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Maastricht University

Faculty of Medicine and Life Sciences
School for Life Sciences
Master of Biomedical Sciences

Master's thesis

The influence of catheter locks on thrombin and fibrin generation assays in haemodialysis patients

Britt Wouters

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences, specialization Molecular Mechanisms in Health and Disease

SUPERVISOR :

dr. Line HEYLEN

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The influence of catheter locks on thrombin and fibrin generation assays in haemodialysis patients

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*Running title: *Coagulation assays and catheter locks in dialysis*

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ABSTRACT

Background: Limited knowledge concerning coagulation in haemodialysis patients causes difficulty in managing their haemostatic disbalance. We investigate thrombotic influences in haemodialysis patients through fibrin and thrombin assays, as coagulation end-products. **Methods:** Trimonthly blood samples were taken from in-centre chronic haemodialysis patients. The thrombin generation (TG) assay adds tissue factor to whole blood samples to stimulate thrombin formation. Russel-Viper Venom factor X (RVV-X) was added to whole blood samples to evaluate fibrin formation over time. **Results:** Formation of fibrin (p=0.004) and thrombin (p=0.0008) was slower in patients with a catheter (n=28) than in patients with a fistula/graft (n=42). Additionally, peak height and total amount of thrombin were lower in catheter patients (p=0.003; p=0.0002). Comparison of citrate (n=16) to heparin catheter locks (n=12) showed heparin to delay fibrin and thrombin formation (RVV-X LT, p=0.0008; TG Lag time, p=0.007; TG time to peak, p=0.007). Heparin decreased TG velocity index and peak (both p=0.04), indicating lower thrombin generation efficiency and potential. A pairwise analysis of patients that switched from citrate locks to heparin locks, confirmed heparin's delaying effects on fibrin and thrombin (RVV-X LT, p=0.04; TG-LT, p=0.005; TG-TTP, p=0.0005). Heparin decreased efficiency (TG-VI, p=0.004), but not peak height (p=0.05). **Discussion:** Significant increases in time-related results indicate heparin catheter locks to have stronger delaying effects on coagulation compared to citrate locks. The lower TG peak and TG-VI indicate that heparin also has a stronger inhibitory effect on the maximal amount of active thrombin. The impact on catheter patency and bleeding risk needs further investigation.

INTRODUCTION

Disease and diagnosis

Chronic kidney disease (CKD) comprises the slowly progressive and irreversible loss in function and structure of the kidney (1). Chronic kidney disease is a worldwide leading cause of death and an estimation of 13.4% (11.7-15.1%) of the worldwide population suffer from CKD, with approximately 10.4% in men aged >20 years and 11.8% in women aged >20 years (2, 3). The worldwide number of patients is estimated at 843.6 million. This prevalence has increased substantially and, subsequently, mortality due to CKD has risen by 41.5% since 1990. Chronic kidney disease often presents as a secondary disease. Risk factors include, but are not limited to, hypertension, diabetes, smoking, obesity, older age, acute kidney injury, etc (4, 5). Moreover, diabetes mellitus type 2 (T2DM) is expected to be the most important risk factor for CKD, although the majority of CKD risk factors are closely related as comorbidities. The ongoing global increase of T2DM and obesity, among others, is an explanation behind the growth in CKD patients, in addition to improved screening and an elevated life expectancy (3). Chronic

kidney disease translates into an impaired filtration of excessive fluids and nutrients and waste products from the blood to the urine at the glomerulus, as the kidneys' functioning unit (6). The CKD-pathogenesis is divided into 5 stages, with stage 1 as least severe and stage 5 as most severe (5). The rate of glomerular filtration (Glomerular filtration rate, GFR) is used as a measure for kidney function and as a diagnostic tool to determine the stage (1-5) and prognosis of CKD in a patient (Figure 1). The estimation of a patient's GFR (eGFR) is done based on a calculation using creatinine in the patient's serum, since the actual measurement of GFR is expensive and extensive laborious (7). A healthy person's GFR approximates 100 to 125 ml/min/1.73 m^2 and decreasing GFR values indicate a higher, worsening stage of CKD (Table 1) (8). Stages 1 and 2 are often due to a gradual decrease of kidney function during the process of natural ageing and the asymptomatic presentation causes them to frequently go undiagnosed (9). Renal impairment is only limited and recommendations are to maintain a healthy lifestyle and monitor the kidney's further decline (4). Stage 3 is associated with an official CKD diagnosis and treatment includes more extensive monitoring (i.e. twice or trice per year instead of once). In addition, CKD increases the risk for development of electrolyte abnormalities, mineral and bone disorders, and anaemia, which requires further clinical screening and treatment (10, 11). Stage 4 is associated with fluid-build up in arms and legs and a referral to a nephrologist is recommended by KDIGO guidelines to plan renal replacement therapy (RRT) (10, 12). When progressing to stage 5 CKD, patients are diagnosed with end stage renal disease (ESRD) (8, 11). Global prevalence of the individual CKD stages (1-5) is calculated at 3.5% (2.8–4.2%; stage 1), 3.9% (2.7–5.3%; stage 2), 7.6% (6.4–8.9%; stage 3), 0.4% (0.3–0.5%; stage 4), and 0.1% (0.1–0.1%; stage 5) (2, 3).

