Effect of a Histidine-Tryptophane-Ketoglutarate solution on coagulation as measured by a Thromboelastogram

A Dissertation

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1 DECLARATION

I, Kasandji Freddy Kabambi, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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2 CERTIFICATE OF APPROVAL

The undersigned have examined the dissertation entitled '**Effect of Histidine-Tryptophan-Ketoglutarate on coagulation as measured by a thromboelastogram**' submitted by **Dr Kasandji Freddy Kabambi**, and hereby certify that it is worthy of acceptance and approve that the above mentioned has fulfilled part of the requirements for the award of the degree of **Master of Medicine in Anaesthesiology** by the University of Cape Town.

Date	1st Examiner
Date	2nd Examiner

3 ABSTRACT

Background

The Histidine-tryptophan-ketoglutarate (HTK) solution is cardioplegic solution that confers myocardial protection during periods of ischemia in heart surgery. It has the advantage of allowing a longer protection and it is especially the preferred cardioplegic solution in complex paediatric heart surgery like transposition of great vessels. This has also been used in organ transplant as a preservative solution. Recently concern has been raised over its safety because of the increased incidence of transplant failure due to thrombosis in individuals whose transplanted organ were preserved with HTK solution.

The main purpose of this study was to establish whether the HTK solution in the dose and volume used for myocardial protection during paediatric heart surgical procedures increases the thrombotic risk of patients.

Patient and methods

This study was an experimental study conducted at the coagulation laboratory of the department of anaesthesia of the University of Cape Town. The human research ethical committee of this university approved this study. Twenty healthy individual were recruited for this study and each one of them read and signed the consent form before being enrolled for the study. The exclusion criteria were any known coagulopathy, liver diseases, alcoholism, kidney disease and intake of any drugs known to impair the coagulation. Ten millilitre of blood was drawn from the volunteer at the coagulation laboratory. The blood was diluted with either HTK solution or St Thomas' cardioplegic solution at 9:1 ratio to mimic the hemodilution due to HTK solution in paediatric population. The effect on coagulation was assessed by means of thromboelastography run on the native blood and each dilution separately.

Results

The difference in the magnitude of change from baseline in r-time between the HTK solution and Saint Thomas' solution (mean difference 0.49 min) (p 0.014) was statistically significant. There was no statistically significant change in MA between the HTK solution and St Thomas' solution (mean difference -2.13 mm) (p 0.165). Compared to native blood there were no significant change in r-time with the HTK solution (mean -0.215 min), the same with St Thomas' solution. Compared to native blood, there was significant change in maximum amplitude with the HTK solution (mean 1.38 mm) the same with St Thomas' solution (mean 3.51). Although the difference in the magnitude of in R time change between HTK solution and St Thomas' was statistically significant, it is clinically not relevant. The data did not show a trend that might become significant with large sample. All variables showed a slight tendency towards decreased coagulation in the diluted samples, but not of sufficient magnitude to be clinically important. Our goal was the use of a dilution that is clinically relevant in the context of paediatric cardiac surgery.

Conclusion

The results of this study suggest the HTK solution at 10% dilution does not cause significant changes in coagulation parameters. In comparison to normal saline, there were a tendency toward hypocoagulation. More research in this field is needed to clarify the pathophysiologic pathways of thrombosis observed in these transplants.

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5 DEDICATION

I dedicate this dissertation to my father, Kasanji Maurice Lembalemba, who did not live long enough to witness this day. To my mother, Madeleine Muadi Tshimbalanga, suffering with a terminal illness when the final words of thesis are being written; You were deprived of education but you believed in it so much that you deprived yourself of everything to ensure you that your children get the best education under the sun. To my spouse Liliane Odia Kabambi, always on my side during times of joy and hardship. And finally to my son, Kaniama Nathan Kabambi, the most efficient antidepressant on earth, may this achievement remind you all the time only sky should be your limit. My love for you is unconditional.

6 ABREVIATIONS

AT III Antithrombin III

CABG coronary artery bypass grafting

CK Creatine Kinase

CPB Cardiopulmonary Bypass

HTK Histidine-Tryptophan-Ketoglutarate

ICU Intensive Care Unit

NYHA New York Heart Association

ROTEM Rotational Thromboelastometry

TAPSE Tricuspid Annular Plane Systolic Excursion

TEG Thromboelastography

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8 PART A: RESEARCH PROTOCOL

Effect of a Histidine-Tryptophan-Ketoglutarate solution on coagulation as measured by a Thromboelastogram.

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8.1 Background

Myocardial protection during the period of ischemia that is associated with open heart surgery is of great concern, since ischemia and the subsequent reperfusion injury may have deleterious effects on myocardial function. During ischemia, cell storage of glycogen and high energy molecules decreases, and acidosis follows. Myocardial protection during the period of ischemia involves the application of techniques designed to protect the heart from injury.

Since its introduction to clinical practice in the mid-1960s, the frequently used and well established technique for myocardial protection during the period of ischemia is the pharmacologically induced and reversible electromechanical diastolic cardiac arrest through the infusion of cold crystalloid cardioplegic solutions(Mirsad Kacila et al., 2006).

The cold crystalloid cardioplegic solution protects the myocardium by means of hypothermia and electromechanical arrest. In so doing, it reduces the myocardial metabolic demand and improves its tolerance towards ischemia and reperfusion injury(Mirsad Kacila et al., 2006). The reduction of metabolic demand preserves myocardial energy molecule reserves and prevents cell membrane disruption, osmotic shift and electrolyte imbalance.(Careaga et al., 2001).

Cardioplegic solutions can be divided into two main groups: the extracellular group (e.g. St Thomas' solution) mirrors the extracellular fluid and contains a high concentration of sodium, calcium and magnesium, whereas the intracellular group (e.g. histidine- tryptophan-ketoglutarate solution) mirrors the intracellular fluid and contains a low concentration of sodium and calcium. Both types have a high concentration of potassium. Both have demonstrated beneficial effects in animal and human models looking at biochemical markers of ischaemia, and there is some evidence that HTK solution may be a superior myocardial protective agent (Careaga et al., 2001).

The histidine- tryptophan-ketoglutarate (HTK) solution was proposed by Bretschneider in the 1970s as a solution for cardioplegia. It is based on intracellular electrolyte composition, to which histidine, mannitol, tryptophan and ketoglutarate have been added. Histidine acts as a potent buffer and delays the reduction of the pH in the extracellular milieu, which is caused by an accumulation of metabolites. Tryptophan acts as a membrane stabilizer, and ketoglutarate acts as

an intermediary in the Krebs cycle and improves anaerobic metabolism for energy production. Mannitol maintains osmotic balance across the membranes and prevents cell oedema. It also acts as a free radical scavenger(Kober et al., 1998).

Several studies have highlighted the efficacy of the HTK solution based on biochemical markers and physiological evaluation in an experimental model and in trials testing clinically relevant outcomes in an adult population (Careaga et al., 2001). Alonso de Begonia et al found that compared to other solutions, the HTK solution provided the best results in terms of hemodynamic and biochemical parameters (Alonso de Begona et al., 1993). Furthermore, HTK solution has been shown to be effective in preventing low cardiac output states, arrhythmias and reducing length of stay in ICU (Careaga et al., 2001).

The HTK solution has an additional advantage of prolonged myocardial protection after a single dose. In lengthy complex paediatric heart surgery, administration of multiple doses of cardioplegia prolongs surgery, increases cellular oedema and therefore myocardial damage (Liu et al., 2008).

