.

Study fluids

After admission to the cardiac surgical ICU, patients were randomized to receive one of the study solutions. The randomization was performed by means of closed envelopes prepared before the beginning of the study by a person not participating in the study. Thereafter, the predetermined dose of study solution was administered at the rate necessary to achieve or maintain pulmonary capillary wedge pressure (PCWP) between 10 and 15 mmHg. The dosing regimen of the study solutions was the following:

Studies I, IV: a single dose of 15 mL kg⁻¹ of 6% HES 130/0.4, 6% HES 200/0.5, or 4% albumin solutions.

Studies II, V: four repeated doses of 7 mL kg⁻¹ (resulting in cumulative doses of 7 mL kg⁻¹, 14 mL kg⁻¹, 21 mL kg⁻¹, and 28 mL kg⁻¹) of 6% HES 130/0.4, 4% gelatin, or Ringer’s acetate solutions (Table 2).

Study III was carried out *in vitro*: venous blood collected from volunteers was diluted by 4% gelatin solution to make 40 vol%. hemodilution.

Table 2. Characteristics of the study solutions.

	6% HES 130/0.4	4% GEL	6% HES 200/0.5	4% ALB	RIN
Study	I, II, IV, V	II, III, V	I, IV	I, IV	II, V
Average MW, kDa	130	30	200	65	-
pH	4.0-5.5	7.1-7.7	3.5-6	7	6.0
Sodium, mmol L⁻¹	154	154	154	154	131
Chloride, mmol L⁻¹	154	120	154	154	112
Acetate, mmol L⁻¹	-	-	-	-	30
Osmolality, mOsm L⁻¹	308	274	308	NA	270

ALB = 4% albumin, GEL = 4% gelatin, HES = hydroxyethyl starch, MW = molecular weight, NA = not available, RIN = Ringer’s acetate

Hemodynamic management and fluid therapy postoperatively

After surgery, PCWP was maintained between 10 and 15 mmHg, and CI over $2.0 \text{ L min}^{-1} \text{ m}^{-2}$. After administration of the fixed dose of study solution, Ringer's acetate was administered in cases of hypovolemia (PCWP < 10 mmHg). An epinephrine infusion was initiated (0.02 to $0.1 \mu\text{g kg}^{-1} \text{ min}^{-1}$) when the CI remained < $2.0 \text{ L kg}^{-1} \text{ min}^{-1}$ despite a PCWP between 10 and 15 mmHg. A norepinephrine infusion (0.03 to $0.3 \mu\text{g kg}^{-1} \text{ min}^{-1}$) was started whenever the mean systemic arterial blood pressure fell below 70 mmHg despite the targeted PCWP and CI.

Red blood cells, fresh frozen plasma, and platelet concentrate transfusion triggers

In clinical studies (I, II, IV, V), red blood cells (RBC) were transfused after CPB if hemoglobin (Hb) concentration fell under 80 g L^{-1} . During CPB, hematocrit was kept between 20 to 25%. In the cardiac surgical ICU, ACT, prothrombin time value, and platelet count were determined if postoperative blood loss exceeded 200 mL h^{-1} . If ACT was prolonged more than 10 s compared with its pre-bypass level, 25 mg of protamine was administered. If prothrombin time was greater than 30 s, 10 mL kg^{-1} of FFP was transfused. If platelet count fell below $50 \cdot 10^9 \text{ L}^{-1}$, $1 \text{ unit } 10 \text{ kg}^{-1}$ platelet concentrate was administered. If the bleeding continued, 1g of tranexamic acid was given.

***In vitro* study protocol (III)**

A venous blood sample was obtained twice from a dozen apparently healthy volunteers, six male and six female. An undiluted citrated blood sample served as a control. The 40 vol% hemodilution performed corresponds to the rapid administration of 2000 mL of gelatin solution to any patient with acute bleeding.

Various coagulation factors were added to the diluted blood samples at room temperature in random order 5 to 10 min prior to analysis:

1. FVIII+vWF, $80 \mu\text{L mL}^{-1}$ (Haemate P®, CSL, Behring, Marburg, Germany) (FVIII+vWF),

2. FXIII, 100 $\mu\text{L mL}^{-1}$ (Fibrogammin®, CSL, Behring) (FXIII),
3. Fibrinogen, 60 $\mu\text{L mL}^{-1}$ (Haemocomplettan P®, CSL, Behring) (Fibrinogen), or
4. FFP, 210 $\mu\text{L mL}^{-1}$ (Finnish Red Cross Transfusion Service, Helsinki, Finland).

Hb concentration, hematocrit, and platelet count were analyzed in all blood samples. To define the impact of fibrinogen and platelets on the changes in blood coagulation, modified TEM was performed with ExTEM and FibTEM reagents.

