An observational study to assess coagulation abnormalities in patients with elevated levels of urea and/or creatinine secondary to renal failure, presenting for renal biopsy – challenging conventional testing using visco-elastic testing

by

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SUBMITTED TO THE UNIVERSITY OF CAPE TOWN

In fulfilment of the requirements for the degree

Master of Medicine (MMed) in Anaesthesia

(AAE7002W)

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

Date of resubmission: 14 October 2018

Supervisor: Dr Malcolm Miller (Department of Anaesthesia, University of Cape Town)

Declaration

I, *Dr Jacques Rodrigues*, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgments 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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Acknowledgements

I would like to express my sincere gratitude and thankfulness to Dr Malcolm Miller, my research supervisor, for the opportunity to work together on this research topic, for the extensive amount of time that has been invested into this research project and for the invaluable source of knowledge and counsel. Also, instrumental to the completion of this work was the guidance provided by Prof M F M James, who kindly assisted with the data interpretation and provided useful insight into the results obtained, as well as generating the graphs under the supporting documents section. Prof R A Dyer also provided much needed assistance with this work, for which I am very grateful.

I am also very appreciative for the assistance granted to us by Dr Erica Jones and the Nephrology unit of Groote Schuur Hospital in identifying suitable candidates for this study.

It is with gratitude that I acknowledge the assistance of Dr Ricky Ginsburg, as an advisory consultant during this study, and Lizel Immelman, the laboratory technologist within the Department of Anaesthesia at Groote Schuur Hospital, who dutifully performed the visco-elastic testing and assisted with data collection and data capturing, as well as assisting with the coordination of the study.

Lastly, I would like to thank Mr Harold Stuurman, who was employed as the department’s general laboratory assistant, who expedited transport of the blood specimens between the laboratories and provided us with general assistance.

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List of abbreviations

AKI	Acute kidney injury
AKIN	Acute Kidney Injury Network
aPTT	Activated partial thromboplastin time
AT III	Antithrombin III
BT	Bleeding time
CFT	Clot formation time
CI	Coagulation index
CKD	Chronic kidney disease
CT	Clotting time
DIC	Disseminated intravascular coagulation/coagulopathy
EDTA	Ethylenediaminetetraacetic acid
F	Factor
GFR	Glomerular filtration rate
INR	International normalised ratio
KDIGO	Kidney Disease: Improving Global Outcomes
K-time	Kinetics (time taken to achieve a certain level of thrombus strength)
MA	Maximum amplitude (the ultimate strength of the fibrin mass)
MCF	Maximum clot firmness
MeSH	Medical Subject Headings
mmHg	Millimetre(s) of mercury
NHLS	National Health Laboratory Services
NKF	The National Kidney Foundation
PT	Prothrombin time

ROTEM®	Rotational thromboelastometry
R-time	Reaction time (time from start of test to initial fibrin formation)
RIFLE	Risk, Injury, Failure, Loss, End-stage kidney disease
SCr	Serum creatinine
TEG®	Thromboelastogram/Thromboelastography
TF	Tissue factor

Part A: Abstract

Introduction

Coagulation abnormalities are well described in patients with elevated levels of urea and/or creatinine secondary to renal failure. These range from hypercoagulable to hypocoagulable states due to a range of mechanisms well described in the literature. Conventional tests of coagulation such as INR and PTT do not adequately assess these disorders of coagulation. Thromboelastography (TEG®) has proven to be a suitable alternative test of coagulation that serves as a dynamic test of global coagulation including assessment of thrombus formation as well as its breakdown. TEG® and ROTEM® assesses the visco-elastic properties of blood in vitro to define in vivo coagulability. The standard of care in our institution to assess the bleeding risk in patients with renal failure (defined by a raised urea and/or creatinine level) presenting for a renal biopsy is to use the conventional tests of coagulation, including a bleeding time if their creatinine is above 300 µmol/L. The aim of this study is to evaluate the conventional standard laboratory tests of coagulation (including a bleeding time where available), TEG® and ROTEM® in assessing coagulation disorders in patients with elevated levels of urea and/or creatinine presenting for renal biopsy.

Methodology

Patients with elevated levels of urea and/or creatinine presenting for a renal biopsy will be identified by the nephrology team responsible for their medical management. Prior to the renal biopsy, these patients will be approached by the study team and reviewed for inclusion into the study. Informed consent will be obtained on agreement to participate in the study. We will collect a blood sample for the TEG® and ROTEM® and this test will be performed by a laboratory technician in the Department of Anaesthesia. The clinician/nephrologist performing the biopsy will not be influenced by the outcome of these viscoelastic tests. A convenience sample of a minimum of 25 patients with renal impairment presenting for a renal biopsy will be included in this study.

Results

A total of 44 adult participants was entered into this observational study. Results for 1 participant were excluded from this study as their biopsy was delayed, allowing their renal function to improve and return to normal with medical management on the day that they presented for a renal biopsy. 43 patients were worked up for a renal biopsy but only 38 patients proceeded to a renal biopsy. Of these, only 31 patients had a bleeding time performed on the day of their renal biopsy. The participants ages ranged from 24 to 69 years and included 24 male and 19 female participants. Renal biopsies were cancelled by the consultant nephrologist in 5 patients on the day of their biopsy. Control samples, from 10 members in the Department of Anaesthesia, fell within the specified range of the various manufacturers. An interesting TEG® result was an average MA result of 74.22 mm (normal range 64 – 72 mm), which lies above the upper limit of normal. Two patients developed a small renal haematoma on ultrasound after the biopsy, with 1 of these patients also developing haematuria.

Conclusion

TEG® and ROTEM® provides a global assessment of coagulation and might be helpful in assessing coagulation defects in patients with elevated levels of urea and/or creatinine presenting for a renal biopsy, with possible extension to the surgical patient with abnormal renal function presenting for a surgical procedure to assess their risk of bleeding, especially in those who are being considered for a regional or neuraxial technique - as this could be an unacceptable risk in this population sub-group .

Part B: Study proposal

As approved by the Departmental Research Committee (Addendum A) and the Human Research Ethics Committee of the University of Cape Town (Addendum B).

Background:

It is well known that patients with renal impairment, either with acute kidney injury (AKI) or chronic kidney disease (CKD) have high levels of urea and other nitrogenous waste products circulating in their blood (Berns & Coutre, 2015; Soyoral et al., 2012; Stammers, Bruda, Gonano, & Hartmann, 1998). The range of coagulation abnormalities, from hypercoagulable to hypocoagulable states, due to several mechanisms (Boccardo, Remuzzi, & Galbusera, 2004) are well described in the literature (Berns & Coutre, 2015; Boccardo et al., 2004; Kaw & Malhotra, 2006; Soyoral et al., 2012; Stammers et al., 1998). Patients with renal impairment "may experience two opposite haemostatic complications: bleeding diathesis and thrombotic tendencies" (Boccardo et al., 2004). Bleeding may range from an insignificant bleed (such as purpura) to a serious gastro-intestinal haemorrhage complicating either AKI or CKD. With the introduction of dialysis and erythropoietin, bleeding complications have decreased (Berns & Coutre, 2015; Boccardo et al., 2004); however, it still impacts on clinical decision making. These patients can present for surgery and can be a challenge to the anaesthesiologist when considering possible interventions.

Conventional tests of coagulation (e.g. bleeding time (BT), INR, PTT) assesses components of the coagulation cascade (Slaughter, 2010); however, they do not adequately assess coagulation abnormalities. Furthermore, they are purely plasma-based tests and thus provide no information on the cell-based aspects of coagulation. These tests thus provide only isolated information on individual components of the coagulation cascade. Thromboelastography (TEG®) and rotational thromboelastometry (ROTEM®) have been shown to be suitable ex-vivo tests, serving as dynamic measures of in vivo coagulation. TEG® is a visco-elastic measure of coagulation and provides a global assessment of whole blood thrombus formation, stabilisation and fibrinolysis that reflects in vivo haemostasis. ROTEM® is a modification of the thromboelastogram. These tests provide the clinician with information on the "coagulation cascade, fibrinogen and the platelets" and their interaction "as an entire unit" (Timmermans, Dolmans, & Kahn, 2009) and can guide the replacement of blood and blood components in the surgical patient (Johansson, Bochsen, Stensballe, & Secher, 2008).

TEG® is generally performed on a single channel that is activated with a contact agent such as celite or diatomaceous earth. ROTEM® routine provides 4 channels: coagulation is stimulated with recombinant tissue factor (similar to the INR, but with cellular elements) called the EXTEM, ellagic acid (similar to the aPTT) called the INTEM, tissue factor plus cytochalasin to remove platelet components and assess the fibrinogen component only (FIBTEM), whilst the 4th channel can be used with heparinase or aprotonin to remove the effects of heparin or fibrinolysis (see below). Thus, ROTEM® may provide more information than TEG® alone, specifically with regard to the contribution of fibrinogen.

TEG® was first described by Hartert in 1948 (Stammers et al., 1998; Whiting & DiNardo, 2014) as a device that provided information about the nature of whole-blood haemostasis, thrombus strength and its breakdown. It provides information on both cellular and non-cellular factors involved in thrombus formation. This point of care system assesses the visco-elastic properties of blood being tested and provided a global assessment of coagulation (Curry & Pierce, 2007).

In this study, we will evaluate the ability of coagulation parameters of thromboelastography and rotational thromboelastometry to identify coagulation abnormalities in patients with elevated levels of urea and/or creatinine secondary to renal failure, presenting for a renal biopsy. We will also collect data concerning standard laboratory coagulation tests (e.g. INR, PTT, BT) but a statistical comparison between methods will not be performed. A convenience sample of a minimum of 25 patients presenting for a renal biopsy, will be tested in this observational case series.

Methodology:

Patients with elevated levels of urea and/or creatinine (renal impairment) presenting for work-up for a renal biopsy at Groote Schuur Hospital will be identified by the renal unit. Work-up for the renal biopsy includes a referral to the Renal Unit, the booking of a biopsy with the renal senior registrar, with a BT to be requested if the creatinine is > 300 µmol/L.

On the day of biopsy, the current protocol regarding haematological criteria for eligibility for renal biopsy is (Addendum C):

a platelet count ≥ 100 x 10^9/mL,

PTT < 40 seconds,

INR ≤ 1.4,

a bleeding time ≤ 10 min, or ≤ 12 min if an emergency biopsy.

The TEG® and ROTEM® devices will be used as an alternative method of assessing the coagulation profile. This overcomes the technical difficulties of performing a bleeding time, as well as providing a more comprehensive in vitro assessment of coagulation. The primary outcome is thus to assess whole blood coagulation, using standard parameters generated by the TEG® and ROTEM® traces. In addition, fibrinogen concentrations (Wolberg & Campbell, 2008) will also be measured.

A representative from the renal unit will inform the study group of patients on the day of the planned renal biopsy. Research participants will include both male and female adult subjects (age > 18) of any ethnicity with elevated levels of urea and/or creatinine presenting for a renal biopsy. These patients will be approached by a representative from the study group and included into the study only once informed consent (Addendum D) has been granted and prior to the provision of any sedation. Exclusion criteria included patient refusal, LMWH/heparin therapy, antiplatelet medication and renal replacement therapy.