The HTK solution is associated with shorter aortic cross-clamping time compared to other solutions. This may shorten the duration of myocardial ischemia and furthermore reduces the frequency of cardioplegia administration. The HTK solution is also used as an organ preservative for organs harvested for transplant (e.g. heart, kidney, pancreas etc.)(Nardo et al., 2005, Agarwal et al., 2006, Feng et al., 2006, Moray et al., 2006, Alonso et al., 2008).

Although the use of HTK is assuming a growing role in cardiac surgery, it has been associated with an increased incidence of graft thrombosis after liver and pancreatic transplants on patients in whom the HTK solution was used as a preservative solution (Viana et al., 2013).

Although there is sufficient evidence in the literature regarding the efficacy of the HTK solution with regard to myocardial protection during the period of ischemia, questions remain unanswered regarding its effect on coagulation. This raises the question of patient selection, as well as the preoperative and postoperative work up on patients in whom the use of HTK is contemplated. Cardiac surgery and cardiopulmonary bypass with the use anticoagulants has multiple effects on coagulation which further confuses the issue. The influence of these factors

on coagulation is well known and documented (Bochsen et al., 2009, Lison et al., 2011, Edelman et al., 2014).

Ruttmann et al have shown that hemodilution using a saline solution induces a hypercoagulable state (Ruttmann and james, 1996). To the best of our knowledge this question has not been the subject of any specific research thus far.

The aim of this study is therefore to assess the effect of the HTK solution on coagulation, as measured by a thromboelastography (TEG) in vitro.

This study aims to determine the effects of HTK on the following TEG parameters:

-reaction time(R)

-clot kinetic time (K)

-angle of clot kinetic

- clot strength (maximum amplitude)

- clot firmness (G)

-lysis time (ly 60).

8.2 Hypothesis

The hypothesis of this study is that the histidine-tryptophan-ketoglutarate (HTK) solution has no effect on coagulation.

8.3 Methodology

Design and Setting

This is an experimental study that will try to replicate the model of dilution due to cardioplegia in a paediatric patient. The study is to be conducted at a tertiary level university hospital.

To create an in-vitro model of dilution due to infusion cardioplegia in a paediatric patient, the whole blood will be diluted to a 9:1 volume ratio with a HTK solution. The dilution of whole blood by 10% volume of HTK solution was selected with the help of the perfusionist at Red Cross War Memorial Hospital as this the typical degree of dilution that is used. It has been established that the dilution due to cardioplegia infusion (here the HTK solution), calculated using total volume (patient volume, circuit volume) haematocrit pre-first dose and the total volume haematocrit post-first dose, can range between 5 and 10% blood volume.

The sample test will be diluted in a similar way with Saint Thomas' cardioplegic solution and subjected to the thromboelastograph (Model 5000, Haemoscope Corp., and Skokie, IL). The Saint Thomas' cardioplegic solution will serve as control. This will help determine the intrinsic role of HTK solution in the enhancement of coagulation if any. It is not a common practice in our centre to suction the Saint Thomas' cardioplegia out of circuit (cardiopulmonary circuit). In doing so, the dilution with Saint Thomas' cardioplegic solution is greater than with HTK solution. Therefore, the 10% dilution that we will use in this study is not out of proportion from what is seen in clinical practice.

Patient Eligibility

Healthy adult volunteers with no morbid conditions and not taking any medication or supplements known to impair coagulation or platelet function (e.g aspirin, heparin, warfarin, dabigatran) will be considered for this study. Any person with known coagulopathy, renal impairment, liver impairment or history of alcohol abuse will be excluded from the study.

Blood Collection

After signing an informed consent form, 10ml of blood will be drawn into citrate-containing tubes and sent immediately to the coagulation research laboratory at D23 at Groote Schuur Hospital

Blood Processing

A TEG (Model 5000, Haemoscope Corp., and Skokie, IL) machine will be utilised to analyse TEG blood samples. The TEG analyzer will be warmed to 37°C. Biological QC I and Biological QC II assays (Haemoscope Corp.) will be performed on the morning of the tests to ensure quality

control. TEG sample analysis will commence within 4 minutes of taking the samples. The TEG result will be analysed by TEG software (version 4.2.3). Results will be read from the software. [12]. A total of 360µl of donors' blood (the native blood or diluted blood) will be mixed with 10 µl of Rapid TEG reagent (Haemoscope) and placed into TEG cups. Clotting will be triggered by the addition of 20µl of 0.2 mole /l $CaCl_2$.

The blood will be drawn into a citrate-containing tube the thromboelastograph (TEG) will be performed on the native blood without dilution and the TEG parameter: R time, alpha angle, K time, maximum amplitude (MA), clot strength (G) and lysis time will be recorded. Thereafter, the dilutions of the whole blood with HTK and Saint Thomas' solution will be used. Firstly, a 10% dilution with HTK solution and TEG will be run on that sample and the following TEG parameters will be recorded: reaction time (R), clot kinetics time (K), angle of clot kinetics (alpha), maximal amplitude (MA), clot strength (G), and percentage of lysis at 30 min (LY30). Secondly, a 10% dilution with Saint Thomas' solution will be used and a TEG will be run on that sample with the same TEG parameters being recorded. This means that in this study, from each volume of blood drawn from the volunteer, three samples will be obtained: the native blood, 10% volume dilution with HTK solution and 10% volume dilution with Saint Thomas' solution.

Data Collection

All data pertaining to a participant will be collected on a standardized form. The following data will be collected: name, age, weight, any medication with potential to affect coagulation (aspirin, warfarin, heparin, dabigatran…), TEG coagulation indices (R and K times, alpha angles, maximum amplitude, LY 30 and G values (which measure the shear strength of the clot).

Primary outcome

-The difference in the magnitude of change in R time and maximum amplitude from baseline between the HTK solution and the Saint Thomas' solution.

Secondary outcomes

-The magnitude of change in R time and maximum amplitude caused by the HTK solution.

-The magnitude of change in R time and maximum amplitude caused by Saint Thomas' solution.

Statistical Analysis

We believe that a 50% drop in R time or 50% increase in maximum amplitude (MA) will be clinically significant for a mean R time of 6 and standard deviation of 2.8, a sample size of 20 is needed to sufficiently power the study. The analysis will be done using the statistica software package. The sample size is in keeping with previous studies done by Ruttmann and James. All the tests will be performed on each of the 20 blood samples drawn from the patients participating in this study, namely the native blood and two dilutions. Data will be collected using a specially designed form and will be saved onto a computer using Microsoft Excel software.

Data will be expressed as mean and standard deviation. The native blood and each dilution will constitute one group each and the student t- test will be used to determine the difference between the dilutions with HTK solution and Saint Thomas' solution group. The significance level of 0.05 will be used to detect statistically significant differences between groups. The statistical analysis will be carried out using statistica software.

8.4 Dissemination of Results

This study will form the basis for the principal researcher's Master of Medicine (Mmed) dissertation. The results will be presented at the Department of Anaesthesia's meeting and the Society of Anaesthesiologists of South Africa. This study will also be submitted to a peer reviewed journal for publication.

8.5 Ethical Considerations

This study is experimental research in the laboratory environment conducted on the blood taken from healthy volunteers. The dilutions will be done in vitro. Before commencing with this study, the researchers will seek the approval of the ethical committee of the University of Cape Town. No ethical challenges are foreseen, given that this study does not expose the participant to known risks.