Transferring this *in vitro* model to *in vivo* conditions, the doses of various coagulation factors in cuvettes corresponded to a single dose of 1000 IU of Haemate P, 1250 IU of Fibrogammin, 3 g of Haemocomplettan P, or 15 mL kg^{-1} of FFP administered to an adult patient of approximately 70 kg of body weight and a blood volume of 5 liters. These doses are recommended for the treatment of moderate or severe bleeding.

Outcome measurements

Major outcome measurements

The major outcomes were maximum clot firmness measured by TEM analysis (ROTEM®; TEM Innovations GmbH, Munich, Germany) (Studies I, II, III) and cardiac and stroke volume index (Studies IV, V) measured by the thermodilution method using a pulmonary artery catheter.

A decreasing MCF is a major risk factor for bleeding after cardiac surgery (Rahe-Meyer *et al.* 2009). Specific reagents in TEM will make it possible to detect the impact of fibrin and platelets on coagulation (Rienhöfer *et al.* 2008). The pulmonary artery catheter is widely accepted in cardiac surgery for the monitoring of hemodynamics. By means of this catheter, it is possible to determine the CO, CI, and SVI as well as pre- (central venous pressure (CVP), PCWP) and postload (pulmonary vascular resistance index [PVRI], systemic vascular resistance index [SVRI]).

Thromboelastometry measurements (I, II, III)

The modified TEM was performed using four commercial reagents which were intrinsic ROTEM (InTEM®), extrinsic ROTEM (ExTEM®), fibrinogen ROTEM (FibTEM®), aprotinin ROTEM (ApTEM®). FibTEM was used to evaluate the impact of fibrinogen on blood coagulation, and ApTEM to detect possible activation of fibrinolysis. The ROTEM instrument was calibrated according to manufacturer's instructions.

Coagulation was initiated with activators by means of a semiautomatic electronic pipette according to manufacturer's instructions. Coagulation was allowed to proceed for 60 minutes in clinical studies, and for 30 minutes in Study III. Automatic ROTEM variables were clotting time (CT, s), clot formation time (CFT, s), α -angle (α , degree), maximum clot firmness (MCF, mm), and clot lysis after 30 and 60 minutes (LY30 and LY60). The effect of platelets on clot strength was assessed as follows: Platelet MCF = ExTEM MCF – FibTEM MCF (Lang *et al.* 2009).

In clinical studies, TEM was performed after surgery, after each dose of study solutions (Studies I, II), and on the first postoperative morning (Study II only). TEM analysis before intervention (after surgery) served as the control for each patient.

Hemodynamic measurements (IV, V)

Before anesthesia, the left or right radial artery was catheterized for measurement of systemic blood pressure. After induction and intubation, the pulmonary artery catheter (Swan-Ganz catheter®, Edwards Lifesciences, Unterschleisheim, Germany GmbH) was placed via an internal jugular vein. Hemodynamic measurements included heart rate (HR), mean arterial blood pressure (MAP), pulmonary artery pressure (PAP), CVP, PCWP, and CO. CO was measured by triplicate thermodilution using ice-water; CI, SVRI, PVRI, and SVI were calculated from these measurements.

Hemodynamic measurements were performed after surgery, after each dose of study solutions, and on the first postoperative morning (Studies IV, V).

Blood sampling and laboratory analyses

Blood sampling

In clinical studies, arterial blood samples were collected before the start of fluid infusion (when the patient reached the ICU), after each dose of the study solution, and on the first postoperative morning via a nonheparinized radial artery catheter. Blood samples for TEM were collected into polypropylene tubes containing 3.2% buffered citrate, which stabilized the sample for a period of 4 hours. This method was determined to be safe without significant changes in TEM in comparison with unstable native blood, and the ROTEM instrument is calibrated for citrate-coagulated blood samples (Zambruni *et al.* 2004).

Laboratory analyses

Hb concentration, hematocrit, and platelet count in arterial blood samples were analyzed by the Cell-Dyn 610 hematology analyzer (Sequilia-Turner Corp., Mountain View, CA). Partial pressure of oxygen (P_aO_2) and of carbon dioxide in arterial blood (P_aCO_2), pH, lactate, chloride, and ionized calcium were measured, and the standard serum bicarbonate concentration and base excess (BE) were calculated with an ABL 825 blood gas analyzer (Radiometer Medical A/S, Copenhagen, Denmark) using the Henderson-Hasselbalch equation and the Siggaard-Andersen nomogram (Siggaard-Andersen 1963). In addition, the SvO_2 was measured, and oxygen delivery ($DO_2 = 10 \times (0.136 \times Hb / 100 + 0.0031 \times PaO_2) \times CO$), consumption ($VO_2 = CO \times avD0_2$), and strong ion difference ($SID = [Na^+] + [K^+] - [lactate^-] - [Cl^-]$) (Stewart 1983) were calculated before and after the each dose of the study solution, and on the first postoperative morning.