Blood sampling will be standardised between the phlebotomists, the blood specimen is to be taken using a Vacutainer (BD Vacutainer Systems, Plymouth, England). Blood sampling will be in accordance with accepted aseptic principles and technique. After the initial 3 mL of blood is discarded, the whole-blood sample is to be collected in a 2 mL syringe and dispatched to the Anaesthesia laboratory immediately, a further 2 samples will be collected in two 4 mL Greiner 9 NC Coagulation Sodium Citrate 3.2% tubes (BD Vacutainer) – 1 sample for the laboratory (NHLS) for fibrinogen testing and the other sample to the Anaesthesia laboratory as a control sample. The TEG® is to be measured on the TEG® 5000 Thromboelastograph Haemostasis Analyser System (Haemoscope) made available to us in the laboratory of the Department of Anaesthesia. ROTEM® will be performed and analysed on the Viking ROTEM® Delta haemostasis analyser. The software for TEG® analysis will be performed on TEG®V4.2.3. For TEG®, measurement of R-time (reaction time), K-time (kinetics), maximum amplitude (MA) and coagulation index (CI) is to be performed for each patient.

Additional tests with ROTEM® will be performed for each patient, and will include clotting time, clot formation time, alpha angle and maximum clot firmness. These additional tests include INTEM (which essentially analyses the intrinsic pathway), EXTEM (analysing the extrinsic pathway), FIBTEM (the addition of a platelet inhibitor prevents platelets from contributing to thrombus formation thus allowing analyses of the functional fibrinogen component) and APTEM (addition of aprotinin to inhibit fibrinolysis).

TEG® and ROTEM® will be performed by the same laboratory technologist, to ensure standardisation of the test procedure.

Statistical analysis will be performed using a standard statistical package (Statistica Version 13, Dell Inc, Tulsa, OK) and data will be evaluated in terms of means, medians, standard deviations and ranges. These measures will be compared with the standard ranges for conventional tests of coagulation, quoted by the instrument manufacturer, to identify possible coagulation abnormalities in this patient group.

Referencing and Guidelines:

The SAMJ author and referencing guidelines have been used as a guideline for this document, however, the references for Part B, C and D are in the APA 6th style. Once the manuscript is presented for publication, the references will be updated to the Vancouver style as specified by the SAMJ. Where a reference has a digital object identifier (DOI) available, these have been included.

Ethical Considerations:

The study is approved by the Ethics Committee of the Faculty of Health Sciences of the University of Cape Town (Addendum B).

Participation in the study will be voluntary, and participants will complete an informed consent form (Addendum D).

Confidentiality and anonymity will be ensured.

Participants will receive a participant number and be further identified throughout the study with the participant number (Addendum E and Addendum F).

All data will be stored under the participant number (Addendum E and Addendum F).

Participants are free to withdraw at any time and without providing a reason.

All patients will receive the normal standard of care, irrespective of participation or non-participation in the study (Addendum C).

Participants will be ensured that they will be taking no extra risk if they agree to be part of the study.

Participants will have no direct benefit from participation in the study, but participation will not hinder nor change the standard of care they will receive.

There is to be no cost, nor compensation for participation in the study.

Funding will be provided by the Department of Anaesthesia.

No conflicts of interest have been identified or declared.

The protocol complies with the Helsinki Declaration of 2008.

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Part C: Structured literature review

Objectives of the literature review

The objectives of this literature review were to review the current published information about coagulation abnormalities in patients with elevated levels of urea and/or creatinine secondary to renal failure, and to include a short review of coagulation and the various tests of coagulation, pertinent to our study, which are currently available. The available literature frequently describes renal dysfunction and bleeding (or "uraemic bleeding") and this predisposition to bleeding is often cited as being caused by an interference in platelet function (Boccardo et al., 2004; Soyoral et al., 2012). The association between renal dysfunction and bleeding has been known for more than 200 years. "Patients with renal impairment are screened for bleeding risk by measuring the bleeding time" (Berns & Coutre, 2015), which is still the practice in our institution, prior to a renal biopsy or some other invasive procedure.

Literature search strategy

A literature search was performed using PubMed and the MeSH database. A search with the terms 'uremia' and 'renal failure' and 'blood coagulation tests' yielded 41 results (Addendum G) and a search with the terms 'uremia' and 'renal failure' and 'blood coagulation' yielded 52 results (Addendum H). Substituting 'uraemia' for 'uremia' yielded no further results. Filters included literature which was published between January 1970 and December 2016, performed in humans and in English. Literature was excluded if it was not originally published in English.

Review and critical appraisal of the literature

Blood Coagulation

No universally accepted definition of coagulation was found in the literature. A proposed definition for normal coagulation of blood (also known as clotting or haemostasis) denotes the biochemical sequence whereby blood changes from a liquid form into a gel-like state, eventually resulting in the formation of a stable blood clot (a thrombus) and the cessation of blood loss. Physiologically, this usually occurs due to the disruption of the integrity of the vascular endothelium and is initiated within seconds in an attempt to limit the loss of blood.

Previously, haemostasis was separated into **primary haemostasis**, which was the formation of the platelet plug at the injury site, and **secondary haemostasis**, also known as the coagulation cascade. Secondary haemostasis was classically divided into three pathways: an extrinsic pathway (the tissue factor pathway), an intrinsic pathway (the contact activation pathway) and a final common pathway. This **classic model of coagulation** was grossly artificial and could explain haemostasis in vitro, however, it did not consider the interaction between primary and secondary haemostasis. The classic model aided the understanding of the pharmacology of anticoagulants and the interpretation of results of laboratory tests which assessed clotting times (e.g. prothrombin time, activated partial thromboplastin time and the INR), however this has been replaced with an updated model which was developed as our knowledge of coagulation improved.

The classic model was an artificial interpretation of coagulation in vitro but did not accurately explain coagulation in vivo. In 2001, a **cell-based model of coagulation** was introduced by Maureane Hoffman and Dougald M. Monroe III (Hoffman & Monroe, 2001). In this model, they proposed “that coagulation occurs not as a "cascade", but in three overlapping stages:

1) **Initiation**, which occurs on a tissue factor (TF) bearing cell” (when TF binds to circulating factor VIIa, and in the presence of other factors leads to the generation of a small amount of thrombin);

2) “**Amplification**, in which platelets and co-factors are activated to set the stage for large scale thrombin generation” (occurring on the surface of activated platelets); and

3) “**Propagation**, in which large amounts of thrombin are generated on the platelet surface” (the thrombin burst). This currently accepted model of coagulation emphasises the pivotal role of TF-FVIIa complex, the importance of platelet activation and function, and the generation of thrombin in the promotion and inhibition of

coagulation (Curry & Pierce, 2007). Two further steps were identified to complete the model:

4) "**Stabilisation**" (in which soluble monomers were crosslinked by FXIIIa to stabilise the thrombus) and

5) "**Inhibition of further coagulation**" (to prevent the pathological extension of the thrombus and thus localising it to the area of injury. (Curry & Pierce, 2007)

The Standard Tests of Coagulation

For these tests to be adequately performed and accurately interpreted, standard guidelines on sampling techniques and blood collection must be adhered to. Poor sampling techniques may adversely affect the laboratory's ability to accurately process the sample and provide accurate results. Whole blood specimens are collected in tubes to which a calcium-chelating agent (EDTA or citrate), in a 9:1 ratio of blood to anticoagulant, has been added to prevent the specimen from clotting. If the specimen tube is underfilled, the plasma volume of the specimen relative to the anticoagulant will be reduced and lead to impaired accuracy of the results obtained from these blood tests, resulting in falsely prolonged results. Routine tests of coagulation are typically performed on platelet poor plasma, which is prepared from whole citrated blood which has been centrifuged (Sultan, 2010). This action negates analysing the contribution of primary haemostasis to overall coagulation.

Fibrinogen level

The reference range is usually 2.0 – 4.0 g/L (but this is dependent on the laboratory and assay performed). Fibrinogen levels were performed on citrated blood by the local National Health Laboratory Service (NHLS).

Several assays are currently available for the estimation of fibrinogen level. The NHLS utilises the Clauss assay (which is used by most laboratories) at present. According to Perry and Todd (2013), in this assay, plasma is diluted (usually 1:10) and then allowed to clot after exposure to a high concentration of thrombin. This test requires a reference plasma where the level of fibrinogen is known, and which is calibrated against an international standard. A calibration curve is generated using the reference plasma in a series of dilutions and the results are plotted on graph paper. There should be a linear correlation between clotting times of the test and reference samples. The time taken for the test specimen to clot is compared to the calibration curve and the fibrinogen level can be deduced.

Prothrombin Time (PT)

This test was first described by Quick in 1935. It is the time required for fibrin strands to form in the sample after tissue factor, phospholipid and calcium has been added to the decalcified, platelet poor plasma.

It measures the so-called extrinsic and common pathways in the classical model of coagulation and may be prolonged in deficiencies of fibrinogen, prothrombin; Factor (F) V, VII and X; DIC, vitamin K deficiency, liver disease and in patients receiving high doses of heparin.

International Normalised Ratio (INR)

This is the most sensitive test for a decrease in FVII, but it may also be affected by the factors which impair the PT. Initially, the term ‘thromboplastin’ was used for the substance that converted prothrombin to thrombin. Tissue thromboplastin was extracted from the brain and other organs and contained a significant amount of tissue factor and phospholipid. The different thromboplastins utilised in PT testing provide several ranges for PT; however, the INR uses a standardised thromboplastin reagent and “it is the ratio of the patient’s PT to a control PT” (Curry & Pierce, 2007), corrected for the sensitivity of the thromboplastin.

Activated Partial Thromboplastin Time (aPTT)

This test measures the functioning of the intrinsic and common pathways of the classic model of coagulation. It is also performed on platelet poor plasma. The aPTT was described in 1953 and initially only the phospholipid concentration was controlled (as opposed to the phospholipid and surface activator concentrations), hence ‘partial thromboplastin’ (phospholipid is present, but there is no Tissue Factor). These phospholipid concentrations could accelerate clotting in normal plasma, but it did not correct clotting times for haemophilic plasma. Current automated aPTT testing utilises micronised silica and ellagic acid as a surface activator (these bind directly to FXII, causing its activation). Kaolin (which was historically used as the activator for this test) is no longer used as its opacity interferes with the optical detection of the endpoint.

The Bleeding Time (BT)

Perry and Todd (2013) described the BT as a tool to predict “the risk of bleeding in relation to surgery”. The BT is dependent on an adequate number of functional platelets and was thus developed to assess platelet function. The reference range is between 2 – 7 minutes; however, this differs depending on the method used. Several methods have been developed, with the DUKE, the IVY and TEMPLATE methods being well described.

The DUKE method (Duke, 1910) was described by the US pathologist William Waddell Duke (1883 – 1945), who was the first to conclusively demonstrate the role of platelets in haemostasis (Soyoral et al., 2012; Tocantins, 1946). This method was described by using a special needle to nick the earlobe. However, due to the high vascularity in this tissue, it is no longer used.