Permission to collect data

This research protocol will first be submitted to the ethics committee of the University of Cape Town for approval. After approval by the ethics committee, permission to conduct the study will be obtained from the Groote Schuur Hospital Medical Superintendent.

Patient confidentiality

Each individual participating in the study will be given a unique study number. Demographic data and personal identification data gathered on each individual will be kept confidential, unless otherwise required by the law. The data collected is the property of the University of Cape Town and will remain on the premises of the university.

Data storage and record safekeeping

The data collected will be entered in an electronic spreadsheet and protected by a password. Only the principal investigator will have access to the password. The data collection sheet will be kept in his locker, which is protected by a key. After the completion of the study, the data collection sheet will be sent to the Department of Anaesthesia for safekeeping.

Informed consent

Each participant will be required to sign an informed consent form. The format of the consent form can be found in the annexure of this protocol. The interviews will be conducted in English, and the informed consent form will include a detailed description of the whole process involved in this study.

Benefit and risk assessment

The individuals participating in this study will risk no harm other than providing a blood sample. The blood will be collected by a qualified member of the anaesthetic department. There is no direct benefit to the patient. Participants in this study will receive no compensation. Rather, it is

envisaged that this study will be of benefit to future patients undergoing cardiac surgery, as surgeons will have a better understanding of the effect of this solution on coagulation.

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9 PART B: LITERATURE REVIEW

9.1 Objectives of the Literature Review

The goal of this literature review, done before drafting the research protocol for this study, was to inform me of the amount of evidence in the literature about the efficacy and adverse effect of HTK solution as a cardioplegic and organ preservation solution, as well as guide me in terms of the best way of conducting this experimental study. The objective was to determine whether or not the HTK solution has a deleterious effect on the coagulation system when used as a cardioplegia solution**.**

9.2 Literature search strategy

For the purpose of greater efficiency during this literature review, I subdivided my search of the literature into three themes as the initial search of the literature combining all the keywords relevant to my study did not yield anything. The themes were

- The HTK solution in myocardial protection during heart surgery
- The HTK solution as preservative solution for organ transplant
- Thromboelastography as a tool to assess effect of hemodilution on coagulation

9.2.1 The HTK solution in myocardial protection during heart surgery

I searched PubMed in January 2015. I used the medical subject heading (Mesh) database to look for the synonyms of the following keywords: Custodiol, heart surgery, myocardial protection, and thrombosis. The combination of the keywords “Custodiol and heart surgery” in PubMed yielded 215 articles. The addition of myocardial protection to the previous combination reduced the number of articles to 78, which I found reasonable to start with. The addition of the keywords “hemodilution”, “thrombosis” and “coagulopathy”, which are related to the complications that may arise, to the search above separately yielded zero articles respectively. This showed me that this is an area that has been poorly researched. I therefore decided to keep the 78 articles as the basis for further selection. I first filtered the articles, removing all studies done on animals, after which 33 articles remained. These articles were then saved on the clipboard of my NCBI account. I then read through the articles and abstracts, discarding articles investigating the effect

of substances other than the HTK solution, and studies investigating robotic surgery. I retained randomised controlled trials and review articles, which resulted in a final count of 26 articles.

9.2.2 The HTK solution as preservative solution for organ transplant

I searched the Mesh database using the keywords “Custodiol”, “organ preservation”, and “organ transplant”. I then used these keywords in PubMed for the literature search. The combination of Custodiol, organ preservation and organ transplant yielded 328 articles. The addition of the keyword “complication” to this previous combination reduced the articles to 37. I found this restriction to be reasonable, as I am interested in the complications arising from the use of HTK solution for organ preservation. The addition of “thrombosis” and “coagulopathy” to the search yielded 1 and 0 articles respectively. I therefore decided to keep the 37 articles for further scrutiny. After removing all the articles reporting on animal studies, and keeping only human studies, I was left with 18 articles, which I saved onto the clipboard.

9.2.3 Thromboelastography as a tool to assess effect of hemodilution on coagulation

For this last theme of my literature search, the keywords “thromboelasto*” and “hemodilution” were used. This initial search yielded 55 articles. The addition of “in vitro” to the search reduced the initial search results to 26. I found this to be relevant because my study is experimental and in vitro. I read through the titles and abstracts of the articles, and then discarded the animal studies and those dealing with ROTEM rather than thromboelastography (TEG) as a means of investigating coagulation. I also discarded the articles investigating other substances and which were not related to hemodilution. After this selection, I found 4 articles to be worth including in my literature review, and I saved them onto my clipboard. I then downloaded all these articles into the Endnotes X7.2.1 citation manager, placing them in a separate folder.

9.3 Quality criteria

Good literature reviews and well-designed experimental studies on the topic of myocardial protection during cardiac surgery and organ preservation for transplantation were found to be the

most useful. Studies reporting on the effect of dilution of blood with different fluids were also found to be useful, especially in the design of this study.

9.4 Summary and interpretation of literature review

9.4.1 The HTK solution in myocardial protection during heart surgery

The HTK solution has been investigated in the areas of coronary artery bypass graft (CABG), valvular heart disease, minimally invasive surgery, and paediatric cardiac surgery

In the case of CABG surgery, Hjelms et al. conducted a study of patients undergoing an elective CABG, with cold HTK solution in one group and intermittent aortic cross-clamping at 30 degrees in the other group as control. The HTK solution showed more consistent results with lower CK-MB elevations, both in terms of peak values and areas under time-enzyme activity(Hjelms and Steiness, 1982). These effects have been reported in cases where the HTK solution was used in high doses and for lengthy surgery. However, Arslan later found that even low dose HTK is protective in CABG surgery, provided that there is a short clamping time (Arslan et al., 2005). While the results of investigations of the HTK solution versus other crystalloid solutions have generally favoured the HTK solution, the comparison with blood cardioplegic solutions has not been that conclusive. Beyersdorf et al randomised 37 patients undergoing coronary revascularisation into 3 groups: the hypothermic ventricular fibrillation group, the intermittent multi-dose group, and the single dose HTK solution group. They found intermittent blood cardioplegia to be better than hypothermic ventricular fibrillation and the HTK solution in terms of preservation of high energy phosphate molecules, incidence of rhythm disturbance, release of serum enzyme (CK-MB) and functional recovery (Beyersdorf et al., 1990).

In the case of valvular heart disease surgery, Schaper et al., in a seminal study, compared the quality of myocardial protection offered by four cardioplegic solutions (Kirsch, Bretschneider, St. Thomas'' Hospital, and Hamburg) in patients undergoing aortic valve replacement and coronary artery bypass graft (CABG) (Schaper et al., 1986). Though they found that the Kirsch

cardioplegic solution did not offer adequate myocardial protection and that none of the solution prevented ischemic injury completely, the HTK solution, along with St Thomas' and Hamburg solution, offered satisfactory myocardial protection. Sakata et al. investigated the HTK solution in the case of mitral valve replacement (Sakata et al., 1998). Among patients undergoing mitral valve replacement, they compared HTK solution and intermittent blood cardioplegia. Their study found that the HTK solution group had a high incidence of spontaneous defibrillation and low need for temporary pacing post-bypass. They concluded that the HTK solution provided more adequate myocardial protection than the cold blood cardioplegia solution in mitral valve replacement. Braathen later found the HTK solution to be as protective as the cold cardioplegia in mitral valve surgery (Braathen et al., 2011). In contrast to the above authors, Gaudino et al. found that the subset of patients with prior right ventricle dysfunction, as assessed by a tricuspid annular plan systolic excursion (TAPSE) < 15, did worse with the HTK solution than warm blood cardioplegia in terms of indexes of right ventricle function (RV ejection fraction, RV end diastolic volume). They were of the view that warm cold cardioplegia is more protective that the HTK solution in cases of mitral valve replacement surgery in patients with prior preoperative right ventricle dysfunction (Gaudino et al., 2013). In light of this, one needs to appreciate that this particular study assessed patients in advanced stages of disease process. RV dysfunction in the setting of mitral valve disease entails a long-lasting process with the high likelihood of RV hypertrophy and diastolic dysfunction. Ventricular hypertrophy is known to put the myocardium at high risk of ischemic injury during periods of cross-clamping (Schaper et al., 1986, Scheld et al., 1985).