Statistical analyses

Number of study subjects was based on observations of MCF in a previous study (Niemi *et al.* 2006). A difference of 15% in MCF was considered significant. Thus, the number of subjects needed was 13 by group in comparison of two groups with an α -error of 0.05 and β -error of 0.2. It also was estimated that 12.8 patients were needed in each group to detect a 20% difference in CI (standard deviation (SD) 0.5 L

min⁻¹ m⁻²) after the end of infusion of the study solution. Data distribution was tested by the Kolmogorov-Smirnov test. Analysis of variance (ANOVA) or the Kruskal-Wallis test was applied to detect any difference between or within groups. For multiple comparisons, Bonferroni correction was used. A paired t-test, Student-Newman-Keuls test, or Wilcoxon test served for paired comparisons. The results are reported as means with SD, or medians with quartiles (25th to 75th percentiles), if data were not normally distributed. The results of CI and SVI are also shown as means with 95% confidence intervals (Study V). The frequencies were tested by χ^2 test. A p value < 0.05 was considered statistically significant. All the statistical measurements were performed by SPSS for Windows (version 15.0).

RESULTS

All patients and volunteers who were initially included completed the study. In Study III all the hemodilutions were successful. In Study V, measurement of CI was impossible in three patients due to technical reasons. All TEM measurements were successful. The results of TEM of healthy volunteers (Table 3) are comparable with those of other studies, which indicates the reliability of the present observations.

Table 3. TEM variables in healthy volunteers (Study III, undiluted sample) (reprinted with permission from Wiley-Blackwell).

		Mean±SD	Range
CT	ExTEM	68.8±13.4	52-95
	(s) FibTEM	61.8±14.8	41-84
CFT	ExTEM	109.7±44.7	61-201
	(s) FibTEM	NA	NA
MCF	ExTEM	58.6±9.1	44-72
	(mm) FibTEM	14.8±4.7	11-22
α	ExTEM	69.0±7.1	55-78
	(°) FibTEM	65.2±8.8	53-78
LY30	ExTEM	98.9±2.3	93-100
	(100-%) FibTEM	99.6±0.7	98-100
G	ExTEM	7507±2702	3872-12674
	(U) FibTEM	890±3432	596-1451

CFT = clot formation time, CT = clotting time, G = shear elastic modulus, LY30 = clot lysis after 30 minutes, MCF = maximum clot firmness, mm = millimeter, NA = not available, s = second, SD = standard deviation, U = unit, α = α-angle, ° = degree

Effects of colloids on whole blood coagulation (I, II)

In the early postoperative period after cardiac surgery, HES 130/0.4, HES 200/0.5, and gelatin solutions impaired whole blood coagulation as measured by TEM. After a dose of 15 mL kg^{-1} of both HES solutions, MCF decreased and CFT increased compared to values for human albumin (in Study I, Figure 1). At 2 h, after all of the study solution was infused, the coagulation disturbances partially recovered (using InTEM and ExTEM reagents). In Study II, MCF decreased and CFT increased even after a small dose (7 mL kg^{-1}) of colloid solutions compared to the effect of Ringer's acetate, and these TEM changes became more pronounced with the larger doses of HES and gelatin solutions used, in a dose-dependent manner (Table 4). On the first postoperative morning, all TEM parameters were comparable among all groups (Study II).

Albumin caused no coagulation disturbances. The use of Ringer's acetate at a dose of 14 mL kg^{-1} raised MCF slightly but significantly above its pre-infusion level (Study II).

RESULTS

Table 4. Study II. Initial fibrin formation and build-up (ROTEM) before (Pre), and after each bolus of the study infusion (7mL kg⁻¹, 14 mL kg⁻¹, and 21 mL kg⁻¹), and on the first postoperative morning (1 POM) using ExTEM reagent (reprinted with permission from Oxford Journals).

	HES 130/0.4	4% gelatin	Ringer's acetate
ExTEM MCF (mm)			
Pre	57.9 (5.6)	56.9 (4.2)	58.5 (5.0)
7mL kg ⁻¹	55.1 (5.6)†	54.9 (4.4)†	59.7 (3.9)*
14 mL kg ⁻¹	51.7 (5.9)†	53.6 (3.6)†	60.4 (4.7)*†
21 mL kg ⁻¹	51.1 (6.2)†	52.3 (3.9)†	61.4 (3.4)*
1 POM (28 mL kg ⁻¹)	59.9 (5.2)	56.6 (4.4)	61.7 (3.4)#
ExTEM CFT (s)			
Pre	110.8 (39.8)	121.2 (32.4)	104.7 (19.2)
7mL kg ⁻¹	141.3 (45.0)†	136.8 (40.2)	101.1 (22.1)*
14 mL kg ⁻¹	169.5 (57.7)†	145.9 (39.7)†	96.0 (18.0)*
21 mL kg ⁻¹	200.1 (89.0)†	163.9 (36.8)†	97.3 (14.2)*
1 POM (28 mL kg ⁻¹)	124.6 (46.4)	135.7 (34.1)	102.1 (18.9)#

Values are means (SD). CFT = clot formation time, HES = hydroxyethyl starch, MCF = maximum clot firmness.