Haemodialysis treatment

Diagnosis of ESRD (stage 5 eGFR <15 ml/min per 1.73 m2) concludes as severely impaired kidney function, in which the ability of the kidney to clear the blood from toxins, waste products, etc. is hampered. Renal replacement therapy is needed in case of progression to kidney failure (typically at eGFR around 7-9 ml/min per 1.73 m2), with a kidney transplant as the treatment of choice. However, during the waiting period for an available kidney, or in case of patients who are deemed unfit for kidney transplantation, patients are put on dialysis as RRT (12). During haemodialysis (HD), the patient's blood is run through an extracorporeal circulation, containing a filtration membrane. The membrane works as an artificial kidney and accounts for removal of accumulating uraemic products from the blood through diffusion to maintain the equilibrium of electrolytes in the patients' body. The purified blood then circulates back into the body (13). Access for HD to the body's blood stream happens through either a native arteriovenous fistula, an arteriovenous graft or placement of a central venous catheter. A fistula, as the most favourable access, is a surgical connection in the arm between an artery and a vein to create a loop in function of a proper circulation. With the change in pressure due to contact with the artery, the vein thickens to enable better punction. When the patients' veins are unfit for fistula-creation, a graft is placed as the functioning loop connection between vein and artery (14). Placement of a catheter is a less favourable choice of dialysis access, since there is a permanent connection between the external environment and the blood, increasing the risk for infection. Additionally to the connection to the external environment, the blood also interacts with the artificial structure of the catheter, which increases the risk for thrombosis (15). However, catheters are often temporarily placed when the surgically formed fistula is not yet developed enough for dialysis, or as definite treatment upon preference of the patient or when unfit for fistula or graft (14, 16).

Cardiovascular risk

Development of CKD increases the risk for development of cardiovascular disease (CVD) (17). Prevalence of CVD in patients without CKD diagnosis is 37.5%, compared to 63.4% (stage 1 and 2), 66.6% (stage 3) and 75.3% (stage 4 and 5) in patients with CKD. The CKD prognosis consortium established that the eGFR and the ratio of Urine Albumin-to-Creatinine in specific have a high correlation with CVD mortality. For example, patients suffering from stage 4 CKD and extreme albuminuria have a 9.49 fold higher risk for CVD mortality in comparison to CKD stage 1 patients with low albuminuria (18). Explaining mechanisms behind this association include the microvascular effects of hypertension and hyperglycaemia in T2DM patients, obesity, glomerular low-grade inflammation and anaemia due to erythropoietin deficiency (19, 20).

In extension to the increased prevalence of CVD and the increased risk for CVD mortality in CKD patients (e.g., due to age, hypertension, T2DM, anaemia, albumin concentration, etc.), these risks are further attenuated by undergoing HD treatment (18). Over 50% of dialysis patients suffer from CVD and approximately 40% of dialysis patients die due to cardiac death (21, 22). Moreover, a meta-analysis indicated the association between the duration of HD and the increased risk for cardiac death (23). Concretely, long-term dialysis patients have a 30 fold higher risk for CVD mortality in comparison to controls matched for age, ethnicity and gender (24). Additional risk factors for CVD in HD patients, compared to CKD patients without HD therapy, include vascular access, contact with biomaterials, haemodynamic stress due to friction and mechanical flow, overall vascular changes and fast removal of fluids, among others (19). Consequential to alteration in platelets and coagulation factors, HD patients often progress towards a pro-thrombotic profile through development and support of atherosclerotic plaques or thromboembolism formation. Unfortunately, these alterations in haemostasis also increase the risk for bleeding; patients with stage 5 CKD have an up to 3.5-fold higher risk for haemorrhages (25). Ultimately, 40-50% of HD patients are reported with bleeding (26). In addition to the dual and paradoxical tendency of HD patients for both coagulation and bleeding, the pathology of cardiovascular events also differs from the general population, causing conservative therapies to be ineffective in HD patients (25). For example, vitamin K antagonists are used as standard anticoagulation treatment in patients suffering from atrial fibrillation in prevention of stroke. Despite the significant and indisputable workings in the overall population, they showed to increase stroke and mortality and induced vascular calcification in the dialysis population (27). Moreover, the cardiovascular protective effect of statins disappears almost completely during HD treatment. In explanation, the atherosclerotic pathology happens through calcification of the media layer of the vessel, instead of cholesterol plaque build-up in the intima layer (28). Lastly, patients undergoing HD showed a higher aspirin resistance in their antiplatelet treatment than the general population, increasing the cardiovascular risk (29).

The effects of haemodialysis on the endothelium and platelet function

An explanation behind the disbalanced haemostasis is the retention of uremic toxins and inflammatory cytokines. Their build-up in plasma is caused by the impaired renal function and, due to their binding to albumin, they are too large for clearance through dialysis (30). The accumulation of these toxins (e.g., p-cresyl, indoxyl sulfate) influence platelet function, subsequently leading to generation of reactive oxygen species, loss of anti-oxidant mechanisms, presence of inflammation and formation of fibrosis. Fibrosis is an important mechanism in development and progression of vascular calcification in HD-specific atherosclerosis, vascular stiffness and congestive heart failure (31). Additionally, fibrosis and vascular calcification can also arise from direct inflammation in the presence of cytokines (e.g., interleukin-6). Indeed, elevated levels of inflammation predict increased vascular calcification and mortality in long-term HD treatment. Moreover, interleukin-6 is correlated to a higher blood viscosity and elevated number and activity of platelets and interleukin-6 inhibitors achieve reduction in biomarkers of thromboses in CKD patients (32). Furthermore, other accumulating uremic proteins (e.g., hippuric acid) have the ability to induce reactive oxygen species in the mitochondria of endothelial cells in the lining of the vessels, promoting oxidative stress and damage/necrosis in these cells (Figure 1) (31). Overall, the continuous state of micro-inflammation in long-term HD patients causes endothelial dysfunction. Consequential to this damage, tumour necrosis factor is activated and stimulates maladaptive endothelial vascular endothelial growth factor expression. This growth factor is highest in ESRD patients on chronic HD and the overproduction leads to altered angiogenesis in these patients (30). Additionally, dysfunctional endothelial cells exert a different control on platelets and their function.