Even in minimally invasive cardiac surgery, the HTK solution provided a safe and long cardioplegic arrest time, without any significant change in the electrocardiogram and release of cytonecrosis enzymes (Savini et al., 2005). However, this study is a retrospective study using a very small sample size with no comparative arm. Careaga focused on clinically relevant outcomes, such as the following: incidence of arrhythmia, inotropic support, and length of stay in the intensive care unit. In this randomised trial, they concluded that HTK decreased the incidence of arrhythmia, inotropic support and length of stay in intensive care unit (Careaga et al., 2001). Viana found the HTK solution to be as safe as tepid blood cardioplegia (Viana et al., 2013).On the other hand, it has been proven that the susceptibility to ischemic injury is correlated to

ventricular mass (Schaper et al., 1986, Scheld et al., 1985). A hypertrophied ventricle (aortic stenosis) is more likely to suffer an ischemic injury during cardiopulmonary bypass than a normal size ventricle. Scheldt found that provided the regional myocardial temperature was kept below 15 degrees, the HTK solution offered adequate myocardial protection to the hypertrophied myocardium (Scheld et al., 1985).

Zhidkov compared the HTK solution and Konsol solution in patients undergoing aortic and mitral valve replacement (Zhidkov et al., 2006), and found no significant difference in basic hemodynamic parameters between the two solutions.

In the field of paediatric cardiovascular surgery, Korun et al. recently investigated the efficacy and safety of the HTK solution compared to St Thomas' solution in children undergoing surgery for congenital heart defects, taking into account apoptotic and proliferative indices analysed histopathologically, as well as clinical variables, (Korun et al., 2013). They found no difference between the two solutions. Interestingly, Liu came to the same conclusion in a retrospective study, and was also able to show that the HTK solution was associated with less cross-clamping time compared to the St Thomas' solution (Liu et al., 2008). Bojan, however, compared the HTK solution and cold blood cardioplegia, and found that cold blood cardioplegia offered better protection than the HTK solution in neonates undergoing complex cardiac surgery (Bojan et al., 2013).

Compared with hypothermic intermittent aortic cross-clamping with myocardial fibrillation, cardioplegic arrest with HTK offered more beneficial effects in procedures with prolonged ischemia (Sunderdiek et al., 2000). Similarly, Braathen found that a single dose of HTK solution was as protective as repetitive cold blood cardioplegia infusion for more than two hours of ischemia (Braathen et al., 2011).

9.4.2 The HTK solution as a preservative solution for organ transplant

From its beginning until today, the field of organ transplant has been blessed with many innovations and technological advances, which have resulted in the expansion of the pool of donors. On the other hand, the demand for more organs still outweighs the supply. For this reason, researchers are looking for ways to increase the number of organs available for transplant

(Voigt and DeLario, 2013). Various preservation solutions are used for solid organ transplants, and their effectiveness is measured by their ability to preserve the organ and the graft survival rate. The most popular preservation solutions used for organ preservation are the University of Wisconsin solution and the HTK solution. The Celsior solution is currently being used more often and could in the near future be an alternative to these solutions. Each solution has its advantages and disadvantages.

With regard to heart transplantation, Reichenspurner retrospectively analysed the data of 160 cases of heart transplants, where the hearts were preserved with UW solution or HTK solution. They found that the 2 groups showed similar results in terms of postoperative exercise tolerance, as assessed by their New York Heart Association (NYHA) status and incidence of arrhythmia. Myocardial preservation with the HTK solution provided satisfying functional results, as long as the ischemic time did not exceed four hours (Reichenspurner et al., 1994). In hepatic transplantation, it is generally accepted that there is are less biliary complications with organs preserved with HTK solution, due to its low viscosity compared to the UW solution. Nardo, in a randomised controlled trial, compared the graft and patient survival in recipients of livers preserved with the HTK solution or with Celsior. They found that the 1-year graft and patient survival was high in the Celsior group. This was a preliminary report of a randomised, multicentre trial, highlighting the result from a single centre. To make a definitive conclusion in this regard, one needs to wait for the completion of the trial (Nardo et al., 2005). Moench found that the ischemic type of biliary complications occurred at the same frequency in HTK-preserved livers as in the case of UW- preserved livers (Moench and Otto, 2006). Although this was a retrospective study, it casts a doubt on the theory that the low viscosity of the HTK solution makes one less prone to an ischemic type of lesion. Meine et al., in a prospective study, randomised livers of deceased donors into a UW solution group and HTK solution group, and found no difference in terms of biliary complications and incidence of graft dysfunction (Meine et al., 2006,). Other authors also found no difference between the two preservation solutions (Meine et al., 2006, Moray et al., 2006, Rayya et al., 2008). However, Mangus, in a study of a large retrospective cohort (n = 698) of standard criteria donor (SCD) and extended criteria donor (ECD) livers at a single centre over 5 years, found the HTK solution to offer better protection

against biliary complications than the UW solution (Mangus et al., 2008). In the setting of kidney transplants, Klaus, in a randomised prospective study, compared the HTK solution and Belzer solution for cadaveric kidney perfusion. They found that the one year graft and patient survival rate was the same for both solutions (Klaus et al., 2007).

While some authors found the HTK solution to be a suitable replacement for the UW solution in the preservation of a pancreas for transplantation (Agarwal et al., 2008), others have cautioned against this enthusiasm, quoting the increased incidence of acute rejection and pancreatitis in pancreas preserved with HTK solution (Alonso et al., 2008). The close analysis of cases of acute rejection pointed to graft thrombosis as the cause. Stewart et al., analysing the united network for organ sharing database of pancreas transplants for a four year period, found that HTK preservation was independently associated with increased risk of graft loss (Stewart et al., 2009). This risk of arterial thrombosis has also been reported recently in a setting of a liver transplant from a deceased donor, even though it did not reach a level of statistical significance (Gulsen et al., 2013). A critical review of the literature thus far would seem to indicate that the HTK solution is effective as an organ preservation solution for solid organ transplants. The report of increased risk of thrombosis is worrisome, however, and warrants further investigation to determine if there is a causal correlation in this regard.