The IVY method (a modification of the Duke Method by Ivy, Nelson and Bucher) (1941) is the traditional format of the test and utilises a blood pressure cuff inflated to 40 mmHg on the upper arm. A lancet or scalpel blade is used to make a stab wound on the palmar aspect of the forearm. The timer is then commenced, and every 30 seconds blood is drawn off with filter paper or paper towel, until the bleeding has stopped. The filter paper can disturb the development of the platelet plug if it encounters the edge of the thrombus, so care should be exercised when blotting blood away.

The TEMPLATE method was developed to standardise the IVY method and involves the same preparation, however, an automatic blade which can make a standard-sized incision (±6mm in length x 1mm in depth) on the palmar aspect of the forearm is utilised.

The BT is not widely used due to difficulties in standardisation of the procedure, the wide variability of results and inter-operator differences; however, it is still routinely performed prior to an invasive procedure (e.g. renal biopsy) in patients with renal dysfunction.

Viscoelastic Tests (TEG® and ROTEM®)

The conventional laboratory tests of coagulation described above (e.g. PT, INR, aPTT, BT) assess components of the “classic coagulation cascade”; however, they do not adequately assess coagulation in its entirety. Although these tests provide valuable information, they can only provide isolated details on individual components, they have a longer turnaround time and require several millilitres of whole blood samples. The standard tests of coagulation need special preparation and highly trained staff and often have to be transported to a laboratory which may not be on-site.

Near-patient or point-of-care testing has been documented since the English physician, Thomas Willis (27 January 1621 – 11 November 1675) described the tasting of the urine from a patient suspected of diabetes as a test for glycosuria (Hobbs, 1996). A point of care test (POCT) is defined by the International Organisation of Standardisation (ISO) as “testing that is performed near or at the site of the patient with the result leading to a possible change in the care of the patient”. “Near-patient test” is the preferred terminology used in the United Kingdom. These medical diagnostic tests provide reliable results faster than those processed through a laboratory, decreasing the turnaround time, ensuring that care is continued with the desired information readily available and capable of influencing rapid decision making (Delaney et al., 1999).

Thromboelastography (TEG®) has been shown to be a suitable near-patient ex vivo test, serving as a dynamic measure of coagulation. The TEG® was developed in Germany during World War II and was first described in the scientific literature by Professor Helmut Hartert, from the University of Heidelberg, in 1948. It is a visco-elastic measure of coagulation that provides a global assessment of whole blood thrombus formation, stabilisation and fibrinolysis which reflects in vivo haemostasis as well as providing information on both cellular and non-cellular components involved in thrombus formation.

Rotational thromboelastometry (ROTEM®) was developed in 1997 by Andreas Calatzis (MD) and Pablo Fritzche (a physicist) as a modification and enhanced form of thromboelastography and is a worldwide TEG® competitor (Fritsma, 2015). These tests can provide the clinician with information about the "interaction of the coagulation cascade, fibrinogen and the platelets as an entire unit" (Timmermans et al., 2009) and be used to guide the replacement of blood and blood components in the surgical patient (Johansson et al., 2008). TEG® is utilised by both anaesthesiologists and surgeons to monitor coagulation factor deficiency, heparin therapy, platelet function and fibrinolysis in cardiac surgery, and over the last 20 years it has proven "vital to the management of coagulopathy secondary to shock and massive haemorrhage in trauma and surgery" (Fritsma, 2015). Limitations with TEG® include its susceptibility to vibrations and interferences with the surface the machine is placed on, whereas with ROTEM® these limitations are negated.

TEG® and ROTEM® may be performed on fresh whole blood or on whole blood which has been collected in 3.2% sodium citrate specimen tubes. The TEG® sample volume is 360µL and for ROTEM® it is 340µL. The TEG® can perform two assays at the same time, while ROTEM® can perform up to 6 additional tests (e.g. INTEM, EXTEM, FIBTEM, APTEM, HEPTAM and ECATEM).

Renal Failure

Renal failure can be divided into an ACUTE, CHRONIC or an ACUTE-ON-CHRONIC pathological process based on the patient's history, presentation and the time frame of the disease process. Much debate was previously encountered as to the precise definitions of these different entities. A lack of standardised definitions made it difficult to compare and interpret information obtained from different centres, and often spawned the generation of several guidelines targeting the same pathology. This practice was invariably driven by funding of professional societies worldwide by the pharmaceutical industry as a matter of self-interest.

In an effort to standardise the definition of each pathological process, KDIGO (Kidney Disease: Improving Global Outcomes) was founded in 2003 “as an independently incorporated non-profit foundation governed by an international executive committee”. It was initially under management of the United States’ National Kidney Foundation (NKF). However, to facilitate its image as the developer of international guidelines targeting the global community, and not only the American populace, KDIGO obtained its status as an independent organisation under self-management on 01 October 2012. KDIGO is the sole international guideline organisation in nephrology and has published 9 clinical practice guidelines to date, with two new guidelines under development.

Their mission statement is to improve the management and outcomes of patients with renal disease by the development and implementation of evidence based clinical practice guidelines.

In March 2012, KDIGO released its clinical practice guideline for ACUTE KIDNEY INJURY (AKI), defined as an abrupt decrease in kidney function. It is a clinical syndrome which encompasses several aetiologies. The definition and staging of AKI are based on RIFLE (Risk, Injury, Failure, Loss, End-stage Renal Disease) and AKIN (Acute Kidney Injury Network) criteria ("Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO Clinical Practice Guideline for Acute Kidney Injury," 2012). AKI is defined by the KDIGO criteria when any of the following are present:

- Increase in SCr (serum creatinine) by ≥ 26.5µmol/l within 48 hours
- Increase in SCr to ≥ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; or
- Urine volume ≤ 0.5 ml/kg/h for 6 hours

and staged for severity (into stages 1, 2 or 3) based on the rise in SCr and reduction in urine output. Protocols have been developed for the identification of the cause of AKI and the management of this disease process.

Chronic Kidney Disease (CKD), as defined by KDIGO, is the presence of abnormalities in kidney structure and function which are present for more than 3 months and has implications for health. It is classified according to the aetiology (based on the presence or absence of systemic disease and the location within the kidney), GFR category and albuminuria category. ("Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease.," 2013).

The Effects of Uraemia on Coagulation

Uraemia does not represent one single uraemic toxin but refers to several toxins (urea, creatinine, parathyroid hormone, β2 microglobulin, polyamines, advanced glycosylation end products, cyanate, phenols and other "middle molecules") which become elevated in individuals with deteriorating renal function. This leads to a clinical syndrome associated with fluid and electrolyte abnormalities, hormonal imbalances and metabolic dysfunction. It is usually associated with the later stages of CKD, but it can occur if loss of renal function is rapid in AKI. A bleeding tendency has been described as characteristic in patients with end-stage renal disease, which is thought to be due to platelet dysfunction resulting in a disturbance in platelet adhesion and aggregation (Soyoral et al., 2012). However, anaemia, dialysis (due to the use of heparin during dialysis) and drug accumulation have also been identified as other plausible causes for the disturbance in haemostasis. Dialysis has been shown to only partly reverse the haemostatic disturbance caused by uraemic toxins.

The need for further research

The exact mechanism by which the coagulation pathway, in a patient with elevated levels of urea and/or creatinine, is impaired remains to be identified and further research in this field is necessary. In our literature search we encountered numerous citations to the bleeding tendency which develops; however, less information regarding the hypercoagulable state is available. Even though patients with renal failure have potential for a range of coagulation abnormalities, these patients are often denied access to interventions due to the risk which the associated pathology introduces. These patients would benefit from having their coagulation monitored with thromboelastography, and where there is doubt regarding platelet function a platelet function analyser may be utilised. TEG® provides a global assessment of coagulation and may prove helpful in assessing coagulation defects in patients with elevated levels of urea and/or creatinine presenting for renal biopsy. The final decision rests with the attending physician who should practice discretion when ordering special investigations, to always question whether an invasive procedure is necessary and to search for the possibility of a non-invasive option.

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Part D: Manuscript

An observational study to assess coagulation abnormalities in patients with elevated levels of urea and/or creatinine secondary to renal failure, presenting for renal biopsy – challenging conventional testing using visco-elastic testing

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Abstract:

Introduction

Coagulation abnormalities are well described in patients with elevated levels of urea and/or creatinine secondary to renal failure. These range from hypercoagulable to hypocoagulable states due to a range of mechanisms well described in the literature. Conventional tests of coagulation such as INR and PTT do not adequately assess these disorders of coagulation. Thromboelastography (TEG®) has proven to be a suitable alternative test of coagulation that serves as a dynamic test of global coagulation including assessment of thrombus formation as well as its breakdown. TEG® and ROTEM® assesses the visco-elastic properties of blood in vitro to define in vivo coagulability. The standard of care in our institution to assess the bleeding risk in patients with renal failure (defined by a raised urea and/or creatinine level) presenting for a renal biopsy is to use the conventional tests of coagulation, including a bleeding time if their creatinine is above 300 µmol/L. The aim of this study is to evaluate the conventional standard laboratory tests of coagulation (including a bleeding time where available), TEG® and ROTEM® in assessing coagulation disorders in patients with elevated levels of urea and/or creatinine presenting for renal biopsy.

Methodology

Patients with elevated levels of urea and/or creatinine presenting for a renal biopsy will be identified by the nephrology team responsible for their medical management. Prior to the renal biopsy, these patients will be approached by the study team and reviewed for inclusion into the study. Informed consent will be obtained on agreement to participate in the study. We will collect a blood sample for the TEG® and ROTEM® and this test will be performed by a laboratory technician in the Department of Anaesthesia. The clinician/nephrologist performing the biopsy will not be influenced by the outcome of these viscoelastic tests. A convenience sample of a minimum of 25 patients with renal impairment presenting for a renal biopsy will be included in this study.

Results

A total of 44 adult participants was entered into this observational study. Results for 1 participant were excluded from this study as their biopsy was delayed, allowing their renal function to improve and return to normal with medical management on the day that they presented for a renal biopsy. 43 patients were worked up for a renal biopsy but only 38 patients proceeded to a renal biopsy. Of these, only 31 patients had a bleeding time performed on the day of their renal biopsy. The participants ages ranged from 24 to 69 years and included 24 male and 19 female participants. Renal biopsies were cancelled by the consultant nephrologist in 5 patients on the day of their biopsy. Control samples, from 10 members in the Department of Anaesthesia, fell within the specified range of the various manufacturers. An interesting TEG® result was an average MA result of 74.22 mm (normal range 64 – 72 mm), which lies above the upper limit of normal. Two patients developed a small renal haematoma on ultrasound after the biopsy, with 1 of these patients also developing haematuria.

Conclusion

TEG® and ROTEM® provides a global assessment of coagulation and might be helpful in assessing coagulation defects in patients with elevated levels of urea and/or creatinine presenting for a renal biopsy, with possible extension to the surgical patient with abnormal renal function presenting for a surgical procedure to assess their risk of bleeding, especially in those who are being considered for a regional or neuraxial technique - as this could be an unacceptable risk in this population sub-group.