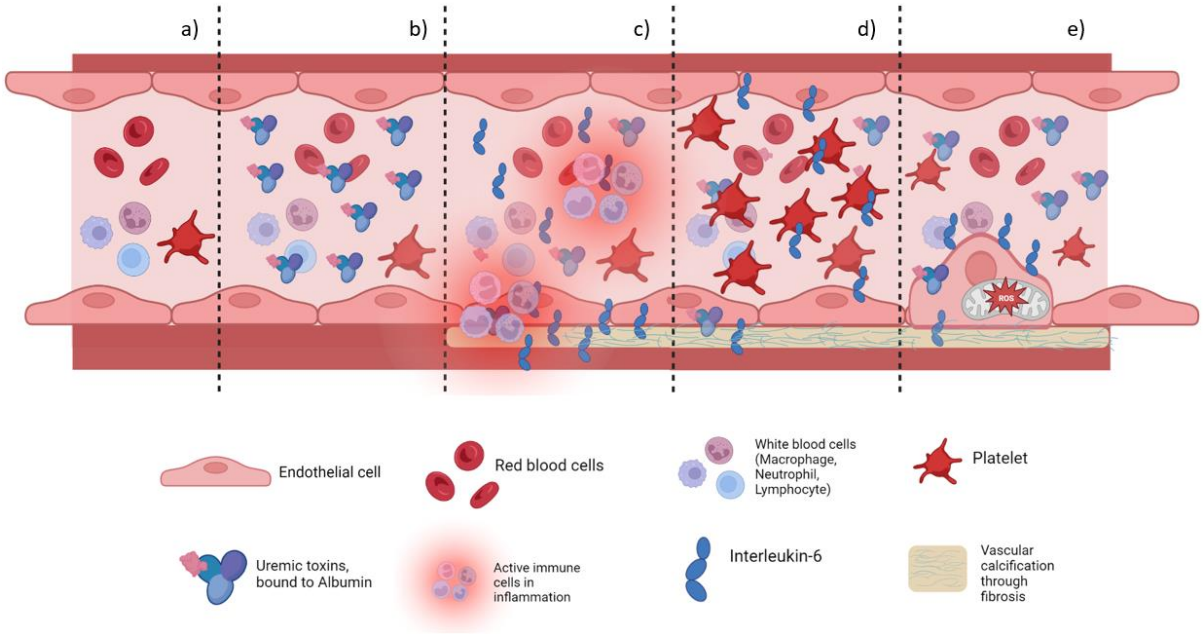

Figure 1: The effects of haemodialysis on the vascular structure in haemodialysis patients. *a) Reference normal vascular structure. b) Build-up of uraemic toxins in the blood of haemodialysis patients due to impaired renal function and their too large size for haemodialysis membranes. c) Inflammation and interleukin-6 cytokines cause vascular calcification in the media layer through fibrosis. d) Increased viscosity and increased amount of platelets via interleukin-6. e) Reactive oxygen species in mitochondria of endothelial cells, causing endothelial dysfunction and vascular damage.*

For example, Von Willebrand factor (vWF) appears to be elevated in HD, causing stronger stimulation towards platelet aggregation and formation of a stronger thrombus (25, 33). Damage to the endothelium also elevates levels of endothelial-associated tissue factor (TF), which initiates thrombin formation and activates extrinsic coagulation pathways (25, 34). Last, inflammation and uremic toxins exert direct effects on platelets by altering structure and subsequently affecting their function (32).

The effects of haemodialysis on the coagulation system

Due to uremic build-up in CKD patients, factors VII, VIII and TF are increased in plasma and promote higher thrombin activation and thrombus formation through factor X (FX) activation (Figure 2) (35). Tissue Factor specifically has an elevated expression in CKD patients due to the inflammatory nature of the disease and the inflammatory effect of uremic toxins. Another elevation in CKD patients is the concentration of D-dimers, which translates into a higher presence of blood cloths than normal, and higher vWF and fibrinogen itself (36). Despite the presence of uremic toxins as a consequence to kidney failure and incomplete filtration by HD, HD also alters coagulation properties in the blood. In explanation, the shear stress caused by the passing of blood through the extracorporeal circulation triggers the activation of coagulation cascades. Specifically, the HD biomaterials activate factor XII (FXII), which mediates a procoagulant and pro-inflammatory response through its contact system. First, active FXII activates factor XI (FXI). Factor XI then promotes proteolytic activation of factor IX (FIX) for formation of the intrinsic tenase complex between FIX and factor VIII (FVIII). FXI also activates factor X, which is essential for the production of thrombin (24). Thrombin then converts fibrinogen to fibrin for formation of the cross-linked fibrin matrix in mature blood cloths (Figure 2). The combination of increased presence and increased activation of thrombin, fibrinogen and FXII subsequently leads to an elevated tendency for thrombus formation in dialysis patients (34). On top of fibrin formation, thrombin is also functional in downstream modulation of coagulation pathways and activation of protease-activated receptor-1 for release of vWF and other adhesion molecules (e.g., E-selectin, P-selectin) (24, 34). In specific, intracellular cell adhesion molecule-1 and vascular cell adhesion molecule-1 are involved in the rolling, adhesion and activation of platelets and leukocytes for thrombogenesis. Additionally, FXII is involved in reciprocal activation with kallikrein for production of bradykinin. Bradykinin acts as a pro-inflammatory mediator