9.4.3 Thromboelastography as a tool to assess the effect of hemodilution on coagulation

It has been suggested that the dilution of blood with saline increases coagulation (Tocantins et al., 1951). Ruttmann and James found this effect to be more pronounced with moderate saline dilution than with colloid dilution (gelatine) for the same concentration. In this experiment, using the TEG as a tool to measure coagulation, the authors found normal saline to induce a hypercoagulable state at 20% dilution (Ruttmann and James, 1996). These results were later confirmed by Egli et al., who found a hypercoagulable state with normal saline at 30 %, but at the same dilution found hypocoagulability with the colloid (Egli et al., 1997). However, Mortier et al., using extreme dilution (50%) with different colloids: hydroxyethyl starch 6%, gelofusine, and dextran 40 found a decrease in R time, which meant an increased coagulability in a solution diluted with hydroxyethyl starch and gelofusine, though dilution with dextran 40 caused extreme hypocoagulability with the colloid. There was a decrease in clot formation rate and maximum

amplitude with the hydroxyethyl starch dilution, while no significant change was noticed with gelofusine (Mortier et al., 1997). It is evident from the review of literature that the dilution with normal saline induces a hypercoagulable state. This enhancement of coagulation is not limited to normal saline, as crystalloid used for priming the cardiopulmonary bypass machine (Ringers lactate) has shown similar results (Srivastava et al., 2008). While some studies have shown a moderate increase in the coagulability of blood with moderate dilution with colloid (Ruttmann and James, 1996), other authors are of the view that moderate and extreme dilution with colloid induces the hypocoagulability of blood (Egli et al., 1997).

In the case of in vivo studies, some authors questioned the hypothesis that fluid induces hypercoagulability, as the scenario is observed in the control group that did not receive fluid challenge (Gorton et al., 2000). However, other authors found the enhancement of coagulation produced by crystalloid to precede the onset of surgery, and colloids not to induce hypercoagulability (Ruttmann and James, 2002). From the review of the literature thus far, it appears that the infusion of crystalloids and the hemodilution that ensues causes some degree of enhancement in coagulation. However, the extent of this is dependent on the amount of hemodilution and the specific fluid that is utilised, based on the colloid having less of an effect than crystalloids.

The question as to what could explain these findings has been the focus of many experimental and clinical studies, which have aimed at exploring this dilemma of some acellular fluid causing the hypercoagulability of blood. Ruttmann et al. (2001) investigated the hypothesis that the enhancement of coagulation seen in hemodilution is mediated through an imbalance between the procoagulant and anticoagulant system. In this specific study, it was found that the correction of antithrombin level to the predilution level corrected the decrease in R time (Ruttmann et al., 2001). Similarly, other authors using antithrombin deficient plasma found that dilution did not cause the enhancement of coagulation in this setting (Nielsen et al., 2004). The lack of enhancement in coagulation found with hydroxyethyl starch could be explained by the fact that HES does not affect the ability of antithrombin to inhibit clot initiation (Nielsen, 2005). In the clinical setting, there has been found to be an inverse correlation between increasing onset of coagulation and decreasing activity of antithrombin with dilution (Ruttmann et al., 2001)

9.5 Areas for further research.

The literature review shows that infusion of crystalloid enhances the onset of coagulation. In the case of cardiac surgery, the priming of the cardiopulmonary (CPB) circuit with crystalloid is a standard procedure. It follows that the hemodilution of blood subsequent to the initiation of CPB enhances blood coagulation, as demonstrated by Srivastava et al. (Srivastava et al., 2008). However, there are many confounding factors to look for in the setting of cardiac surgery, because the CPB machine itself and the drugs used preoperatively and intraoperatively have an influence on the coagulation system. It would be therefore understandable that the infusion of another crystalloid in terms of the cardioplegia solution would be an additive confounder in the setting of this complex relationship between drugs, fluid, and machine, thereby affecting the coagulation system.

The HTK solution is a cardioplegia solution which already has a long history in cardiac surgery. Its efficacy in terms of myocardial protection during periods of ischemia is well documented. It has also been extensively used for organ preservation prior to transplant. Concerns have been raised recently over the increased risk of thrombosis in organs preserved with the HTK solution. Thus far, however, studies investigating its impact on the coagulation system in the setting of cardiac surgery are lacking.

Thromboelastography (TEG) is a technique for the assessment of coagulation using its viscoelastic properties, and was developed by Hartert in 1948. It had limited clinical use for many years, until 1974. Since then, it has been improved, applied and validated in liver transplants and cardiac surgery. Hemodilution is encountered in clinical medicine in different settings. The effect of the hemodilution of blood crystalloids or colloids on coagulation has been extensively studied using the TEG as a tool of assessment. In the literature, however, studies assessing the effect of a cardioplegia solution in general or the HTK solution in particular on the coagulation system could not be found. The HTK solution differs from other crystalloids used for replacement and as a cardioplegic solution because of its unique composition mimicking the intracellular milieu. Whether or not this difference will be enough to prevent it from enhancing

coagulation as much as the other crystalloids is yet to be demonstrated. The aim of this study is to attempt to fill this knowledge gap.

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10 PART C: PUBLICATION-READY MANUSCRIPT

10.1 TITLE PAGE

The effect of Histidine-Tryptophan-Ketoglutarate solution on coagulation as measured a thromboelastogram

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10.2 ABSTRACT

Background

Histidine-tryptophan-ketoglutarate (HTK) solution is a cardioplegic solution and confers myocardial protection during periods of ischaemia in heart surgery. It has the advantage of providing longer myocardial protection and is often the preferred cardioplegic solution in complex paediatric heart surgery, such as the transposition of great vessels, as it allows for a longer cross-clamp time. It has also been used in organ transplantation as a preservative solution for liver, kidney and pancreas transplantation. Recently, concern has been raised over its safety, due to the increased incidence of transplant failure caused by thrombosis in individuals whose transplanted organs were preserved with HTK solution. Though many studies have used the thromboelastogram (TEG) to investigate the effects of other fluids (crystalloid and colloids) on coagulation, no study has yet investigated the effects of cardioplegic solutions on coagulation.

The purpose of this study was to determine whether or not the HTK solution in the dose and volume used for myocardial protection during paediatric heart surgery increases the thrombotic risk of patients.

Methods

Twenty healthy individuals were recruited, and each one read and signed the consent form before being enrolled for the study. The exclusion criteria were any known coagulopathy, liver disease, alcoholism, kidney disease and the intake of any drugs known to impair coagulation.

Ten millilitres of blood were drawn from the volunteers at the coagulation laboratory and divided into three samples. One sample was left undiluted and the two others were diluted with either HTK solution or St Thomas' cardioplegic solution, which served as a control, at a ratio of 9:1. This ratio was chosen to mimic the hemodilution due to HTK solution in the paediatric population undergoing cardiac surgery. The effect on coagulation was assessed by means of a thromboelastograph performed on the native blood, as well as the diluted sample. It was postulated that a 50% drop in R time or 50% increase in maximum amplitude (MA) would be clinically relevant. The primary outcome of this study was the difference in the magnitude of

change in r-time, k-time and maximum amplitude from baseline between the HTK solution and the Saint Thomas' solution. The secondary outcomes were the magnitude of change of the TEG parameter from baseline in the HTK and Saint Thomas' solutions.

Results

The difference in the magnitude of change from baseline in reaction r- time between the HTK solution and Saint Thomas' solution (mean 0.49 min ± 0.82) ($p<0.05$) was statistically significant. However, for maximum amplitude (MA), no statistical difference was noticed (mean -2.13 mm) ($p = 0.165$). Compared to native blood, there were no significant changes in the r-time, k-time, alpha angle and MA with the HTK solution. However, the Saint Thomas' solution caused changes in all the TEG parameters that were statistically significant.

Conclusion

Although the difference in the magnitude of change in r-time between the HTK and St Thomas' solutions was statistically significant, it was not clinically relevant. The data did not show a trend that might become significant with a large sample. All variables showed a slight tendency towards decreased coagulation in the diluted samples, but it is not of sufficient magnitude to be clinically important. Perhaps with a greater dilution (e.g 20%), one may see significant changes, but the goal of this study was to use a dilution that is clinically relevant in the context of paediatric cardiac surgery. This study does not provide any evidence that the 10% dilution with HTK solution can lead to the hypercoagulability of blood. More studies are needed to clarify the pathophysiology of the increased incidence of thrombosis observed in these patients after their transplants.