One-way ANOVA, and T-test. *p < 0.05 Ringer's acetate vs. HES and gelatin, #p < 0.05 RIN vs. gelatin, p < 0.05 gelatin vs. HES and Ringer's acetate, +p < 0.05 Ringer's acetate vs. HES (Bonferroni post hoc test), †p < 0.05 between pre and 7 mL kg⁻¹, 14 mL kg⁻¹, 21 mL kg⁻¹ and 1 POM (T-test)

Mechanism of coagulation disturbance after use of colloids (I-III)

No differences appeared in MCF or CFT between ExTEM and Ap-TEM reagents, nor any changes in platelet MCF (ExTEM MCF - FibTEM MCF) after administration of any of the study solutions (Studies I, II).

All the clot lysis parameters before and after use of these solutions fell within the normal range in the postoperative period (Studies I, II).

In Study III, the 40 vol% dilution with gelatin reduced MCF and raised CFT. FFP caused a partial correction of ExTEM values. The effect on TEM of fibrinogen, FVIII+vWF, or FXIII was minimal.

In the FibTEM tracing, all the coagulation factors improved MCF, but the MCF was significantly higher after administration of fibrinogen or FFP than after FVIII+vWF and FXIII (Table 5).

RESULTS

Table 5. Results of MCF and CFT (Study III) (reprinted with permission from Wiley-Blackwell).

	MCF (% of undiluted value)	CFT (% of undiluted value)
ExTEM Undiluted control	100.0	100.0
+Gelatin 40%	76.3 (65.9-80.0)# *+	270.3 (229.2-404.9)#
+Fibrinogen	74.0 (62.8-76.5)#*+	312.7 (279.1-363.8)#*
+FVIII+vWF	67.2 (50.6-73.8)#*	320.9 (268.1-467.7)#*
+FXIII	68.1 (58.3-72.9) #*	303.7 (271.4-396.2)#*
+ FFP	80.8 (76.4-83.5)#	226.7 (214.4-253.3)#
FibTEM Undiluted control	100.0	NA
+Gelatin 40%	32.5 (27.4-45)# *†+	NA
+Fibrinogen	66.9 (53.0-81.8)#*	NA
+FVIII+vWF	48.5 (37.4-53.7)#*†	NA
+FXIII	46.7 (38.1-69.6)#†	NA
+ FFP	57.1 (47.8-73.1)#	NA

Medians (25th to 75th percentiles) are shown. CFT = clot formation time, FFP = fresh frozen plasma, FVIII+vWF = coagulation factor VIII+von Willebrand factor, FXIII = coagulation factor XIII, MCF = maximum clot firmness, NA = not available.

Student-Newman-Keuls test. *p < 0.05 compared with FFP, †p < 0.05 compared with fibrinogen, +p < 0.05 compared with FVIII+vWF and FXIII, #p < 0.001 compared with undiluted sample

In Studies I and II, Hb concentration, hematocrit, and platelet count were decreased after infusion of HES 130/0.4, HES 200/0.5, and gela-

tin solution, but remained unchanged in the Ringer's and albumin groups. On the first postoperative morning, all these parameters were comparable between the groups, except that Hb concentration in the albumin group was significantly lower ($p = 0.046$) than in the HES 200/0.5 group (Study I).

Effects of colloids and crystalloids on hemodynamics (IV, V)

All colloids raised CI and SVI and maintained hemodynamics successfully in the early postoperative period. The effects of 15 mL kg^{-1} of HES 130/0.4 and HES 200/0.5 on CI and SVI were similar and significantly higher than those of albumin solution immediately after completion of the infusion (in Study IV, Figures 1, 2). In Study V, the effect of HES 130/0.4 on CI and SVI was stronger than that of the gelatin and Ringer's acetate solutions at a dose of 7 mL kg^{-1} . At this stage, no differences emerged in CI and SVI between the gelatin and Ringer's groups. When the dose of colloid increased (14 mL kg^{-1} to 21 mL kg^{-1}), the effect of gelatin on CI and SVI was comparable to that of HES 130/0.4 solution, and superior to that of Ringer's acetate. Ringer's acetate changed neither CI nor SVI. On the first postoperative morning, the hemodynamic values in all the groups were similar (Tables 6, 7). No low-output syndrome appeared postoperatively, and use of vasoactive agents between groups in the studies was similar.