and promotes angiogenesis (Figure 2). Overall, active FXII is elevated in HD patients and these increased levels are associated with adverse cardiovascular outcomes (e.g., myocardial infarction, coronary heart disease, atherosclerosis) and with venous thrombosis and stroke through products FXI and prekallikrein (24, 26). On top of the FXII-derived contact system, HD-associated shear stress also activates the innate complement activation immune system in the blood of HD patients. For example, C3 of the complement activation system is significantly increased during the first 30 minutes of dialysis. C3 itself promotes a denser cloth structure, but also increases the micro-inflammatory profile in HD patients. These results also indicate an association between CVD risk and complement activation. Therefore, all systems are closely correlated in a vicious circle of vascular damage (24).

Problem statement and perspectives

Treatment of CVD and its complications have been significantly improved in the general population, but these benefits have not yet been established in HD patients (22). Especially due to limited knowledge concerning specific coagulation an thrombotic tendencies of this population and their dual profile of clothing and haemorrhaging. The difficulty in coagulation studies in HD patients up till now is foremost the complexity of underlying comorbidities, disorders and factors behind their development. Other drawbacks include the exclusion of specific population factors, a small sample size and the difficulty in measuring these changes (24, 25). Additionally, measurements in presence or changes of coagulation factors mostly have been done during the course of a dialysis session or before and after the first dialysis session and do not evaluate the long-term effects of chronic HD treatment (24, 37). With our longitudinal design, we hope to provide new perspectives on these long-term effects. Moreover, with the use of a thrombin generation assay, we aim to provide insights in the thrombotic tendency and discourse in HD patients, instead of only the presence of coagulation factors and platelet count. Lastly, from inclusion of platelet-based assays, we hope to gain more insights on the role of platelets in the haemostatic disbalance of haemodialysis patients.

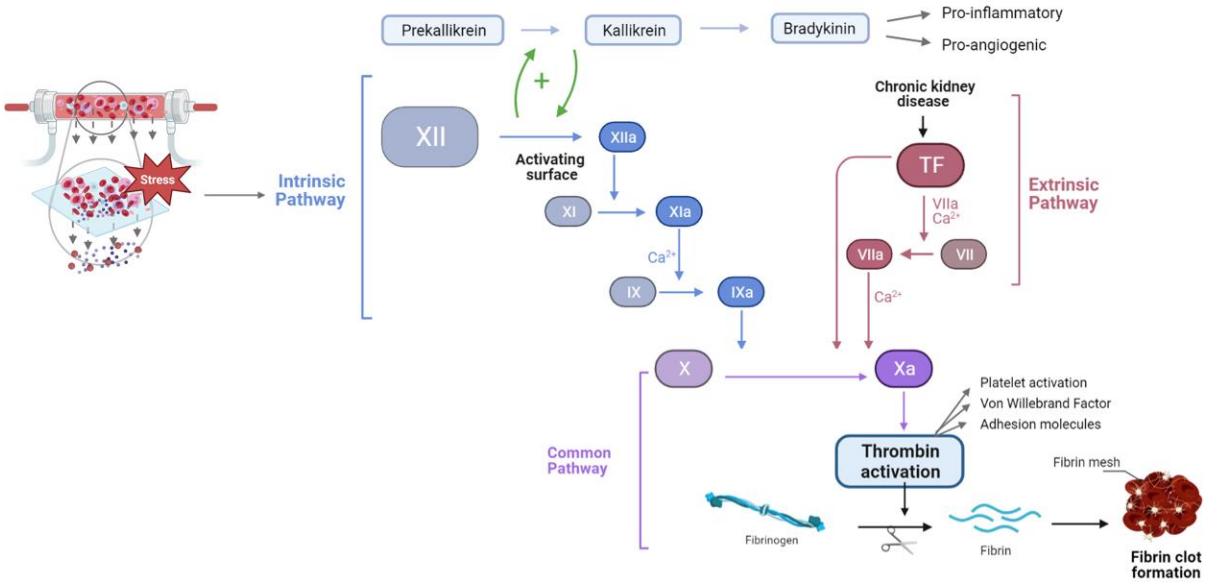

Figure 2: Activation of coagulation systems in haemodialysis patients by shear stress and friction by the biomaterials in tubes and membranes in the haemodialysis machine, focussed on the factor XII derived contact activation system. *Coagulation factors indicated by Roman numerals, Ca^{2+}, calcium; TF, Tissue Factor.*

EXPERIMENTAL PROCEDURES

Study design – The COTCH study is a monocentric, prospective, longitudinal cohort study conducted in Ziekenhuis Oost Limburg (ZOL), Genk (Belgium), by the nephrology department. The study protocol was designed by the principal investigator, Dr. Heylen, L. The study design was approved by the medical ethics committee of ZOL in November 23, 2021 and started March 2022. The aim of the study is to evaluate blood coagulation and thrombotic tendencies and link these to cardiovascular outcomes to evaluate the effect of CHD treatment on the thrombotic profile and risk for CVD. Study results were not used to guide clinical care of haemodialysis patients.