MAIN TEXT

10.3 Introduction

Crystalloid cardioplegic solutions are commonly classified according to two main groups: one has a composition similar to the extracellular milieu, whilst the other, whose composition is similar to the intracellular milieu, is mainly represented by the HTK solution. The HTK or Bretschneider's solution was introduced into clinical practice for use as a cardioplegic in 1975.[1] The electrolyte composition of the HTK solution mimics the electrolyte constituents and concentration of intracellular fluid. Histidine, tryptophan, ketoglutarate, and mannitol have been added to the solution for specific physiological goals. Histidine acts in a buffer capacity, limiting ischemia-induced acidosis, whilst ketoglutarate improves ATP production. Tryptophan is used as a membrane stabiliser and the osmotic property of mannitol reduces cellular oedema.[2] Several studies have reported the efficacy of HTK solution in coronary artery bypass graft (CABG) surgery,[3] even when low doses were used. [(4)] In valvular heart surgery, HTK solution has been proven to provide protection against ischaemic damage during cardiac surgery.[5-7] In paediatric cardiac surgery, HTK solution was shown to be as protective as St Thomas' solution, [8] with the advantage of decreased cross-clamping time due to the decreased need for redosing the cardioplegia.[9] The use of HTK solution in the field of organ transplantation has reported good results with liver, [10-12] kidney, and pancreatic transplants. [13, 14] Recently, this enthusiasm has been tempered by reports of a higher risk of graft thrombosis in both liver and pancreas transplantations. [11, 15-17]

The thromboelastogram (TEG) involves two mechanical parts: a cuvette and a piston. The cuvette, which contains the loaded blood sample, oscillates 4º 45' in each direction in a four-second cycle period at 37º C. The piston is suspended in the blood sample by a torsion wire, which is transduced to a chart recorder. The motion of the cuvettes starts affecting the piston only when fibrin starts forming, cross-linking the piston to the cuvette. [18] The viscoelastic property of the forming clot is then transmitted through the piston to the recording software system. Several parameters can be measured from the trace, including: reaction time (r); coagulation time (k); alpha angle (α); and maximum amplitude (MA). The reaction time is the interval from the start of recording to the time at which the amplitude of the tracing is 2 mm. It represents the thrombin generation time and is affected by the low concentration or dysfunction

of clotting factors. Coagulation time (k) is the time between the end of r time and when the amplitude of the trace reaches 20mm. It is influenced by clotting factors, fibrinogen and platelet function. The alpha angle (α) is formed from the slope of the tangential line from r-time to k-time. It represents the clot formation rate and is affected by fibrinogen concentration and platelet function. The maximum amplitude (MA) is the greatest amplitude achieved on the TEG trace. It represents clot strength and reflects the property of platelets, fibrinogen and factor XIII.[19]

10.4 Objectives

The purpose of this study was to establish whether HTK solution, in the dose and volume used for myocardial protection during paediatric cardiac surgery, increases the thrombotic risk to patients. This study aimed to determine the effects of HTK on coagulation, as measured by the following TEG parameters: reaction time(r), clot kinetic time (k), angle of clot kinetic (α), and maximum amplitude (MA).

10.5 Material and Methods

Approval from the Human Research Ethics Committee of the University of Cape Town was sought and obtained before the enrolment of volunteers. This in vitro study sought to investigate the coagulation changes that occur during hemodilution due to cardioplegia in a paediatric population undergoing open cardiac surgery. Twenty volunteers participated in this study, after signing a written informed consent. Exclusion criteria were: pre-existing conditions known to affect coagulation; anticoagulants, antiplatelets; and non-steroidal anti-inflammatory medications.

A TEG (Model 5000, Haemoscope Corp., and Skokie, IL) machine, warmed at 37°C, was utilised to analyse the blood samples. The TEG analyser was calibrated using Biological QC I and Biological QC II assays (quality control assay from Haemoscope Corp.) to ensure quality control. To mimic an *in-vitro* model of dilution due to infusion cardioplegia in a paediatric patient, the whole blood was diluted to a 9:1 volume ratio with a HTK solution. This ratio was

selected based on observations of the dilution of blood after the infusion of a cardioplegia solution during open cardiac surgery at Red Cross War Memorial Children's Hospital, as the typical dilution due to HTK solution can range from 5 to 10% blood volume. The blood was diluted in a similar way with the Saint Thomas' cardioplegic solution, which served as a control, and was subjected to the thromboelastograph in order to help determine the intrinsic role of HTK solution in the enhancement of coagulation, if any.

The blood samples were collected into citrate-containing tubes. Ten millilitres of blood were collected, of which the first four millilitres were discarded to minimise possible tissue factor activation. The remaining six millilitres was divided and placed in three different tubes. The first tube was left undiluted and used to measure baseline coagulation parameters on the TEG. The remaining two samples were diluted with either HTK or Saint Thomas' solution. The physicochemical characteristics of the two cardioplegic solutions are provided in Table I below. From each tube, 330 µl of blood were pipetted and mixed with 10 µl of kaolin containing TEG reagent and 20 µl of 0.2 mol/l calcium chloride in a disposable cuvette to make up the 360 µl solution, which was placed in the calibrated TEG analyser. The coagulation process was analysed within four minutes following blood sampling and the following TEG parameters were recorded using TEG software (version 4.2.3): reaction time (r); clot formation time (k); clot formation rate (α angle) and maximum amplitude (MA), first on the undiluted blood, then on the 9:1 dilution with HTK solution, and lastly on the 9:1 dilution with Saint Thomas' solution. The TEG was performed on each sample and the same parameters were recorded

The primary outcome of this study was the difference in the magnitude of change in r-time, k-time and maximum amplitude from baseline between the HTK and Saint Thomas' solution. The first secondary outcome was the magnitude of change in r-time and maximum amplitude caused by the HTK solution, and the second secondary outcome was the magnitude of change in r-time, k-time, alpha angle and maximum amplitude caused by the Saint Thomas' solution.

Table I: Composition of cardioplegia solutions

	HTK solution	Saint Thomas' solution
Na (mmol/l)	15	144
K^+ (mmol/l)	9	20
Mg^{2+}(mmol/l)	4	16
Ca^{2+} (mmol/l)	0.015	2.2
Histidine (mmol/l)	198	0
Tryptophan (mmol/l)	2	0
Ketoglutarate (mmol/l)	1	0
Mannitol (mmol/l)	30	0
pH	7.02-7.20	5.5-7.0
Osmolality (mosm/kg)	280	300-320

10.6 Statistical Analysis

The researchers postulated based on the study by Ruttmann et al., that a 50% drop in r-time or 50% increase in maximum amplitude (MA) would be clinically significant. For a mean r-time of six minutes and standard deviation of 2.8, a sample size of 20 volunteers would be needed to conduct a successful study.

Statistical analysis was done using the Statistica software package (version 12, statsoft Inc., Tulsa, USA, licensed for the University of Cape Town, South Africa). Results were expressed as the mean ± standard deviation (SD). The native blood and the dilutions constituted one group each. The mean values before and after dilution were compared using a Students paired *t* test. The significance level of 0.05 was used to detect statistically significant differences between the groups.