CVP and PCWP were held successfully at target level, and MAP did not differ between the groups during the entire study period. Only after a cumulative dose of 21 mL kg^{-1} , PCWP was higher in the HES 130/0.4 group than in either the gelatin or Ringer's group ($p = 0.03$).

RESULTS

Table 6. Cardiac index after infusion of 7, 14, 21, or 28 mL kg⁻¹ of the study fluid (reprinted with permission from SAGE).

	HES n = 15	GEL n = 14	RIN n = 13	95% CI HES vs. GEL	95% CI HES vs. RIN	95% CI GEL vs. RIN
Pre L min ⁻¹ m ⁻²	2.1 (0.5)	1.9 (0.5)	2.0 (0.7) p = 0.702#	-0.18-0.50	-0.40-0.40	-0.60-0.29
7 mL kg⁻¹ L min ⁻¹ m ⁻²	2.8 (0.6)+	2.4 (0.5)+	2.2 (0.4) p = 0.031#	0.00-0.80*	0.11-1.00*	-0.20-0.50
14 mL kg⁻¹ L min ⁻¹ m ⁻²	3.1 (0.8)+	2.8 (0.6)+	2.2 (0.3) p = 0.002#	-0.20-0.80	0.46-1.30*	0.30-1.00*
21 mL kg⁻¹ L min ⁻¹ m ⁻²	3.4 (1.0)+	2.9 (0.6)+	2.3 (0.53) p = 0.004#	-0.09-1.10	0.50-1.70*	0.20-1.00*
28 mL kg⁻¹ L min ⁻¹ m ⁻²	3.1 (0.7)+	2.9 (0.5)+	2.8 (0.4)+ p = 0.507#	-0.26-0.60	-0.15-0.69	-0,24-0,44

Values are means (SD). CI = confidence interval, GEL = gelatin, HES = hydroxyethyl starch, Pre = before infusion of study solution, RIN = Ringer's acetate.

*p < 0.05, +p < 0.005 in comparison with Pre within group (T-test), #ANOVA test served for comparison between groups

RESULTS

Table 7. Stroke volume index after infusion of 7, 14, 21, or 28 mL kg⁻¹ of the study fluid (reprinted with permission from SAGE).

	HES n = 15	GEL n = 14	RIN n = 13	95% CI HES vs. GEL	95% CI HES vs. RIN	95% CI GEL vs. RIN
Pre mL m ⁻²	25.4 (4.8)	22.0 (5.5)	23.3 (8.6) p = 0.377#	-0.77 - 6.80	-3.10-7.10	-6.30-4.30
7 mL kg⁻¹ mL m ⁻²	34.1 (6.7)+	28.1 (6.2)+	25.8 (7.2) p = 0.006#	1.30-10.70*	2.90-13.10*	-2.90-6.90
14 mL kg⁻¹ mL m ⁻²	37.3 (8.1)+	31.9 (7.2)+	25.4 (4.1) p = 0.000#	-0.60-10.60	7.30-16.70*	2.70-11.30*
21 mL kg⁻¹ mL m ⁻²	42.8 (16.2)+	33.8 (7.3)+	26.7 (6.4) p = 0.004#	-0.20-18.20	7.00-25.00*	2.00-12.00*
28 mL kg⁻¹ mL m ⁻²	36.1 (7.1)+	33.4 (8.0)+	34.2 (7.5)+ p = 0.601#	-2.50-8.50	-3.30-7.30	-6.70-4.70

Values are means (SD). CI = confidence interval, GEL = gelatin, HES = hydroxyethyl starch, Pre = before infusion of study solution, RIN = Ringer's acetate.

*p < 0.05, +p < 0.005 in comparison with Pre within group (T-test), #ANOVA test served for comparison between groups

SvO₂ was higher in the HES 130/0.4 group than in the Ringer's group (Study V), with no difference in DO₂ and VO₂ between these groups.

No differences in acid-base equilibrium appeared between any of the study solutions. SID was slightly higher after infusion of 14 mL kg⁻¹ or more of gelatin solution than it was with HES or Ringer's acetate solutions (Study V).

Blood loss and fluid balance

Chest tube drainage in all groups was similar. None of the single clinical studies detected differences in blood-product use postoperatively, but when Studies I and II were analyzed together, the cumulative use of RBC on the first postoperative morning was higher after the administration of gelatin (1.47 ± 2.0 U/patient) than with Ringer's acetate or albumin ($p < 0.015$). The use of RBC, however, was similar after HES and gelatin administration. Use of FFP or platelet concentrate did not differ between the study groups intra- and postoperatively. The fluid balance, including postoperative crystalloid solutions administered and use of vasoactive drugs, was similar in all study groups. Urine output was higher in both HES groups ($p < 0.035$) than in the Ringer's acetate or gelatin group (Table 8).