Background:

It is well known that patients with renal dysfunction (either Acute Kidney Injury (AKI), Chronic Kidney Disease (CKD) or acute-on-chronic kidney disease) have elevated levels of urea and other nitrogenous waste products circulating in their blood (Berns & Coutre, 2015; Soyoral et al., 2012; Stammers et al., 1998). The range of coagulation abnormalities from hypercoagulable to hypocoagulable states, due to several postulated mechanisms (possibly due to an imbalance in procoagulant and anticoagulant factors, platelet interaction in patients with elevated levels of urea, platelet-vessel wall interactions, platelet abnormalities, anaemia, thrombocytopaenia, drug interactions, or renal replacement therapy) (Boccardo et al., 2004), are well described in the literature (Berns & Coutre, 2015; Kaw & Malhotra, 2006; Soyoral et al., 2012; Stammers et al., 1998). Patients with renal dysfunction may therefore “experience two opposite haemostatic complications: bleeding diatheses and thrombotic tendencies” (Boccardo et al., 2004). Bleeding may range from an insignificant bleed (such as purpura) to a serious gastro-intestinal haemorrhage complicating either AKI or CKD. With the introduction of dialysis and erythropoietin, bleeding complications have decreased (Berns & Coutre, 2015; Boccardo et al., 2004; Soyoral et al., 2012); however, it still impacts on clinical decision making and limits invasive procedures in renal failure patients. These patients can present to surgery and can be a challenge to the anaesthesiologist when considering possible interventions.

Conventional tests of coagulation (e.g. INR, PTT, BT) assesses components of the coagulation cascade (Slaughter, 2010); however, they do not adequately assess coagulation abnormalities. These tests only provide isolated information on individual components of the coagulation cascade.

Thromboelastography (TEG®) and rotational thromboelastometry (ROTEM®) have been shown to be suitable ex vivo tests, serving as dynamic measures of coagulation. TEG® and ROTEM® are visco-elastic measures of coagulation and provide a global assessment of whole blood thrombus formation, stabilisation of the thrombus, as well as fibrinolysis - which reflects in vivo haemostasis. ROTEM® is a modification of thromboelastography. These tests provide the clinician with information on “the coagulation cascade, fibrinogen and the platelets” and their interaction “as an entire unit” (Timmermans et al., 2009) and can guide the replacement of blood and blood components in the surgical patient (Johansson et al., 2008).

TEG® was first described by Hartert in 1948 (Stammers et al., 1998; Whiting & DiNardo, 2014) as a device that provided information about the nature of whole-blood coagulation, the strength of the thrombus and its breakdown. It provided information on both cellular and non-cellular components involved in coagulation. This point of care system assesses the visco-elastic properties of blood being tested and provides a global assessment of coagulation (Curry & Pierce, 2007). The machine consists of a pin and oscillating cup (cuvette) (Stammers et al., 1998). 360 uL of whole blood is placed in a heated cup (at 37°C) and a pin is suspended from a torsion wire into the sample. The torsion wire is connected to a transducer and its movement is transduced and interpreted by a computer. The cup is subjected to oscillations through an angle of 4°45' with the duration of each rotation cycle lasting 10 seconds. As the blood coagulates, fibrin strands form between the blood in the cup and the pin. The rotation of the cup is passed to the suspended pin, which in turn begins to rotate with the oscillating cup. A transducer, capable of sensing and amplifying these changes in the force, passes this information through a microprocessor to be interpreted and displayed by a computer as a TEG® profile. Specific patterns (Curry & Pierce, 2007; Whiting & DiNardo, 2014) are represented in Addendum I.

ROTEM® (or TEM®) is an enhancement of TEG® and it provides a similar trace to that of TEG®, however different coagulation activators are utilised. Instead of the signal being transmitted via a torsion wire, with ROTEM® the signal from the pin is transmitted via an optical detector system. The cup (cuvette) remains immobile, while the pin slowly oscillates within the whole blood sample of 340µL, and as the thrombus forms the speed of oscillation of the pin decreases. The speed of oscillation of the pin is detected by an optical detector system which utilises a light source and a mirror mounted on the pin. Additional tests with ROTEM® include:

INTEM: Phospholipid and ellagic acid are added to the blood sample as activators. It is similar to the aPTT and thus essentially analyses the INTRINSIC pathway. It provides information on factor deficiencies and can detect the presence of anticoagulants.

EXTEM: Tissue factor is added as the activator. The information provided by this test is similar to that obtained by Prothrombin Time. It essentially analyses the EXTRINSIC pathway.

FIBTEM: Cytochalasin D (a platelet inhibitor) is used in this test together with tissue factor. The contribution of platelets to coagulation is thus removed. It is possible to analyse the functional FIBRINOGEN component only.

APTEM: Aprotinin is added to the blood sample. It effectively inhibits fibrinolysis.

HEPTEM: Heparinase is added which deactivates heparin.

ECATEM: Ecarin is added to the sample. This test is a sensitive test to the presence of direct thrombin inhibitors.

A typical ROTEM® trace for a normal individual is included in Addendum J.

In this study, we attempted to evaluate the ability of coagulation parameters of the thromboelastograph (TEG®) and rotational thromboelastometry (ROTEM®) to identify coagulation abnormalities in patients with elevated levels of urea and/or creatinine secondary to renal failure, presenting for a renal biopsy. We also collected conventional coagulation tests (e.g. BT, INR, PTT) but statistical comparisons between these methods were not to be performed. A convenience sample of a minimum of 25 patients presenting for a renal biopsy, was to be tested in this observational case series. The primary endpoint was an observational study of the coagulation disorders present in this sample of patients.

Methodology:

Patients elevated levels of urea and/or creatinine (renal impairment) presenting for work-up for a renal biopsy at Groote Schuur Hospital were identified by the nephrologist in the Renal Unit who informed the study investigators (from the Department of Anaesthesia) about potential candidates. Work-up for a renal biopsy included a referral to the Renal Unit, the biopsy could only be booked with the renal senior registrar and (as per the "criteria") a BT was only performed if the creatinine was > 300 µmol/L.
On the day of biopsy, the current protocol regarding haematological criteria for eligibility for a renal biopsy included (Addendum C):

- A platelet count ≥ 100 x 10^9/L
- PTT < 40 seconds
- INR ≤ 1.4
- bleeding time ≤ 10 min, or ≤ 12 min if an emergency biopsy.

These "criteria" are observed as a guideline only, with the final decision for or against proceeding with the biopsy made by the consultant nephrologist on the day of the biopsy.

The TEG® and ROTEM® devices will be used as an alternative method of assessing the coagulation profile. This overcomes the technical difficulties of performing a bleeding time, as well as providing a more comprehensive in vitro assessment of coagulation. The primary outcome is thus to assess whole blood coagulation, using standard parameters generated by the TEG® and ROTEM® traces. In addition, fibrinogen concentrations (Wolberg & Campbell, 2008) will also be measured.

A representative from the renal unit will inform the study group of patients on the day of the planned renal biopsy. Research participants will include both male and female adult subjects (age > 18) of any ethnicity with elevated levels of urea and/or creatinine presenting for a renal biopsy. These patients will be approached by a representative from the study group and included into the study only once informed consent (Addendum D) has been granted and prior to the provision of any sedation. Exclusion criteria included patient refusal, LMWH/heparin therapy, antiplatelet medication and renal replacement therapy.

Informed consent (Addendum D) was obtained on the day of the scheduled biopsy, prior to the provision of premedication. Blood sampling was standardised, blood being drawn using a Vacutainer (BD Vacutainer Systems, Plymouth, England). The initial blood draw of 3 mL was discarded, where after a whole-blood sample was collected in a 2 mL syringe and dispatched to the Department of Anaesthesia's laboratory. A further 2 samples were collected in two 4 mL Greiner 9 NC Coagulation Sodium Citrate 3.2% tubes (BD Vacutainer) – 1 sample being sent to the local laboratory (NHLS) for fibrinogen testing and the other sample to the study laboratory as a secondary sample. The TEG® was measured on the TEG® 5000 Thromboelastography Haemostasis Analyser System (Haemoscope) made available to us in the laboratory of the Department of Anaesthesia. ROTEM® was performed and analysed on the Viking ROTEM® Delta haemostasis analyser. The software for TEG® analysis was performed on TEG®V4.2.3. TEG® parameters included measurement of R-time (reaction time), K-time (kinetics), maximum amplitude (MA) and coagulation index (CI) and was performed for each patient.

ROTEM® was also performed for each patient as well, which included clotting time (CT), clot formation time (CFT), alpha (α) angle and maximum clot firmness (MCF). These additional tests included INTEM (essentially analysing the intrinsic pathway), EXTEM (analysing the extrinsic pathway), FIBTEM (the addition of a platelet inhibitor, which prevents platelets from contributing to thrombus formation thus allowing analyses of the functional fibrinogen component) and APTEM (addition of aprotinin to inhibit fibrinolysis).

Both TEG® and ROTEM® were performed by the same laboratory technologist, to ensure standardisation of the tests. Ten staff members from the Department of Anaesthesia provided blood as a means of standardising quality control during the study. All the results from the control samples fell within the specified ranges of the manufacturers.

Statistical analysis was performed using a standard statistical package (Statistica Version 13, Dell Inc, Tulsa, OK) and data were evaluated in terms of means, medians, standard deviations and ranges. These measures were compared with the standard ranges for conventional tests, quoted by the instrument manufacturer, to identify possible coagulation abnormalities in this patient population (Pearson correlation).

Results:

A total of 44 adult participants was entered into this observational study. Results for 1 participant (098/2013/26) were excluded from this study, as their biopsy was delayed which allowed their renal function to improve and return to normal, with medical management, on the day that they presented for a renal biopsy. 43 patients were worked up for a renal biopsy but only 38 patients proceeded to a renal biopsy. Of these, only 31 patients had a bleeding time performed (which should only be performed if creatinine > 300 µmol/L as per the protocol) on the day of their renal biopsy. The age range of the participants in this study ranged from 24 to 69 years (with an average age of 41.3 years) and included 24 male and 19 female participants. Renal biopsies were cancelled by the consultant nephrologist in 5 patients for several reasons (Table 1: Cancellation of BiopsyTable 1) on the day of their biopsy. An interesting TEG® result was an average MA result of 74.22 mm (normal range 64 – 72 mm), which lies above the upper limit of normal. Two participants in the study developed a small renal haematoma on ultrasound after their biopsy (098/2013/26 was excluded from the study), with 1 of these patients also developing haematuria.

All the results from the control samples, which comprised of 10 members from the Department of Anaesthesia, fell within the specified ranges of the various manufacturers.