Study population – The patient population consists of patients undergoing CHD treatment at ZOL Genk. All patients treated with CHD in March 2022 were invited to participate (Figure 3). Chronic haemodialysis patients at other locations, home dialysis patients or patients who undergo haemodialysis in the evening shift at ZOL were excluded from the study due to practical measures. For patients who started CHD treatment at ZOL during the course of the study, information about the study was provided at the consultation before start of CHD treatment. Patients who started haemodialysis because of acute kidney failure were not eligible to be included in the study. Nevertheless, if the kidney failure persisted and haemodialysis treatment became chronic, patients could be invited for follow-up blood samples. Other exclusion criteria included the inability to provide informed consent. For the patient population to optimally represent the clinical situation, there were no exclusion criteria based on comorbidities or administered medication. Patients were removed from the periodic sample collection treatment if haemodialysis treatment was stopped (e.g., in case of kidney transplantation, or recovery of kidney function) (Figure 3). If desired, patients are free to be removed from the study without consequences.

Sample collection - Every twelve weeks (three months) during an organised, periodic sample collection at predefined dates, a blood sample was taken. From *de novo* ESDR patients who started CHD during the study, an additional baseline blood sample was taken at the start of their first HD session. When a participant underwent a kidney transplantation, a follow-up blood sample was taken three months after transplantation (Figure 3). All blood samples were drawn before starting the HD session. The vascular dialysis access was used for sampling to limit excessive puncturing. In case of catheter access, the catheter was flushed after removal of the lock according standard of care. Blood samples were analysed by the Synapse Research Institute (Maastricht, The Netherlands).

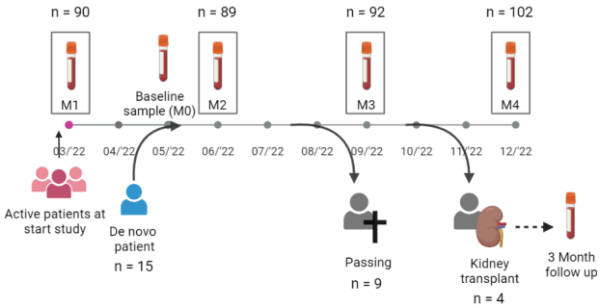

Figure 3: Study design of the COTCH study. *M, measurement.*

Sample processing

Thrombin generation: Thrombin Generation (TG) was performed on whole blood through semi-automated tests on the Calibrated Automated Thrombinography assay to determine thrombotic risk and influences of thrombotic and coagulant factors. Plasma was put into a 96-well plate. The wells were provided with recombinant tissue factor (TF) for stimulation of thrombin and fibrinogen formation through factor X-activation and calcium was added to the sample for TG activation (Figure 2). By treating the substrate for with fluorescent protein complementary to thrombin, the amount of thrombin formed was monitored for 60 minutes, with a measurement every 10 seconds. Factors obtained from thrombin generation were endogenous thrombin potential (ETP), peak height, lag time, time to peak (TTP) and velocity index (VI). Endogenous thrombin potential was the total amount of activated thrombin during TG. The peak height was considered as the maximal amount of thrombin active at the same time during TG. Lag time concluded the time needed until the first thrombin generation. Velocity index (VI) was calculated as the amount of thrombin at peak over the time needed to reach this peak (Figure 4) (38, 39). For every patient, TG was executed three times and final values were calculated as average over the three measurements. Normal values of a healthy control population are TG-ETP=1955±485 nM*min; TG-TTP=10.4 min (10.1-12.0 min); TG-Lag time=4.3 min (3.8-5.0

min); TG Peak=196±56 nM. For this thesis, we only had the summary statistics, not the raw data of a healthy population. Therefore, we were not able to investigate the difference between the haemodialysis patients and healthy individuals. Samples which were not able to generate thrombin, were given dummy values to be included in the statistical analysis. Replacement values were based on cut-offs during measurements and conclude TG-ETP=0 nM*min; TG-TTP=60 min (i.e., the maximum time of the test); TG-Lag time=60 min; TG Peak=0 nM; TG-VI=0 nM/min.

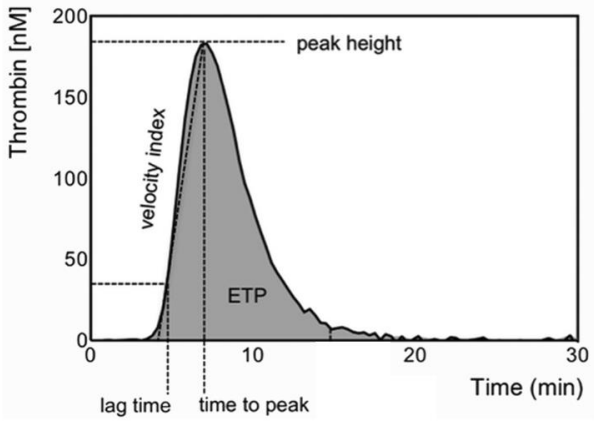

Figure 4: Variables evaluated during the Thrombin Generation assay, with activation by tissue factor and administered calcium at 0 minutes and 0 nM thrombin. *ETP, endogenous thrombin potential; nM, nanomolar; min, minutes (40).*

Platelet-assay TRAP-6: Thrombin Receptor Activator for Peptide-6 (TRAP-6) was added to the whole blood sample to selectively activate Protease-Activated Receptor-1 (PAR-1) to mimic thrombin-mediated PAR-1 activation. After stimulation of the pathway, platelet aggregation is measured in relative light units (RLU), as proportional quantification of present ATP (41). Measured variables in the TRAP-6 assay were Maximal Luminescence (MaxLum, RLU) as the peak amount of activated platelets and Velocity (RLU/sec) as the rate of platelet activation (42).