10.7 Results

Twenty healthy volunteers participated in this study. The mean age was 34.7 years and the standard deviation was 3.07 years. The male to female ratio was 11:9.

Table II: Magnitude of change in TEG parameters after dilution

	Change with HTK	**Change with St Thomas'**	**P-value***
r(min ± SD)	-0.21±1.08	-0.71±0.93	0.01**
k(min ± SD)	-1.05±0.46	-0.3±0.37	0.08
alpha(º± SD)	1.36± 6.98	2.86±4.31	0.42
MA(mm ± SD)	1.38±4.97	3.51±3.96	0.16

Control: native blood; HTK solution: Histidine-Tryptophan-Ketoglutarate; St Thomas' solution: Saint Thomas' Hospital solution; *paired *t* test; **statistically significant.

Table III: Thromboelastogram parameter values in Control vs. HTK solution dilution

	baseline	**HTK solution**	**P-value***
r(min±SD)	7.54±0.99	7.76±1.19	0.38
k(min±SD)	2.08±0.44	1.90±0.48	0.32
Alpha(º ± SD)	61.21±4.84	59.85±7.09	0.39
MA(mm±SD)	61.47±4.20	60.09±5.59	0.23

Control: native blood; HTK solution: Histidine-Tryptophan-Ketoglutarate; *paired *t* test

Table IV: Thromboelastogram parameter values in control vs. St Thomas' solution dilution

	baseline	**St Thomas' solution**	**P-value***
r(min±SD)	7.54±0.99	8.25±1.01	0.0030**
k(min±SD)	2.08±0.44	1.90±0.48	0.0021**
Alpha(º ±SD)	61.21±4.84	59.85±7.08	0.0079**
MA(mm±SD)	61.47±4.20	60.09±5.59	0.0008**

Control: native blood; St Thomas' solution: Saint Thomas' Hospital solution; *paired *t* test; **statistically significant.

A comparison of the magnitude of change in r-time, k-time, alpha angle and maximum amplitude from baseline between the HTK diluted blood and the St Thomas' diluted blood was performed to highlight any changes specific to the HTK solution. There was a hypocoagulant effect of the Saint Thomas' solution compared to the HTK solution, as seen by an increase in r- time that was statistically significant. There was a decrease in k-time and increase in alpha angle and maximum amplitude that were not statistically significant (Table II). The TEG parameters of the HTK solution diluted blood and St Thomas' solution diluted blood were compared with the undiluted blood, which served as the baseline values. The dilution with the HTK solution resulted in a slight decrease in r-time, k-time, alpha angle and maximum amplitude. These decreases in the TEG parameters were marginal and did not reach a level of statistical significance (Table III). Surprisingly, the dilution with St Thomas' solution showed a net hypocoagulant effect, as noted by an increase in r-time and decrease in k-time, alpha-angle and maximum amplitude, which was statistically significant even though the magnitude of the change was not sufficient to be clinically relevant (Table IV).

10.8 Discussion

This is the first study that has investigated the in vitro effect of HTK and Saint Thomas'' cardioplegia solutions as a medium of dilution on coagulation. This study compared the magnitude of change in coagulation between blood diluted with HTK solution and St Thomas' solution. The study has shown that at this level of dilution, neither of the solutions resulted in a decrease in r-time, nor an increase in k-time, alpha angle and maximum amplitude. This is in contrast to what is expected when considering the HTK solution, which is a reported cause of thrombosis in the literature. [17, 20] In contrast, the dilution of the whole blood with the HTK solution or Saint Thomas' solution at 9:1 showed a trend towards hypocoagulability that was statistically significant with the St Thomas' dilution. Previous studies using other crystalloids and / or colloids have found evidence of hypercoagulability, which was not substantiated by this study.[18, 21-23]

Logic would seem to imply that the hemodilution of blood with fluids will cause a decreased viscosity, and therefore a hypocoagulable state. However, many authors have found the opposite results. In 1951, Tocantin *et al*. found evidence of hypercoagulability after the dilution of blood with crystalloid.[21] Later, Narita *et al*. (1979) found an initial hypercoagulability after serial dilution with different volume expanders before hypocoagulability could be detected.[24] Ruttmann, using *in vitro* dilution with normal saline and Haemacel, reported hypercoagulability in both, thereby concluding that the nature of fluids was not a factor that influenced the hypercoagulability of the diluted blood.[18] The results of this study can be explained firstly by the level of dilution, which was 10%, which may be too small to cause hypercoagulability. Previous studies have used greater dilutions than this study. The rationale for the researchers' choice was to use a dilution that was in keeping with the level of dilution due to cardioplegia seen in paediatric cardiac surgery. Secondly, the composition of the HTK solution varies quite considerably from other crystalloid solutions used thus far in dilution studies, since its sodium content is low (refer Table I). The researchers chose the *in vitro* study in an attempt to eliminate all the extraneous factors that could influence the coagulation process, such as: surgical stress response; tissue injury; dilution with cardiopulmonary bypass priming solution; and

heparinisation, which could confound results in an *in vivo* study. Any change could therefore be solely explained as the consequence of the addition of the cardioplegia solution to the normal blood. The limitation of the study is that it is an experimental study in a controlled laboratory environment, which means that the results may not be easily extrapolated to the clinical setting, in which other factors may confound the results. Furthermore, though the TEG measures the whole process of coagulation from initiation phase to fibrinolysis, as well as taking to account the contribution of plasmatic and cellular elements of coagulation, the contribution of vessels is not assessed by it. The vessels are a major contributor to the coagulation process as a whole, and any damage to its integrity may compromise the balance in the coagulation system, thereby enhancing the coagulability of blood. Therefore, this study may not rule out the contribution of vessels to the increased incidence of thrombosis noted in the literature. Further studies using greater dilution are warranted to investigate this trend towards hypocoagulability with HTK that was observed in this study, as well as *in vivo* studies, in order to determine whether or not these results can be replicated in the clinical setting.

10.9 Conclusion

Previous studies have shown that the haemodilution of blood with crystalloids may cause a hypercoagulable state. The disruption of the anticoagulation system, with decreased activity of antithrombin III (AT III), is suspected to be a cause of this phenomenon. The effect of the dilution of blood with cardioplegia solutions has not been investigated yet. Our results confirm that a 10% plasma dilution with HTK solution has no significant effect on coagulation, and no trend that might become statistically significant with a bigger sample size was found. However, the Saint Thomas' solution at 10% dilution has a hypocoagulant effect on several aspects of the coagulation process, from the initial fibrin formation with increased r-time, to the rapidity of fibrin build-up and cross-linkage with increased k-time, and speed of clot formation with increased alpha angle, and the decrease in clot strength (MA). From this study, we can conclude that HTK solution does not have a prothrombotic effect on whole blood coagulation at a 10% dilution, as measured by TEG. Further studies in this field are required to conclusively determine the effect of the greater dilution of blood with the HTK solution on the coagulation system. If the

present results were to be corroborated, this would mean that HTK solution is safe to use in cardiac surgery, and the search for the cause of thrombosis, as reported in the literature, should focus on other risk factors.

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11 APPENDIX

11.1 Appendix 1. Data Collection Form

DATA COLLECTION SHEET

NAME:
AGE:
WEIGHT:
DATE:

COMORBID DISEASE: YES NO

ARE YOU TAKING ANY OF THESE MEDICATIONS?