Table 1: Cancellation of Biopsy

Patient	Reason for cancellation
098/2013/19	The patient had a left mononephric, horseshoe kidney on U/S and biopsy was declined.
098/2013/23	The biopsy was postponed on 2 occasions (first due to a low haemoglobin and then due to a prolonged bleeding time). The patient was then lost to follow-up.
098/2013/36	The biopsy was postponed due to thrombocytopaenia. The patient deceased prior to the follow-up date.
098/2013/40	The patient had bilateral echogenic kidneys and the biopsy was deemed unnecessary.
098/2013/109	The patient was found to have a dilated cardiomyopathy with a poor ejection fraction (31%) and was rejected from further work-up for the renal replacement programme.

The normal range for the platelet count in the laboratory (NHLS) was 178 – 400 x 10^9/L. Of the 43 patients, the range for the platelet count was 95 – 863 x 10^9/l, with the average platelet count being 346.53 x 10^9/L.

One fibrinogen level was not performed by the NHLS due to incorrect information being captured by the NHLS (laboratory error). This result was therefore discarded. Thus, only 42 fibrinogen results were available for evaluation. The normal range for fibrinogen is 2.0 – 4.0 g/dL. The range in the study was 1.4 – 6.0 g/dL, with the average being 3.61 g/dL. There is no correlation between FIBTEM MCF and fibrinogen concentrations (Pearson’s correlation r = 0.12).

The normal range for bleeding time is 2.30 – 9.50 min. Only 31 patients had bleeding times performed, as their creatinine levels exceeded the upper acceptable limit (creatinine > 300 µmol/L). The average bleeding time for these 31 patients was 8.05 min, with the range being 3.5 – 15.0 min.

The normal range for creatinine in the NHLS laboratory is 64 – 104 µmol/L. The average creatinine value was 607.84 µmol/L, with the range being 98 – 1486 µmol/L in our study population.

The normal range for the R time is 4 - 8 min. The average R time was 7.13 min, with the range for the results being 1.8 – 11.9 min.

The normal range for the K time is 0 - 4 min. The average K time was 1.62 min, with the range for the results being 0.8 – 4.4 min.

The normal range for the Maximum Amplitude (MA) is 64 – 72 mm. The average MA was 74.22 mm (which lies above the upper limit of normal), with the range for the results being 36.5 – 87.9 mm.

These results presented above are summarised in Table 2, with the full results sheet available as Addendum K in Part E: Supporting documents section.

10 patients included in the study were diagnosed with End Stage Renal Disease (ESRD) after their biopsy, by the Department of Nephrology at Groote Schuur Hospital.

Table 2: Comparison of results with TEG®

	Number of patients	Normal range	Average	Range in study results
Platelet count (units: x 10^9/L)	43	178 – 400	346.53	95 – 863
Fibrinogen (units: g/dL)	42	2.0 – 4.0	3.61	1.4 – 6.0
Bleeding time (units: min)	31	2.30 – 9.50	8.05	3.5 – 15
Urea (units: mmol/L)	43	2.6 – 7.0	24.07	4.9 – 61.4
Creatinine (units: µmol/L)	43	64 – 104	607.84	98 – 1486
R time (units: min)	43	4 - 8	7.13	1.8 – 11.9
K time (units: min)	43	0 – 4	1.62	0.8 – 4.4
MA (units: mm)	43	54 – 72	74.22	36.5 – 87.9
Alpha angle (units: degrees)	43	47 – 74	69.2	36.5 – 81.0

Table 3 summarises the results of the ROTEM® analysis. Of note, are the remarkable increases in the INTEM MCF, EXTEM MCF and FIBTEM MCF results, particularly in view of the absence of correlation between these results and the fibrinogen concentrations.

Table 3: Comparison of results with ROTEM®

	Normal range	Mean (SD)	Above Range (n)	Below Range (n)
INTEM CT (units: s)	100-240	158.8 (33.1)	0	1
INTEM CFT (units: s)	30 – 110	45.4 (29.5)	2	9
INTEM MCF (units: mm)	50 – 72	81.8 (2.9)	43	0
EXTEM CT (units: s)	38 – 79	74.2 (32.9)	12	0
EXTEM CFT (units: s)	34 – 159	47.3 (19.8)	0	5
EXTEM MCF (units: mm)	50 – 72	80.8 (3.5)	43	0
FIBTEM MCF (units: mm)	9 – 25	79.2 (5.3)	43	0

Discussion:

The performance of a renal biopsy remains a high-risk procedure and bleeding can have devastating consequences. It is essential to assess bleeding risk appropriately in these patients presenting for a renal biopsy. The exact coagulation disturbance in patients with renal impairment remains elusive. It has been proposed that the coagulopathy may be due to the consequences of elevated levels of urea on platelet function (Boccardo et al., 2004). Even though excessive post-surgical bleeding in patients with renal impairment is well documented (Berns & Coutre, 2015; Boccardo et al., 2004; Pivalizza, Abramson, & Harvey, 1997; Timmermans et al., 2009), there is also an associated increase in the incidence of thrombotic phenomena. In a study on coagulation in uraemic patients using TEG® (Pivalizza et al., 1997), the group could demonstrate a hypercoagulable state in the TEG® indices. A study performed by Edwards, Morgan and Donnelly (1993) could also demonstrate hypercoagulability in uraemic patients using TEG® (Timmermans et al., 2009). The main finding of our study suggested a tendency towards a hypercoagulable state in many of the patients with elevated levels of urea and/or creatinine. The TEG® data showed that most patients had a normal onset of coagulation but that the MA and alpha angle both suggest a tendency towards hypercoagulation. The ROTEM® data showed a similar trend with both INTEM and EXTEM showing enhanced maximum clot firmness (MCF). The most interesting ROTEM® observation is the increase in the FIBTEM MCF (results for all participants were above the upper limit of normal). This is difficult to explain but possibly indicates that factor-based coagulation (as opposed to cell-based) is markedly enhanced in these patients. We could speculate that platelet function is normal or perhaps slightly diminished, but that factor-based coagulation is accelerated (possibly explained by the raised FIBTEM MCF). The mechanism for this may either be due to the fact that fibrinogen is an acute-phase protein whose activity is stimulated by the disease or that there is a relative lack of one of the inhibitory components, possibly antithrombin III. It is also possible that endothelium-derived thrombomodulin-driven regulation is impaired, but this is not examined in ex-vivo studies such as this study. Whatever the mechanism, the finding is very interesting given the problems that the renal dialysis teams experience with thrombosis of arterio-venous shunts. Analyses of the standard coagulation data, on average, fall within normal limits. Average fibrinogen levels were noted to be normal to only slightly elevated (Addendum L). However, several patients in our study (16 out of 42 participants with fibrinogen results) had elevated fibrinogen levels. A further interesting observation from the standard coagulation tests is that there is a weak, non-significant, correlation between rising creatinine and platelet count (Addendum M) and a somewhat stronger, significant correlation between creatinine and bleeding time (Addendum N). This helps to explain the rather confusing paradox that is so widely reported in patients with renal failure, that although they may demonstrate a bleeding tendency, they may also be at increased risk of venous thrombosis. Several limitations of this study are identified. The lack of a comparison group with randomisation to compare visco-elastic testing to conventional tests of coagulation. This study was purely observational, and the biopsy only proceeded if the consultant nephrologist on duty on the day of biopsy deemed that the biopsy was necessary. The functioning of platelets in patients with renal failure needs to be further assessed with some form of platelet function analyser – a subject for future research.

Conclusion:

This prospective observational study of patients with elevated levels of urea and/or creatinine presenting for renal biopsy demonstrated that the coagulation status as depicted by visco-elastic tests revealed no evidence of bleeding risk and tends to suggest that large numbers of these patients are, in fact, hypercoagulable as almost 70% of the participants in this study had a maximum amplitude above the upper limit of normal. This was further substantiated by the elevation in fibrinogen levels and the enhanced fibrinogen contribution to coagulation as illustrated by the ROTEM® results, although the absence of significant correlation between these results tends to argue against a cause-and-effect phenomenon.

The strengths of this study are that conventional testing and visco-elastic testing were analysed side-by-side in an observational manner. The patients were followed up for bleeding complications post procedure. The incidence of bleeding post renal biopsy was considered low (2 out of 38 patients developed a haematoma); however, this complication would be unacceptable in patients receiving regional or neuraxial techniques. Conventional bleeding times showed no correlation with visco-elastic testing. The overall finding was that the coagulation profile of patients with renal failure showed a normal distribution using visco-elastic testing. The fibrinogen levels were surprisingly normal to slightly elevated in the entire cohort which underpins the enhanced coagulation seen in these patients (Addendum L).

This pilot study is firmly hypothesis generating and paves the way for a comparative trial to evaluate conventional tests of coagulation and the visco-elastic tests to evaluate bleeding and/or the bleeding risk in patients with impaired renal function. There were no notable serious haemorrhagic complications post-biopsy in the patient group, who participated in this study, when they presented for their renal biopsy. The normal to elevated fibrinogen levels noted in some of the participants - could possibly account for the enhanced coagulation profile seen in some participants in this study. Platelet function is of concern in these patients but unless fibrinogen levels are low, the findings from this pilot study suggest that coagulation may not be affected.

Ethical considerations:

The study was approved by the Ethics Committee of the Faculty of Health Sciences of the University of Cape Town (Addendum B).

Participation in the study was voluntary, and participants completed an informed consent form (Addendum D).

Participants received a participant number and could only be further identified throughout the study with the participant number ensuring confidentiality and anonymity. All data was stored under the participant number (Addendum E and Addendum F).

Participants could withdraw at any time and without providing a reason.

All patients continued to receive the normal standard of care, irrespective of participation or non-participation in the study (Addendum C).

Participants were assured that they would be at no extra risk if they agreed to participate in the study.

Participants received no direct benefit from participation in the study but participation neither hindered nor changed the standard of care they received.

There was no cost, nor compensation for participation in the study.

No conflicts of interest were identified or declared.

The protocol complied with the Helsinki Declaration of 2008.

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Part E: Supporting documents

Addendum A: Departmental Research Committee Approval

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

As Principal Investigator of this research I am aware of a potential conflict of interest. Please describe and provide a plan to manage the conflict of interest in the space below:	

8. Declarations and Signatures

This application will not be processed unless all the required declarations and signatures are completed according to the Committee's Standard Operating Procedures.

8.1 Head of Department or Division
My signature confirms that:

i. The researcher(s)/student(s)/supervisor(s) have the skills, training, experience and time to undertake this research.
ii. There are adequate resources (e.g. equipment, space, support services) to perform this research.

Signature of Head	[signature]	Date	31 01 2013
Print name	R.A. MER		

Note: Where the PI is also Head of Department, confirmation must be obtained from an authorised designee. PIs may not approve their own research.

8.2 Chairperson of the Departmental Research Committee (DRC)
My signature confirms that:

i. This research has undergone peer review by a person(s) experienced in the field of study.
ii. This research is well-designed and scientifically sound.
iii. Where relevant, all methodological issues have been resolved to the satisfaction of the peer reviewer(s).
iv. If conducted according to the protocol, this research is expected to yield valid and useful findings.