Platelet assay RVV-X: Russel Viper Venom-Factor X (RVV-X) initiates cloth formation through activation of factor X after being added to the whole blood sample (43). In addition to RVV-X, a fixed amount of calcium was added to the sample as mediator of the coagulation pathway (44). Clotting times were then recorded based on the formation of fibrin out of fibrinogen and represented as Lag time and time to Vmax (TTV). Lag time is the period between addition of RVV-X to the sample and the first cloth formation. Time to Vmax is evaluated as the time period needed to reach full saturation of the sample by RVV-X and represents the reaction rate. Replacement values for samples which were not able to generate fibrin, conclude RVV-X lag time=1800; RVV-X TTV=1800.

Data collection - For pseudonymisation, all participants were assigned a study code (e.g., COTCH_0001), which was only accessible for primary investigators in function of patient tracking and was not shared during sample processing or analysis. After enrolment, the participant's demographics and clinical background information were collected from the electronic patient database, HIX, used in ZOL. Collected information at enrolment included patient's age, BMI, gender, presence of T2DM, CVD, arterial hypertension, active smoking, liver failure, active malignancy, history of peritoneal dialysis or kidney transplant (Table 1a). Documented information at each participated blood sample collection included time in dialysis from start up until the measurement (days), dialysis technique, dialysis access, catheter lock and the prescription of anticoagulant and antiplatelet medication (Table 1b). For aggregation of the participant's pseudonymised code, background information, outcome events and results during sample collections, the electronic data capture system Castor was used. Registration, incorporation and maintenance of data was appointed to the masters student under supervision of the primary investigator. Blood samples were biobanked at University Biobank Limburg (UBiLim) in case of permission by the participant.

Statistical analysis – Shapiro Wilk tests were used to check continuous variables for normality. In case of normal distributions, results were presented as mean±standard deviation and tested with Pearson correlation or student T-test. Variables non-compliant to normality were presented as median (quartile one – quartile three) and were tested with Spearman correlation or Wilcoxon signed rank. Distribution of categorical variables was tested with a Chi square test. Statistical significance was determined with R Studio as $p<0.05$.

RESULTS

Patient population

Up to measurement (M) 4 (20/12/2022), 125 patients were enrolled into the COTCH study. Due to circumstances, four patients did not participate in any of the organised measurements and were excluded from the analysis. The liver is responsible for production of proteins involved in haemostasis and coagulation pathways. Therefore, five other patients were excluded from this analysis due to liver disease. 93 of the remaining 116 patients were enrolled at the start of the study, of whom 90 participated in M1. Based on previous literature and observation in own analyses, patients on anticoagulant medication between dialysis sessions were excluded to limit bias on results of coagulant assays. In specific, during M1, six patients were excluded for vitamin K antagonist treatment and 14 other patients took low molecular weight heparins in addition to CHD (Figure 5).

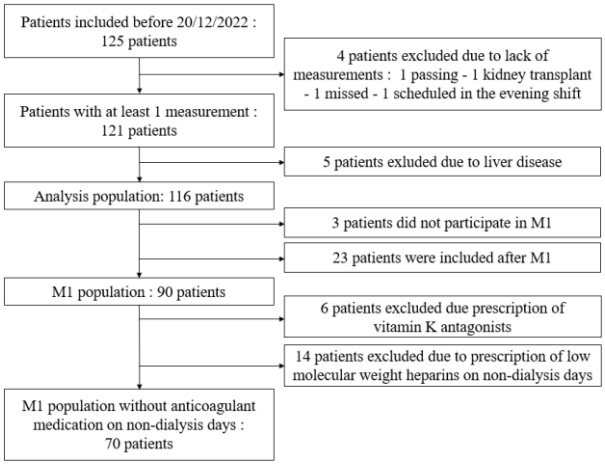

Figure 5: Flowchart of exclusion and final inclusion of patients from the COTCH-study in this analysis.

Of the total analysis population (n=116), 71 (61%) of participants were male. The median age and BMI at inclusion were 76.5 years and 25.4 kg/m^2 respectively. 18 (16%) participants actively smoked at time of enrolment. 80 (69%) and 49 (42%) participants had CVD and T2DM, respectively, at the time of inclusion. Of the 80 participants with CVD, 9 patients were only diagnosed with atrial fibrillation and 67 patients presented with atherosclerotic disease. Atherosclerotic disease concludes diagnosis with at least one of the following; coronary disease, cerebrovascular, peripheral or abdominal vascular disease. The majority of the population (91%) suffers from arterial hypertension (Table 1a).

Table 1a: Cohort demographics. *Kg, kilograms; m, meter; BMI, Body Mass Index; PD, Peritoneal Dialysis; NTX, kidney transplantation.*

	Population (n=116)
Age (years, median (Q1-Q3))	77 (68-82)
BMI (kg/m^2, median (Q1-Q3))	25.4 (22.2-29.8)
Gender (male, n (%))	71 (61%)
Active smoking (n (%))	18 (16%)
CV disease (n (%))	80 (69%)
Coronary disease (n (%))	48 (41%)
Heart failure (n (%))	22 (19%)
Cerebrovascular disease (n (%))	23 (20%)
Peripheral, abdominal disease (n (%))	31 (27%)
Atrial fibrillation (n (%))	30 (26%)
Arterial hypertension (n (%))	105 (91%)
Diabetes mellitus (n (%))	49 (42%)
Active malignancy (n (%))	3 (3%)
History of PD (n (%))	4 (3%)
History of NTX (n (%))	8 (7%)