RESULTS

Table 8. Postoperative fluid balance and cumulative number of transfused blood products on the first postoperative morning.

	HES 130/0.4 n = 30	GEL n = 15	RIN n = 15	HES 200/0.5 n = 15	ALB n = 15	p all groups
RBC transfusion (U/patient)	0.77 (1.1)	1.47 (2.0)*	0.23 (1.6)	0.87 (0.64)	0.4 (0.63)	0.047
FFP transfusion (U/patient)	0.33 (1.5)	0.13 (0.52)	0.29 (1.1)	0.2 (0.78)	0.0 (0.0)	0.881
PLC transfusion (U/patient)	0.93 (2.5)	1.3 (2.9)	1.1 (4.3)	1.1 (2.8)	0.0 (0.0)	0.717
Ringer's acetate (mL 18 hours ⁻¹)	1628 (1500)	1704 (1077)	1300 (918)	1701 (895)	1620 (820)	0.903
Chest tube drainage (mL 18 hours ⁻¹)	923 (329)	1099 (420)	921 (367)	989 (510)	934 (230)	0.62
Chest tube drainage (mL kg ⁻¹ 18 hours ⁻¹)	11.4 (5.3)	15.0 (6.7)	12.7 (4.3)	11.7 (5.8)	12.8 (5.2)	0.311
Urine output (mL 18 hours ⁻¹)	3732 (1321)†	2923 (1040)	2509 (753)	3919 (1644)†	3143 (782)	0.005

Values are means (SD). ALB = 4% albumin, FFP = fresh frozen plasma, GEL = 4% gelatin, HES = hydroxyethyl starch, PLC = platelet concentrate, RBC = red blood cells, RIN = Ringer's acetate.

One-way ANOVA, and T-test. *p < 0.015 in comparison with RIN and ALB (Bonferroni post hoc test), †p < 0.035 in comparison with GEL and RIN (Bonferroni post hoc test)

DISCUSSION

Colloids and crystalloids after cardiac surgery: coagulation

This study showed that the new HES and gelatin solutions have a comparable effect on whole blood coagulation as measured by modified TEM. HES and gelatin slightly reduced clot strength and prolonged CFT. These changes in TEM, however, were not reflected in postoperative blood loss. Postoperative bleeding on the first postoperative morning was similar in the HES, gelatin, albumin, and Ringer's acetate groups.

Earlier studies have demonstrated that the use of HES solutions with a higher degree of substitution (0.6-0.7) predisposes patients to increased bleeding after cardiac surgery (Wilkes *et al.* 2001, Kuitunen *et al.* 2004). Therefore, HES solutions with lower MW and a lower degree of substitution have been developed (i.e., HES 130/0.4). The coagulation disturbances caused by modern HES solutions do not seem to be related to increased postoperative bleeding after various types of major surgery (Langeron *et al.* 2001, Kozek-Langenecker *et al.* 2008). The present study demonstrated that the effects of HES and gelatin solutions on blood loss are similar. Neither colloid caused increased postoperative chest tube drainage in comparison with Ringer's acetate or albumin. This finding contradicts with results of previous study after cardiac surgery, in which the use of gelatin was associated with lower need for allogenic blood transfusion than did use of HES (Van der Linden *et al.* 2004).

Neither the crystalloid nor the albumin solutions disturbed blood coagulation when used postoperatively, which is in agreement with previous findings. The clot strength in the Ringer's acetate and albumin groups was significantly higher than in the HES or gelatin groups. Despite the slight changes in TEM, the postoperative bleeding was similar in all study groups. Several studies have demonstrated that the use of both modern HES and gelatin solutions is safe in regard to coagulation. Because of the slight but negligible effect on blood coagulation, however, the use of modern HES and gelatin solutions after cardiac surgery must be considered carefully in those patients with hemostatic disturbances.

Mechanisms of colloid-induced coagulopathy

Both HES and gelatin solutions can impair blood coagulation (Wilkes *et al.* 2001, Niemi and Kuitunen 2005). They interact with various coagulation factors, disturb fibrin meshing, and produce hemodilution (Hiippala 1995, Petroianu *et al.* 2000, Boldt *et al.* 2002). The similar impairment of TEM with use of both InTEM and ExTEM reagents suggests that HES and gelatin affect both intrinsic and extrinsic coagulation pathways. They alter the strength of the whole blood clot (decreased MCF), and fibrin formation (prolonged CFT). Therefore, contact activation of blood coagulation is inhibited by colloids, which may be important in the presence of heparin (i.e., during CPB or postoperatively when a residual effect of heparin is suspected). After cardiac surgery, it is also very important to preserve tissue factor-induced coagulation and minimize any risk for increased postoperative bleeding and reoperation.