Signature of Chairperson	[signature]	Date:	31 01 2013
Print name	R A MER		

Note: Where the PI is also the Chairperson of the DRC, confirmation must be obtained from an authorised designee. PIs may not approve their own research.

7 October 2010 Page 8 of 10 FHS013

8.3 Student supervisor (if research is for a degree)
My signature confirms that:

i. The student researcher has adequate training and resources to complete the research in the allocated timeframe.
ii. The research has scholarly merit.
iii. The level of risk inherent in the study is commensurate with the student researcher's experience and the extent of oversight that I will provide.
iv. I will meet the student on a regular basis to monitor progress and address any problems that may arise during the study.
v. If applicable, I will ensure that the research undergoes continuing review as required by the HREC.
vi. If applicable, I will ensure that the student researcher reports unanticipated problems or serious adverse events to the HREC.
vii. I will arrange for an alternative faculty supervisor to take responsibility for this research during periods of absence such as sabbatical or annual leave.

Signature of Supervisor	[signature]	Date:	4/2/2013
Print name	M. Miller		

Note: The supervisor and student researcher are jointly responsible for the ethical conduct of this research from inception to dissemination of findings.

8.4 Principal investigator
My signature confirms that:

i. Information in this application is true and accurate.
ii. I will begin the research only after HREC approval is obtained.
iii. I accept full responsibility for the conduct of this research and the protection of participants' rights and welfare.
iv. I will conduct the research according to all ethical, regulatory and legal requirements laid down in the HREC's Standard Operating Procedures.
v. I will provide progress reports to the HREC as requested, including a final closing report at the end of the research.
vi. I will notify the HREC in writing if any change to the research is proposed and await approval before proceeding with the proposed change except when urgently necessary to protect participants' safety.
vii. I will notify the HREC in writing immediately if any adverse event or unanticipated problem occurs during the research.
viii. I will allow an audit of my research if requested by the HREC.
ix. I have the time, training, experience and resources to oversee this research.

Signature of PI	[signature]	Date:	4/2/13
Print name	M. Miller		

Addendum B: HREC Approval

HREC Ref 098/2013 – 1Mar2013

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences
Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/research/humanethics/forms

1 March 2013

HREC REF: 098/2013

Dr M Miller
Department of Anaesthesia
Ward D4
NGSH

Dear Dr Miller

PROJECT TITLE: OBSERVATIONAL STUDY TO ASSESS COAGULATION ABNORMALITIES IN PATIENTS WITH URAEMIA SECONDARY TO RENAL FAILURE, PRESENTING FOR RENAL BIOPSY

Thank you for responding to the issues raised by the Faculty of Health Sciences Human Research Ethics Committee in your letter dated 21st February 2013.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study however, please correct minor errors in the consent form as follows:

1. Background (pg 1, para 3) – "There is" should be "There are"
2. Please remove "to the patient" from the "Risks" subheading.

Approval is granted for one year till the 15th March 2014

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/research/humanethics/forms)

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

Please quote the HREC. REF in all your correspondence.

Yours sincerely

pp T Burgess

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

s.thomas

Addendum C: Renal Biopsy Work-up Protocol

Renal Biopsy Work Up

Biopsies are performed every Tuesday and Thursday afternoon at 14h00 in C10 Ultrasound department. Biopsies are only done once the Renal Unit has been consulted.

Checklist:

Pre-biopsy:

1. Book biopsy with Renal Senior Registrar – have information on clinical details/indication/urgency
2. Book bleeding time for day of biopsy: x4129-only if creat>300umol/L
3. Obtain written, witnessed consent for the procedure
4. Fill in renal biopsy forms:
   "renal biopsy form" (PD546) in duplicate – obtainable in E13
   NHLS pathology form in duplicate

On Biopsy Day:

1. Check coagulation (early ie, before 08h00, or even the day before if they have not received heparin):
   Platelets $\geq 100 \times 10^9$/mL
   PTT < 40 seconds
   INR $\leq$ 1.4
   Bleeding time: $\leq$ 10 min, or $\leq$ 12min if an emergency biopsy

Bleeding time will **not** be performed unless all the bloods have been done.

2. Check BP $\leq$ 140/90 mmHg
3. Confirm that patient is suitable for biopsy with Renal Senior Registrar before 11h30 – he/she will then book the biopsy with C10 Ultrasound department
4. Pre-med @ 13:00 — Valium 10mg orally
   Pethidine 50mg IMI (75mg for larger pts)
   Atenolol 50mg orally (unless contra-indicated)
5. Ensure all consent and biopsy forms are sent with the patient

NB. Please write the results of the bleeding parameters at the top of the consent form.

Post Biopsy:

Call the renal registrar if there is marked haematuria or unexplained haemodynamic instability. Patients should be reviewed by a renal registrar (ideally the registrar who did the biopsy) before discharge.

GSH Renal Unit, June 2010

INSTRUCTIONS TO WARDS AFTER RENAL BIOPSY

1. Strict bed rest supine x 24 hours.
2. ½ hourly BP and pulse x 6 hours.
3. Fluids only x 4 hours.
4. Observe for bleeding from puncture site.
5. Samples of urine to be kept in separate specimen bottles at bedside to check for bleeding.
6. Contact the Nephrology Registrar on call and the patient's doctor if any of the following are present:
    a. Macroscopic haematuria
    b. Bleeding from biopsy site
    c. Abdominal pain
    d. Patient becomes restless
    e. Increasing pulse rate and/or dropping BP

Name: ____________________ Speed Dial: __________

Signature: ________________ Date:________ Time:___________

AFTER HOURS:
On Call Nephrology Registrar:________________ Speed Dial: ________

Addendum D: Consent Form

Informed consent form

TITLE: **Observational study to assess coagulation abnormalities in patients with uraemia secondary to renal failure, presenting for renal biopsy**

Researcher contact details

Principal investigator: Dr MGA Miller
Department of Anaesthesia
Ward D23
NGSH
Tel: 021 404 5003
malcolm.miller@uct.ac.za

You are being invited to take part in a research study. Before you decide to participate in this study, it is important that you understand why the research is being done and what it will involve. Please take the time to read the following information carefully. Please ask the researcher if there is anything that is not clear or if you need more information.

Background:

Patients with kidney failure often have a high level of chemicals and other proteins circulating in their blood – this is known as uraemia. This condition may lead to clotting abnormalities like excessive bleeding or too much clotting.

Patients with kidney failure, needing a biopsy, currently are given a test called a bleeding time. This test is used to find out if the patient is able to clot normally. This is important to make sure that the patient will not bleed abnormally during the biopsy. This test is performed bedside by a qualified medical practitioner according to strict rules and regulations.

There are also a number of specialised tests commonly performed in the laboratory to measure a patient's ability to clot normally. Even though these tests give a lot of useful information, they do not always give a complete picture of possible clotting abnormalities.

Thromboelastography (TEG) is a test that is able to give more detailed information than these standard lab tests. It gives a picture of clotting from the time the clot starts forming until its natural breakdown.

The purpose of this study is:

The reason for this study is to compare the standard bleeding time test used to measure clotting in patients needing kidney biopsies, with the newer TEG test.
At the time of your blood being taken for the usual testing, we will take an extra 10ml of blood to use for this new TEG test. The usual tests will still be performed. The result of the new test will not affect your treatment in any way.
We would like to have 25 patients willing to take part in this study.

Risks:

You will not be taking any extra risk when you agree to take part in this study. We will only take 10ml of blood extra over and above the blood you will give for the routine tests required for your biopsy. The blood will be taken by a qualified person in the renal unit at Groote Schuur Hospital and will be taken according to standard sterile procedures.

Benefits:

You will have no direct benefit from your participation in this study. We do however hope you learn more about the value of this TEG test. This information will hopefully improve our understanding of clotting abnormalities and may therefore be used to improve the general care offered to patients with kidney failure who need biopsies in the future.

Voluntary Participation:

Your participation in this study is completely voluntary. It is up to you to decide whether or not to take part in this study. If you do decide to take part in this study, you will be asked to sign this consent form. You are free to withdraw at any time and without giving a reason.

If for any reason, you do not want to take part in this study, your decision will not affect your treatment in any way. You will still receive the same treatment you would have if you decided to take part.

Costs to Patient:

There are no costs to you for your participation in this study.

Compensation:

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

Consent:

I, ____ (patient's name and surname), confirm that I have read and understood the information and have 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 a reason and without cost. I understand that I will be given a copy of this consent form.

Signature ________________________________ Date __________________

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

Dr MGA Miller

(Principal investigator)

021 404 5003

Lizel Immelman

(Sr Medical Tech at Dept Anaesthesia Coagulation Research lab)

021 406 6144

Prof M Blockman

(Chairperson UCT HSF Human Ethics)

021 406 6492

Addendum E: Data Collection Form

Case report form

March, 2013

UCT Anaesthetics Coagulation Research Laboratory D23)

Case report form

Observational study to assess coagulation abnormalities in patients with uraemia secondary to renal failure, presenting for renal biopsy.

Participant reference number: **098/2013/**

Control participant ☐ Test participant ☐

Patient personal details

Patient sticker

Ethnical group ____________________

Clinical details

Diagnosis ____________________

Medication ____________________

Is the patient receiving dialysis? ____________________

When was the last treatment? ____________________

Biopsy date ____________________

Does the patient have oedema? ____________________

Specimens taken:

2ml unheparinised syringe ☐

4,5ml citrate tube (blue top) ☐

2ml citrate tube (blue top) ☐

Specimens taken by ____________________

Date____________________ Time____________

Signature____________________

Results obtained:

FBC ☐ Bleeding Time ☐

U&E ☐ INR ☐

BUN, Creat ☐ PTT ☐

Fibrinogen ☐

Follow-up

____

____

____

Date:____________________ Signature:____________________

For any queries please contact:

Dr M. Miller (Principle investigator) 021 404 5003

Lizel Immelman (Snr Medical Technologist) 021 406 6144

Addendum F: NHLS Fibrinogen request form

TRIALS ONLY

GROOTE SCHUUR HOSPITAL
DEPARTMENT OF CLINICAL LABORATORY SCIENCES

LAB TRIAL NO. T 699	Fibrinogen Study
Location: T 0 6 9 9 *Fibrinogen Study*	PID: 0 9 8 - 2 0 1 3 - Sex: Initials: (first, middle, last) DOB: d d m m y y
Specimen: BLOOD Date taken: d d m m y y Time taken: H Taken by:	

Investigator: Dr Malcolm Miller	Tel: 021 404 5003	Account:
Co-ordiantor: Lizel Immelman	Tel: 021 406 6144	ZSTU 601

HAEMATOLOGY
tel. (021) 404-4129

CITRATE (blue top)	✓	Fibrinogen	FBGN

T699   H vers 1.1   16-May-13

NB. Fill in non-shaded areas only. No amendments allowed - specifically, no additional tests may be added.

Addendum G: Literature review 1

1. Predictive factors associated with adverse events in patients with toxic colitis: an analysis of the National Surgical Quality Improvement Project. Dayama A, Sugano D, Stone ME, McNelis J. Am J Surg. 2015 Nov;210(5):852-858.e1.