There are no significant distributional differences in dialysis variables between measurements. M1 included 90 participants, M2 and M3 respectively included 89 and 92 participants, and M4 102 participants. Dialysis time was described as the number of days on dialysis between start of treatment and participation to the sample collection. On average over all measurements, 16% of participants were treated with hemodiafiltration compared to conventional haemodialysis, and 43% of participants used a catheter as dialysis access instead of fistula or graft. In the catheter group, citrate catheter locks were used more prevalent than heparin catheter locks. At the end of June 2022, a shortage occurred in urokinase (45). Urokinase is a thrombolytic agent, acting through activation and stimulation of plasmin towards fibrinolysis (46). It has been known to be safe and effective for breakdown of formed thrombi at the catheter site. Due to the depletion in urokinase reserves, patients with a catheter on citrate locks were forced to transfer to heparin locks, explaining the sudden increase in heparin locks after M2 (from 39% to 93%). The majority of patients (95%) were treated with low molecular weight heparins (LMWH, e.g., enoxaparin) as anticoagulant medication during dialysis and an average of 17% of participants received an additional LMWH-treatment at home on non-dialysis days.

Table 1b: Dialysis variables for each organised measurement. *M1, measurement 1 (March 2022); M2, measurement 2 (June 2022); M3, measurement 3 (September 2022); M4, measurement 4 (December 2022); HDF, Hemodiafiltration; LMWH, low molecular weight heparins.*

	M1 (n=90)	M2 (n=89)	M3 (n=92)	M4 (n=102)
Time since start haemodialysis treatment (days, median (Q1-Q3))	1021 (585-1715)	1076 (488 - 1798)	1067 (495 - 1719)	964 (304 - 1771)
Technique (HDF, n (%))	12 (13%)	11 (12%)	17 (19%)	18 (18%)
Vascular access (catheter, n (%))	41 (46%)	41 (46%)	42 (46%)	46 (45%)
Catheter lock (heparin, n (%))	15 (37%)	17 (42%)	40 (95%)	42 (91%)
LMWH on dialysis days (yes, n (%))	86 (96%)	85 (96%)	87 (95%)	96 (94%)
LMWH on non-dialysis days (yes, n (%))	14 (16%)	15 (17%)	15 (16%)	19 (19%)
Antiplatelet therapy (yes, n (%))	56 (62%)	56 (63%)	58 (63%)	65 (64%)
Vitamin K antagonists (yes, n (%))	6 (7%)	7 (8%)	4 (4%)	8 (8%)

On average, 7% of participants per time point were prescribed vitamin K antagonists as oral anticoagulant medication. The latter two groups were excluded from analysis, as anticoagulant therapy disturbs the coagulant assays and, therefore, causes skewed results (47) (Table 1b).

Comparing fistula to catheter dialysis access in the M1 cohort population

A significant difference in coagulation potential between types of vascular dialysis access was observed during analysis of M1 assay results. The catheter group (n=28) was compared to the group with graft and fistula combined, in whom blood was sampled through a direct vascular punction (n=42). First, patients with a fistula access achieved first fibrin formation after 552 seconds (515-624 sec) and patients with a catheter achieved this after 634 seconds (564-811 sec) (p=0.005) (Figure 6a). Additionally, the RVV-X TTV is higher as well in patients with a catheter access (738 seconds (630-909 sec)) than in patients with a fistula access (630 seconds (567-702 sec)). In translation, based on medians, patients with a catheter access needed more time to reach full saturation of fibrin generation after stimulation by RVV-X (p=0.004). This indicates a slower reaction rate for fibrin in patient samples through catheter access (Figure 6b).

This delay in coagulation was also observed in the evaluation of thrombin after triggering by tissue factor (TF) during the thrombin generation assay. Formation of the first thrombin molecule had a median of 3.8 minutes (3.4-4.2 min) for patients with a fistula and 4.3 minutes (4.0-5.0 min) for patients with a catheter. Hence, comparison of TG lag time medians shows a slower formation of the first thrombin molecule in patients with a catheter access than in patients with a fistula (p=0.0007) (Figure 6c). The time to reach the thrombin peak tended to be slower as well in the catheter group, but this did not reach statistical significance (fistula, 10.7 min (10.1-12.2 min); catheter, 12.0 min (10.7-12.9 min); p=0.09) (Figure 6d).

In addition to the delay in formation of thrombin, the ability to form thrombin also decreased in samples of patients with a catheter access instead of a fistula. The total amount of thrombin formed during the thrombin generation assay, shown in TG-ETP, decreased from 2394.5 nM*min (±618.6 nM*min) in fistulas to 1770.2 nM*min (±677.6 nM*min) in the catheter group (p=0.0003) (Figure 6e). Furthermore, the height of the thrombin peak was also lower in the catheter group (162.7±63.8 nM) compared to the fistula group (207.3±50.2 nM) (p=0.003) (Figure 6f). Lastly, the efficiency to reach this thrombin peak was shown in the velocity index. Samples from patients with a catheter access showed a decreased TG-VI (fistula, 29.4 nM/min (21.7-36.7 nM/min); catheter, 20.4 nM/min (17.9-30.7 nM/min)). In explanation, patients with a catheter access needed a longer time period to reach the same peak amount of thrombin or could generate a lower thrombin peak on the same time period as the patients with a fistula as dialysis access, but this did not prove to be significant (p=0.07) (Figure 6g).