The decreased MCF with FibTEM reagent demonstrates the impairment of the fibrin component of clot strength, which is in accordance with a previous study (Fenger-Eriksen *et al.* 2009). Therefore, the correction of colloid-promoted coagulopathy by fibrinogen is logical. This idea is also supported by the findings of the *in vitro* part of the present thesis (Study III).

No differences appeared in clot strength between ExTEM and ApTEM reagents, which indicates an absence of abnormal fibrinolysis after the use of colloid solutions. Several studies have reported that both CPB and use of colloids *per se* may activate fibrinolysis (Nielsen 2006), and use of antifibrinolytic drugs has been considered (Sedrakyan *et al.* 2006). The results of the present thesis do not support routine use of fibrinolysis inhibitors in patients undergoing elective cardiac surgery. Furthermore, in one study, tranexamic acid had no effect on HES-induced coagulation disturbance after cardiac surgery (Kuitunen *et al.* 2006). On the other hand, findings of this study could imply that TEM might be not sufficiently sensitive to show mild fibrinolysis.

This study demonstrated that the use of third generation HES, gelatin, albumin, and Ringer's acetate solutions causes no changes in platelet MCF postoperatively. All the platelet MCF parameters were within the normal range. In this study, only those patients undergoing elective surgery were investigated. They had neither any coagulation disturbances nor medications which could elevate bleeding risk within

5 days prior to surgery. Preoperatively, platelet count was also within the normal range. On the other hand, CPB-induced platelet dysfunction can predispose patients to increased bleeding postoperatively. In the present study, neither modern HES nor gelatin altered platelet MCF, even after CPB, which is a very important property of colloid solution administered after cardiac surgery. The minimal effect of HES 130/0.38-0.45 on platelets has also been demonstrated (Franz *et al.* 2001).

The present thesis showed that the colloid solution gelatin impairs blood coagulation *in vitro* after 40 vol% hemodilution. The fibrinogen concentrate improved blood coagulation, as did FFP. Surprisingly, the effect of solely FVIII+vW and FXIII on coagulation impairment was minimal, which is in contrast to previous findings (Nielsen *et al.* 2004, Haas *et al.* 2008). Those *in vitro* studies investigated profound hemodilution (up to 60%), at which point the activity of coagulation factors decreases dramatically. Therefore, the addition of single coagulation factors improved the quality of the clot. It is possible that after 40% hemodilution, which is closer to the clinical situation (i.e., during CPB), the amount of coagulation factors for the clotting process was sufficient, and the effect of administration of solely FVIII+vW and FXIII was minimal.

Colloids and crystalloids after cardiac surgery: hemodynamics

This study demonstrated that the use of rapidly degradable HES solutions after elective cardiac surgery is an effective method to maintain postoperative hemodynamics, which is in agreement with previous findings (Gallandat Huet *et al.* 2000, Verheij *et al.* 2006a, Kuitunen *et al.* 2007). Many studies demonstrate the effectiveness of rapidly degradable HES solutions, but discussion is continuous about the side-effects of these fluids. In the light of recent studies, the effect of HES 130/0.4 on renal function seems to be neutral (Mahmood *et al.* 2007) even in those patients with renal failure (Boldt *et al.* 2007).

Despite its relatively low MW, third generation HES 130/0.4 is an effective plasma expander. In the present study, after the administration of HES solutions, CI and SVI increased significantly, even after a small dose of this fluid (7 mL kg^{-1}), and this effect was greater than

after use of gelatin, albumin, or Ringer's acetate. The greatest effect of the single small dose of HES 130/0.4 (7 mL kg⁻¹) could be explained by the MW of the HES molecule (130 kDa), which is more than four times as high as that of gelatin.

Surprisingly, this study showed that the effect of a single dose of gelatin solution (7 mL kg⁻¹) on hemodynamics was comparable to that of Ringer's acetate. This finding is novel. When gelatin was administered repeatedly, its effect on CI and SVI was more pronounced and, at this stage, it was comparable with that of HES 130/0.4 (Van der Linden *et al.* 2004). After cardiac surgery, patients are usually overloaded with fluids (i.e., CPB priming, vasoactive agents, antibiotics). The power of the single small dose of HES 130/0.4 to raise the CI and SVI is therefore clinically very important.

Additionally, recent studies show that the side-effects of modern HES solutions on renal function, coagulation, and inflammation are comparable with those of the gelatin solution (Mahmood *et al.* 2007, Boldt *et al.* 2008).