2. Risk factors for postoperative bleeding in ABO-incompatible kidney transplantation. Kim MH, Jun KW, Hwang JK, Kim JI, Chung BH, Choi BS, Kim YS, Yang CW, Moon IS. Clin Transplant. 2015 Apr;29(4):365-372

3. The safety of thoracentesis in patients with uncorrected bleeding risk. Puchalski JT, Argento AC, Murphy TE, Araujo KL, Pisani MA. Ann Am Thorac Soc. 2013 Aug;10(4):336-341.

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5. Invasive pneumococcal pneumonia is the major cause of paediatric haemolytic-uraemic syndrome in Taiwan. Lee CS, Chen MJ, Chiou YH, Shen CF, Wu CY, Chiou YY. Nephrology (Carlton). 2012 Jan;17(1):48-52.

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19. Recombinant human erythropoietin to correct uremic bleeding. Viganò G, Benigni A, Mendogni D, Mingardi G, Mecca G, Remuzzi G. Am J Kidney Dis. 1991 Jul;18(1):44-49.

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Addendum H: Literature review 2

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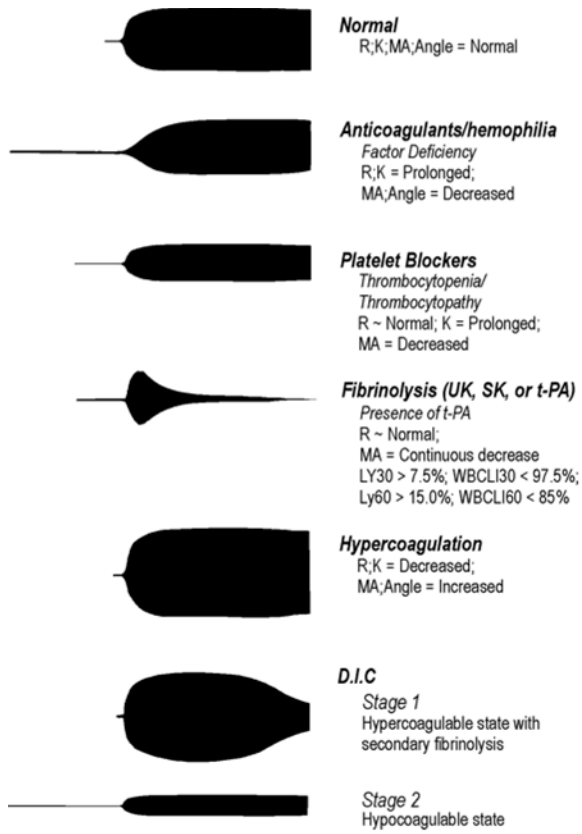
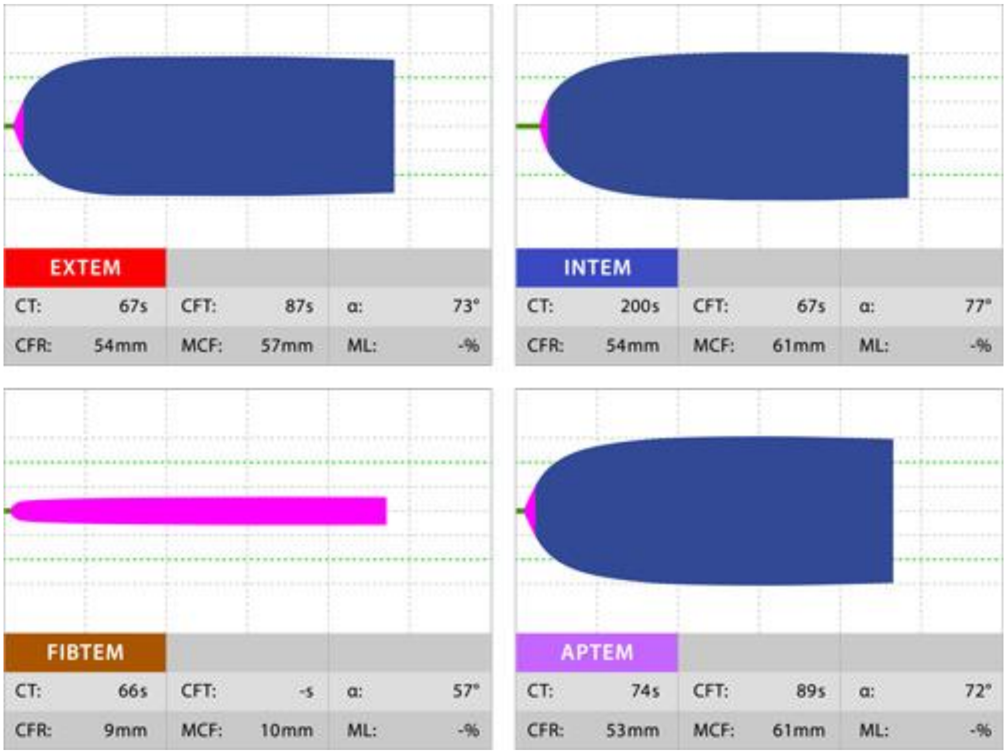
Addendum I: TEG® Trace



A depiction of a normal TEG® trace as compared to traces seen in various coagulopathic states

Addendum J: ROTEM® Trace



ROTEM® trace for a normal patient

(permission to use ROTEM® trace provided by Frederike Fritsch Product Manager, PBM for rotem.de, instrumentationlaboratory.com)

Addendum K: Complete results sheet

Participant	Hb	Platelets	INR	PTT (Pt/Control)	Fibrinogen	Bleeding Time	Urea	Creatinine	R Time	K Time	MA	CI	Bx Date
	12.0 - 15.0 g/dL	178 - 400x10⁹/L		23.0 - 38.0	2.0 - 4.0g/dL	2.30 - 9.50min	2.6 - 7.0mmol/l	64 - 104umol/l	4 - 8min	0 - 4min	54 - 72 mm	-3 to 3	
098/2013/01	7.7	661	1.02	31.4/30.9	2	5	5.6	196	6.9	0.8	79.6	3.1	10-Apr
098/2013/02	12.4	417	1.12	31.1/29.9	5.4	ND	15.7	165	6.7	1.2	84.7	3.5	18-Apr
098/2013/03	14	508	0.98	58.5/29.3	4.8	4	10	98	7.4	1.2	81.3	2.4	18-Apr
098/2013/04	5.5	361	0.99	27.8/31.1	2.5	14	9.2	172	5.1	1.1	78.9	3.9	18-Apr
098/2013/05	11.1	405	0.84	31.8/29.9	5.7	ND	7.7	101	6.3	1.2	86.8	4	2-May
098/2013/06	5.9	481	1.03	33.7/29.7	3.9	ND	41.4	906	6.1	1.3	84.1	3.7	21-May
098/2013/07	9.9	223	0.97	32.8/28.6	3.2	5	61.4	579	6.6	1.3	73.9	1.7	9-May
098/2013/08	7.5	211	0.94	33.9/29.8	1.5	7.45	21.6	621	1.8	1.1	78.2	6	9-May
098/2013/09	7.5	436	0.97	32.7/27.4	4.3	ND	12.6	139	6.7	1.2	82.5	3.2	9-May
098/2013/10	10.5	513	1.27	34.3/30.0	4.6	12.5	32.8	713	7.1	1.4	82.8	2.3	23-May
098/2013/11	11.4	229	1.18	36.7/30.0	3.3	10	30.3	907	10.5	2.6	76.9	-1.7	23-May
098/2013/12	7.2	403	0.98	26.6/29.3	2.9	ND	25.7	445	5.3	1.4	85.4	4.3	23-May
098/2013/13	9.1	323	1.1	24.7/29.6	3	15	43.3	1062	5.1	0.8	84.1	5	6-Jun
098/2013/14	8.7	327	1.15	28.7/36.0	4.1	6.5	21	333	8.6	1.6	87.9	2.4	4-Jun
098/2013/15	9.2	510	1.07	32.1/30.6	3.4	10.5	23.3	1249	10.8	1.6	79.3	-0.5	11-Jul
098/2013/16	6.9	165	1.24	30.8/29.6	ND	ND	24.9	845	7.8	2.5	75.3	0.1	16-Jul
098/2013/17	8.8	273	0.93	29.9/30.3	2.7	9.2	27	1184	7.4	1.6	78.6	1.7	16-Jul
098/2013/18	7.1	288	1.07	34.5/31.0	3.2	12.5	8.9	679	6.6	1.3	82.9	2.1	15-Aug
098/2013/19	6.4	230	0.84	29.3/21.9	1.7	5	40.4	670	4.8	1	76	3.8	ND
098/2013/20	6.4	118	0.97	36.5/30.5	4.5	11.5	22.7	568	8.1	4.4	68.8	-3.1	22-Aug
098/2013/21	15	442	1.03	31.9/30.2	3.5	5	17.5	447	10.5	2.7	77.1	-1.8	3-Sep
098/2013/22	11.9	266	1.09	30.0/29.1	3.9	5	29.7	556	6.7	1.4	71.7	78.1	10-Sep
098/2013/23	5.2	386	1.06	57.1/30.0	4	15	24.8	1281	6.4	0.8	79.8	87.3	ND
098/2013/25	10.3	154	1.08	36.7/29.4	2.8	8	40	1188	9.8	3.2	52.4	71.1	26-Sep
098/2013/31	11	249	0.85	31.2/30.6	5.1	6	10.9	378	3.8	1	75.6	80.9	17-Oct
098/2013/34	6.6	361	1.27	32.7/30.5	4.4	8	52.3	1486	6.8	1.8	71.1	89.3	10-Dec
098/2013/36	8	95	0.83	20.3/30.7	1.4	4	19.3	536	4.8	2.3	59.2	62.8	ND
098/2013/39	13.4	343	0.96	26.7/30.0	1.9	ND	19.2	161	7.7	1.8	66.9	72.7	31-Oct
098/2013/40	7.8	411	1.14	28.4/28.7	1.8	ND	4.9	131	6	1.3	72.1	78.1	ND
098/2013/41	9	268	0.9	28.3/29.6	3.2	4.5	26	749	10.5	1.5	71.1	78.1	05/11, 07/11
098/2013/42	15.1	308	0.95	25.9/29.0	2.8	ND	8.7	145	8.3	2.9	36.5	66.1	31-Oct
098/2013/43	6.9	399	0.95	30.6/30.3	5.2	9	45.5	1334	6.9	2.4	67.6	86.2	5-Nov
098/2013/44	9.1	375	1.01	31.6/29.6	2.9	ND	7.7	189	11.9	2.5	58.5	69.4	5-Nov
098/2013/45	8	863	1.19	41.4/30.2	3.8	8	41.2	1161	6.8	1.5	73	89	17-Dec
098/2013/103	6	271	1.05	31.5/28.9	2.5	6.5	17.8	643	4.9	1.1	75.3	69.4	10-Apr
098/2013/105	7.7	647	1.05	31.9/30.6	6	5	26.7	923	7.9	1.2	74.6	85.3	20-May
098/2013/106	8.2	406	1	27.3/31.0	4.6	ND	21.3	207	7.2	1.2	74.6	82.3	3-Jul
098/2013/107	6.6	134	1.05	34.3/33.8	3.6	10	6.5	373	6.4	1.4	69.9	72.8	10-Jul
098/2013/108	5.3	187	1.49	34.9/33.8	2.6	10	20.6	570	7.1	1.2	75	74.5	10-Jul
098/2013/109	9.3	221	0.98	35.2/33.8	4.5	3.5	30.2	723	11.1	2.4	59.2	76.4	ND
098/2013/110	9.5	557	1.17	ND	5.4	9	35	615	6.2	0.9	78.7	85.4	24-Jul
098/2013/111	11.9	147	0.96	34.5/31.5	4.8	5	29.1	597	8.2	1.8	65.8	67.8	24-Jul
098/2013/112	10.8	329	0.86	28.0/33.3	4.2	ND	14.8	112	5.2	0.8	78	76.9	24-Jul