ASPIRIN: YES NO

WARFARIN: YES NO

HEPARIN: YES NO

RESULTS OF EXPERIMENTS

DILUTIONS	R-Time	K-Time	Alpha Angle (degrees)	Maximum Amplitude	G value	Lysis 30
Native Blood						
Dilution with HTK solution						
Dilution with St Thomas solution						

11.2 Appendix 2. Consent form

INFORMED CONSENT FORM

TITLE: EFFECT OF A HTK SOLUTION ON COAGULATION AS MEASURED BY A THROMBOELASTOGRAM

Principal investigator: Dr K F Kabambi

Registrar: Department of Anesthesia

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You are hereby invited to participate in this research study. However, we would like to familiarize you with what this research is all about before you decide whether or not to participate in it. Please read this document carefully and feel free to ask us any questions related to this subject or any concern you may have.

Background

The HTK solution is a fluid that doctors use to protect the heart during cardiac surgery. When a person has a heart disease that requires surgery, the surgeon will open the thorax. However, in order for him to operate on the heart, open it and fix the problem, he first needs to stop the heart from beating and protect the heart muscle from dying during the surgery by using a

cardioplegia solution (one of them being the HTK solution). A special machine known as a cardiopulmonary bypass machine takes over the role of the heart and allows blood to continue to flow throughout the body.

Although these solutions help to protect the heart, there is a possibility that they could interfere with blood clotting. In this study, we want to determine the effect of these solutions on clot formation using a special machine called a thromboelastograph. This machine gives us a more detailed picture of the clotting process, from when it begins till the time that the clots break down.

Purpose of the study

We will take no more than 10ml of blood directly from you directly. This study is a laboratory study, and no other tests than the clotting test will be performed on your blood. During this test, we will mix your blood with different amounts of the solution, in order to see how it affects the clotting of blood. No blood results will be revealed to you or any other person. We would like to have 25 people willing to take part in this study.

Risk to the participant

You will incur no risk as a result of this study, other the pain of collecting your blood. As mentioned before, the rest of the study will be done in the laboratory and will not require your participation. The blood will be taken by anesthetic personnel.

Benefit to the participant

This study will not benefit you as a participant. We hope that the results of this study will help us to better understand the effect of this solution on blood clotting, and this will in turn help us to improve the care of patients undergoing cardiac surgery.

Voluntary participation

Your participation in this study is on a voluntary basis. You are free to agree or refuse to participate, and can withdraw your consent at any time during the research process without giving reasons. If you agree to participate in this study, we will need you to sign the attached consent form.

Cost of participation

There is no cost to you as a participant and there is also no compensation due to you for participating in this study.

Compensation

There is no compensation to you for your participation in this study.

CONSENT

I ..... (Name & surname), confirm that I have read and understood the information provided to me and had the opportunity to ask questions. I understand that my participation is voluntary and that I am free to withdraw at any time without giving reasons and without any cost. I understand that this study holds no benefit for me as an individual and I need not expect any material gain.

Signature ..... Date .....

Should you have any questions or complaints about the research or any related matters, please contact:

Dr KF Kabambi

(principal investigator)

0214045001

Dr G Wilson

(supervisor)

0213585003

Prof M Blockman

(chairperson UCT HSF human Ethics)

0214066492

11.3 Appendix 3. Ethics Approval Letter

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics
Committee

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www.health.uct.ac.za/fhs/research/humanethics/forms

08 December 2014

HREC REF: 898/2014

Dr G Wilson
Anaesthesia
D26
NGSH

Dear Dr Wilson

PROJECT TITLE: EFFECTS OF HISTIDINE-TRIPTOPHANE-KETOGLUTARATE SOLUTION ON COAGULATION AS MEASURED BY THROMBOELASTOGRAM (MMed candidate-Dr K Kabambi)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study. •

Please add that it is the Human Research Ethics Committee in the Informed Consent Form. **Approval**

is granted for one year until the 30th December 2015.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period. (Forms can be found on our website:
www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

We acknowledge that the student, Dr Kasandji Freddy Kabambi will also be involved in this study.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

Signed

PROFESSOR M BLOCKMA
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

11.4 Appendix 4. Author Guidelines

South African Journal of Anaesthesia and Analgesia

Instructions to authors

Online Submissions

Thank you for choosing SAJAA in which to publish your paper. Please consult the author guidelines below in order to prepare a manuscript that is illegible for further review. All submissions must be made online.

How to submit your paper online:

1. Registered authors must login to submit a paper
   - REGISTER HERE if you do not have a username and password
   - LOGIN HERE if you have already registered with SAJAA
2. Select **Author**
3. Click on **CLICK HERE TO FOLLOW THE FIVE STEPS TO SUBMIT YOUR MANUSCRIPT**
4. Follow the five steps to submit your paper

- To view a video on how to submit a paper online CLICK HERE
- To download instructions to authors, CLICK HERE

Review policy and timelines

1. Immediate notification if submitted successfully

2. Notification within 3 weeks if not accepted for further review
3. Notification within 3 months if accepted for publication, if revisions are required or if rejected by both reviewers.
4. Publication within 6 months after submission.

Author Guidelines

Aims, scope and review policy

The *SA Journal of Anaesthesia and Analgesia* aims to publish original research and review articles of relevance and interest to the anaesthetist in academia, public sector and private practice. Papers are peer reviewed to ensure that the contents are understandable, valid, important, interesting and enjoyed. All manuscripts must be submitted online.

SAJAA is indexed in EMBASE and it is accredited by the Department of Education for the measurement of research output of public higher institutions of South Africa (SAPSE accredited).

Article sections and length

Original research:			3200-4000			words
Reviews:			2400-3200			words
Case Studies:	1800	words	plus	3		photographs
Scientific Letters: 1200-1800 words						
Letters to the Editor: 400-800words						

The following contributions are accepted (word counts include abstracts, tables and references):

Title page

all articles must have a title page with the following information and in this particular order: Title of the article; surname, initials, qualifications and affiliation of each author; the name, postal address, e-mail address and telephonic contact details of the corresponding author and at least 5 keywords.

Abstract

All articles should include an abstract. The structured abstract for an Original Research article should be between 450 and 600 words and should consist of four paragraphs labelled Background, Methods, Results, and Conclusions. It should briefly describe the problem or issue being addressed in the study, how the study was performed, the major results, and what the authors conclude from these results. The abstracts for other types of articles should be no longer than 250 words and need not follow the structured abstract format.

Keywords

All articles should include keywords. Up to five words or short phrases should be used. Use terms from the Medical Subject Headings (MeSH) of Index Medicus when available and appropriate. Key words are used to index the article and may be published with the abstract.

Acknowledgements

In a separate section, acknowledge any financial support received or possible conflict of interest. This section may also be used to acknowledge substantial contributions to the research or preparation of the manuscript made by persons other than the authors.

References

Cite references in numerical order in the text, in superscript format (Format> Font> Click superscript). Please do not use brackets or do not use the foot note function of MS Word.

In the References section, references must be typed double-spaced and numbered consecutively in the order in which they are cited, not alphabetically.

The style for references should follow the format set forth in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://www.icmje.org) prepared by the International Committee of Medical Journal Editors. Abbreviations for journal titles should follow Index *Medicus* format. Authors are responsible for the accuracy of all references. Personal communications and unpublished data should not be referenced. If essential, such material should be incorporated in the appropriate place in the text.

List all authors when there are six or fewer; when there are seven or more, list the first six, then ";et al."; When citing URLs to web documents, place in the reference list, and use the following format: Authors of document (if available). Title of document (if available). URL. (Accessed [date]).

The following are sample references:

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A comprehensive reference guide can be found at:
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Tables

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