The present thesis failed to demonstrate any difference in acid-base status postoperatively between the study groups. Only a slight increase in SID was observable after infusion of more than 14 mL kg⁻¹ of gelatin solution (Study V), and this fact could be explained by the chloride amount's being lower than with the HES or Ringer's acetate solutions. On the other hand, both pH and BE remained unchanged in all the study groups. This slight increase in SID thus had no clinical relevance, even along with a relative large dose of colloids (28 mL kg⁻¹).

Blood loss and fluid balance

The present thesis detected no difference in postoperative bleeding or fluid balance between groups on the first postoperative morning. The similar effect of HES 130/0.4 and gelatin on postoperative blood loss has already been demonstrated (Van der Linden *et al.* 2005). Moreover, the modern HES and gelatin solutions have been proven safe regarding bleeding in comparison with Ringer's or albumin solutions (Tigchelaar *et al.* 1997, Boldt *et al.* 2009).

The findings of a similar fluid balance diverge from those in an investigation (Tølløfsrud *et al.* 1995) claiming that a larger volume of

crystalloids than of colloids is necessary to maintain hemodynamics (CI, PCWP, CVP). The Tølløfsrud group used colloids intraoperatively, and fluid retention was greater in the crystalloid group only at the end of surgery, but not at 48 hours postoperatively. Unfortunately, the investigators did not report the fluid balance on the first postoperative morning. In the present study, a fixed similar dose of study solutions was administered. Therefore, it may have been the case that the fluid balance between these groups was comparable.

Limitations of the study

As to limitations of the present study, it includes a relatively small number of patients undergoing different types of cardiac surgery. However, despite these differences, the patients experienced similar surgical trauma and received similar postoperative intensive care. All the procedures were performed through full median sternotomy using cannulation of the aorta and right atrium / vena cava. Therefore, the condition of these patients perioperatively can be considered comparable.

Because of the small population and short observation time (first postoperative day), this study did not aim to detect differences in clinical outcome.

The limitation of Study III is its lack of standardization of hemodilution. The aim of this model was to achieve conditions that were, as far as possible, comparable to the real clinical situation during the administration of FFP or specific coagulation factors in patients receiving a colloid solution. Both *in vivo* with intact blood vessels and *in vitro*, administration of FFP inevitably results in a lower hematocrit than does the administration of single coagulation factors.

This study did not aim to detect differences in renal or other organ functions. However, in all groups, creatinine levels on the first postoperative morning were similar.

In the present study, the same patient population took part in Studies I and IV, and Studies II and V. The major outcomes were, however, predetermined for each study individually, and the power was calculated for both MCF and CI in these studies. Therefore, it may be justified to report two clinically very important topics in separate stu-

dies. The treatment of coagulation and hemodynamics was intraoperatively similar in all the patients.

Clinical implications

In accordance with previous conclusions, the recommendation is to use the modern HES solutions for intravascular volume replacement in patients after elective cardiac surgery with preserved left ventricular function. HES 130/0.4 is the solution most effective hemodynamically, and any coagulation disturbances it caused were comparable with those of gelatin. However, the effect of a small single dose of gelatin on hemodynamics was significantly inferior to that of HES 130/0.4 solution.

The use of fibrinogen or FFP, but not single coagulation factors (FVIII+vW and FXIII), is recommended for the treatment of colloid-induced coagulopathy.

The use of modified TEM may improve the diagnostics of mechanisms of coagulation disturbances, and TEM is recommended to guide in the treatment of bleeding patients.

CONCLUSIONS

The present study showed that the effects of rapidly degradable HES and gelatin solutions on blood coagulation measured with TEM are similar. After elective cardiac surgery, the single small dose of HES 130/0.4 had a slightly greater effect on hemodynamics (CI and SVI) in comparison with gelatin. Specifically it was demonstrated that

1. Modern HES and gelatin solutions impaired TEM, but the changes in blood coagulation did not elevate postoperative bleeding, based on similar postoperative chest tube drainage. The effect of the natural colloid 4% albumin and crystalloid Ringer's acetate on blood coagulation was minimal.
2. Neither HES nor gelatin impaired platelet function nor induced fibrinolysis after elective cardiac surgery.

Fibrinogen concentrate improved gelatin-induced coagulopathy *in vitro*, but effects of FVIII+vW and FXIII on coagulation changes detected by TEM were minimal.

3. A single dose of HES solution had a slightly greater immediate effect on CI and SVI than did gelatin, albumin, or Ringer's acetate solutions even at a small dose (7 mL kg^{-1}). At this dose, the effect of gelatin solution on hemodynamics was lower than that of HES, and it was comparable to that of Ringer's acetate. With repeated administration of gelatin at doses of 14 mL kg^{-1} or more, its effect on CI and SVI began to be comparable to that of HES 130/0.4.
4. None of the study solutions changed acid-base equilibrium clinically significantly.

On the first postoperative morning, both hemodynamics and TEM parameters were similar in all the patients independent of the fluid used.

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