Participant	Intem				Extem				Fibtem				Aptem			
	CT	CFT	Angle	MCF	CT	CFT	Angle	MCF	CT	CFT	Angle	MCF	CT	CFT	Angle	MCF
098/2013/01	153	25	77	85	41	32	70	83	43	38	37	84	45	30	76	84
098/2013/02	222	34	77	83	61	35	81	83	52	31	47	84	58	40	81	82
098/2013/03	193	42	75	82	54	35	80	83	45	35	37	84	47	34	78	83
098/2013/04	152	39	73	82	51	44	74	81	46	52	32	82	46	44	74	82
098/2013/05	156	28	82	84	49	25	85	85	46	23	52	85	53	25	82	85
098/2013/06	149	35	77	83	42	34	77	83	39	37	41	84	43	34	78	83
098/2013/07	240	51	70	80	75	45	71	81	66	72	30	78				
098/2013/08	157	40	64	83	38	39	67	84	37	37	32	85	38	37	68	85
098/2013/09	162	29	81	84	55	29	83	84	48	30	56	84	52	32	83	83
098/2013/10	172	38	80	83	87	35	80	83	65	48	40	80	63	45	80	81
098/2013/11	152	45	73	81	54	53	75	80	51	71	29	80	58	52	72	79
098/2013/12	135	39	80	82	67	41	78	82	53	41	59	82	57	44	77	81
098/2013/13	125	33	79	83	68	34	77	83	47	30	40	85	60	34	78	83
098/2013/14	151	27	82	85	52	28	82	84	45	23	58	85	46	24	82	85
098/2013/15	171	31	77	84	58	34	78	83	53	38	36	82	50	34	79	83
098/2013/16	175	44	71	81	63	37	73	82	61	39	38	83	25	71	73	77
098/2013/17	145	44	69	81	72	46	71	80	48	41	37	84	51	43	70	81
098/2013/18	186	33	78	83	53	38	81	82	48	34	43	83	49	38	79	82
098/2013/19	137	68	68	76	53	81	69	74	49	246	21	74	68	70	66	76
098/2013/20	150	40	76	82	78	49	75	80	66	39	43	83	65	40	75	82
098/2013/21	194	34	73	83	57	36	77	83	56	45	31	82	63	45	73	81
098/2013/22	160	39	70	82	57	39	75	82	60	50	33	81	62	46	74	81
098/2013/23	155	24	81	85	49	28	82	84	47	21	54	86	53	22	81	85
098/2013/25	172	68	66	76	95	68	68	76	84	156	30	73	51	112	65	70
098/2013/31	119	24	79	85	88	35	81	83	78	30	47	84	99	34	80	83
098/2013/34	154	46	71	81	100	55	65	79	19	301	23	63	96	43	74	81
098/2013/36	110	126	56	70	76	145	56	65	68	nr	15	71	74	132	57	66
098/2013/39	177	49	62	80	50	59	66	78	47	1076	25	78	46	47	67	80
098/2013/40	156	44	71	81	59	46	74	81	52	67	28	78	27	73	6	78
098/2013/41	151	38	75	82	55	42	76	81	62	82	32	76	64	45	77	81
098/2013/42	230	64	68	77	47	58	72	78	53	323	22	77	69	55	73	79
098/2013/43	157	36	80	83	83	47	81	82	75	68	49	76	68	76	80	79
098/2013/44	169	35	73	83	65	39	78	82	58	59	32	79	93	48	78	81
098/2013/45	165	22	87	86	138	37	85	84	120	56	65	80	115	46	87	83
098/2013/103	140	59	67	78	48	64	67	79	80	nr	17	76	30	73	68	76
098/2013/105	141	38	84	84	68	40	86	83	66	44	58	81	64	39	86	83
098/2013/106	154	31	78	83	185	65	82	79	180	115	32	68	194	79	81	77
098/2013/107	170	52	70	81	159	54	73	79	126	122	29	69	106	74	74	76
098/2013/108	138	53	66	80	123	58	70	78	107	82	28	76	118	53	70	79
098/2013/109	161	45	71	82	122	61	75	78	104	81	30	76	108	68	76	77
098/2013/110	197	29	83	84	130	48	86	82	115	67	49	77	100	57	87	82
098/2013/111	210	28	80	81	111	71	70	76	111	113	29	71	106	69	70	76
098/2013/112	141	29	74	84	78	40	77	82	72	77	35	75	67	42	79	82

*ND = Not done

*as per renal biopsy workup protocol: bleeding time only performed if Creatinine > 300umol/L

098/2013/16: no fibrinogen result: incorrect test requested by the lab

098/2013/17: post-Bx scan revealed a surgical haematoma. Not for RRP/dialysis. Deceased 21/07/2013

098/2013/19: Bx cancelled as patient was found to have a left mononephric, horseshoe kidney on U/S

098/2013/23: Bx postponed x 2 (decreased Hb and prolonged BT). Was commenced on ARV's in ward, will need viral suppression prior to RRP. Renal team to f/u and decide if Bx indicated

098/2013/26: no immediate complications post-biopsy but small haematoma noted at lower pole. Stable at review.

098/2013/36: Bx postponed dt decreased plt count. Pt deceased 20/11/2013 (prior to attempt at Bx)

098/2013/40: Bx cancelled by Prof Swanepoel. Echogenic kidneys on U/S - no need to biopsy.

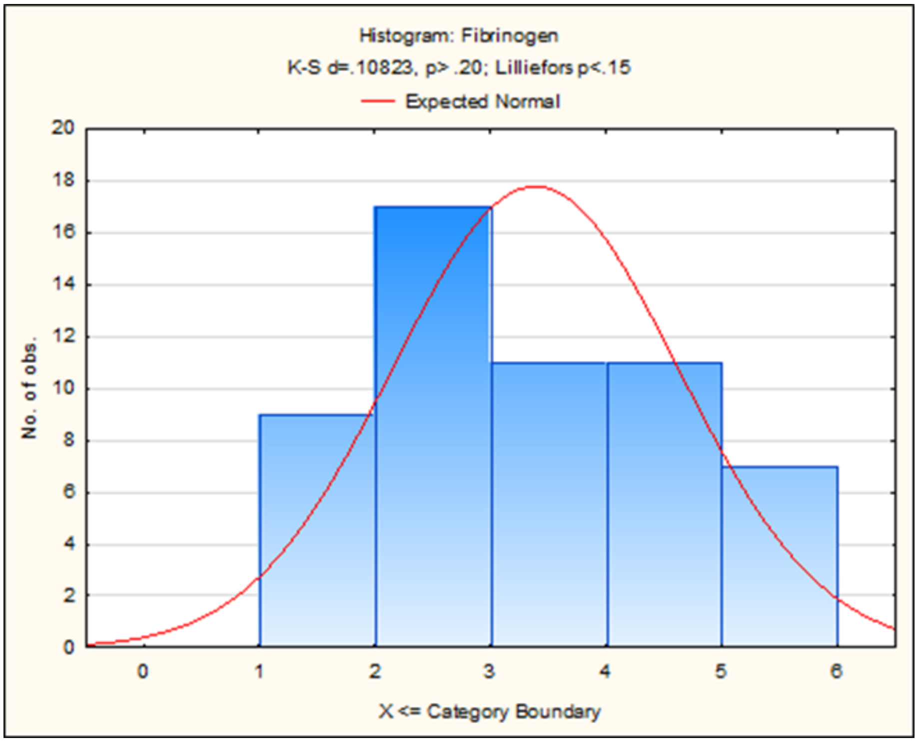
098/2013/41: Several attempts at Bx on 2 separate occasions. Unsuccessful on both attempts.

098/2013/42: Haematuria, flank pain post-Bx. Haematuria cleared. Perinephric haematoma L kidney on U/S. F/U U/S 11/11/13

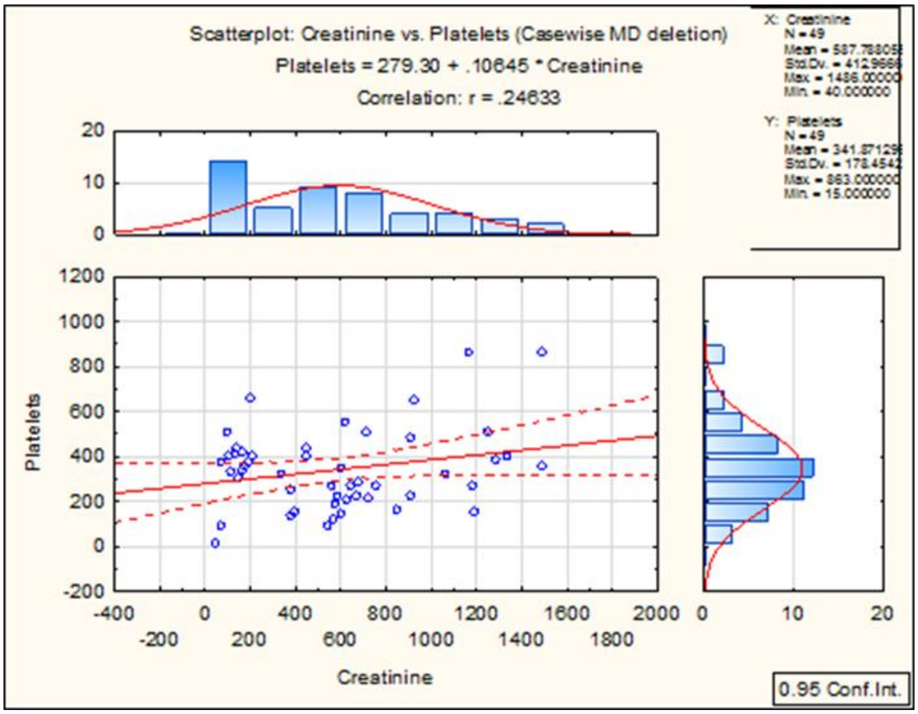
098/2013/101: No record of patient/No case report form

094/2013/104: No TEG results for this case

Addendum L: Fibrinogen graph



Addendum M: Creatinine vs Platelets graph



Addendum N: Creatinine vs Bleeding Time graph