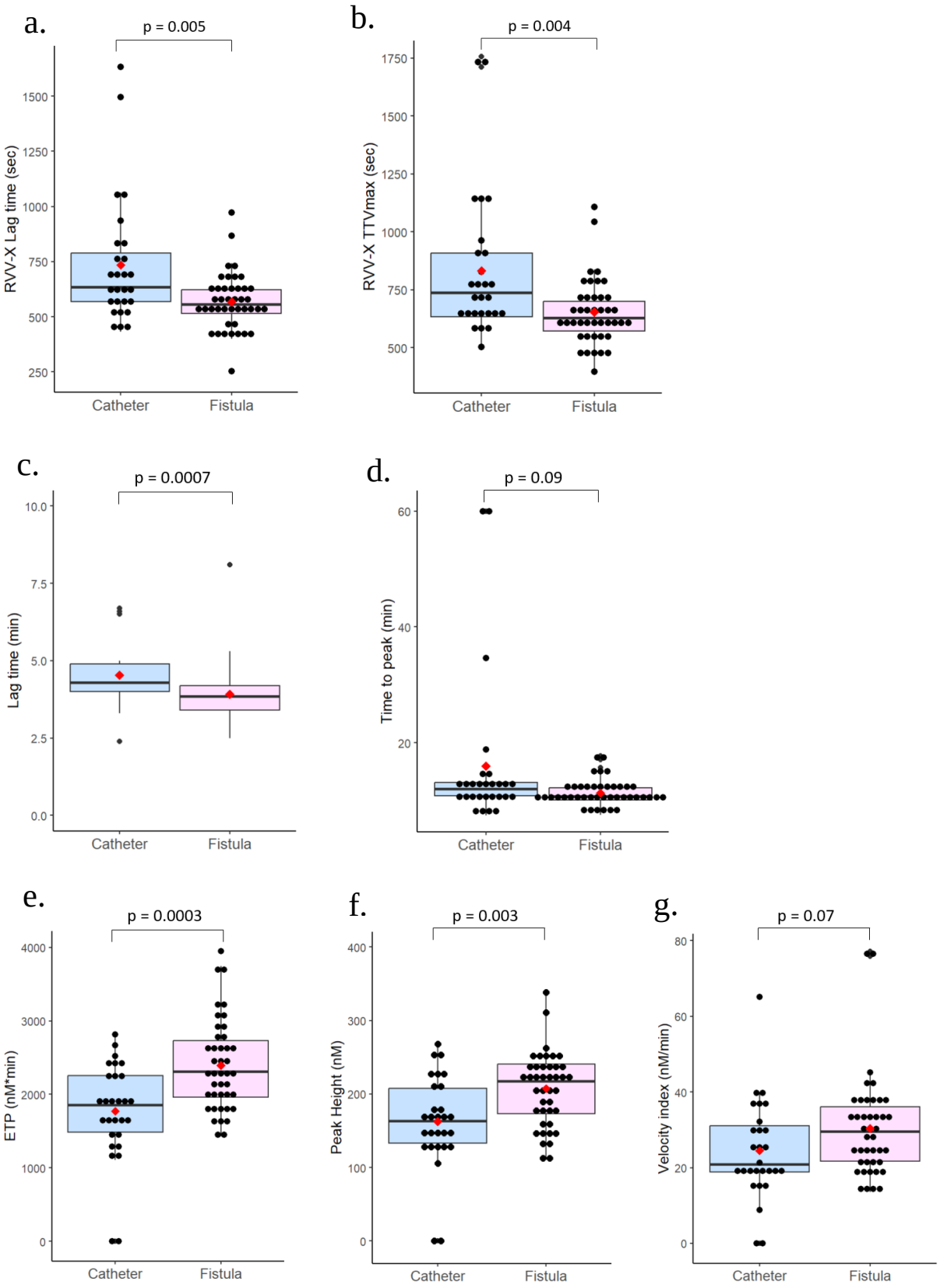

Figure 6a-g: Comparison of fibrin and thrombin generation in RVV-X and thrombin generation assays between catheter dialysis access (n=28) and fistula dialysis access (n=42)*. The group average is displayed as a red dot. a. Time until the first fibrin formation after RVV-X administration. b. Reaction rate of fibrin formation after RVV-X administration. c. Time until the first thrombin formation after triggering by tissue factor, presented in a boxplot in exclusion of extreme outliers for better visualisation of the effect d. Time needed to reach the peak amount of thrombin after triggering by tissue factor. e. Endogenous thrombin potential as the total amount of thrombin formed after triggering by tissue factor. f. Peak amount of thrombin formed after triggering by tissue factor. g. The peak amount of thrombin over the time needed to reach this peak after triggering by tissue factor. RVV-X, Russel Viper Venom-Factor X; TTVmax; time to Vmax; sec, seconds; min, minutes; nM, nanomolar.*

Figure 7a summarizes the overall thrombin generation of patients with a fistula access and patients with a catheter access, presented as averages of all measurements per group. It can be concluded for the catheter group to have a more limited thrombin generation, shown in a lower curve, and a slower thrombin formation, shown in the more postponed location of the curve. In addition, despite the delayed peak, the average curve of catheter patients is smaller compared to the curve of fistula patients. Therefore, the total period of thrombin generation appears to be shorter in catheter patients. However, the exact time period of thrombin generation was not quantified in this analysis. Additionally, the blue curve of catheter patients is extended further over time, while thrombin generation in fistula patients ends more abruptly (Figure 7a).

Despite distinctions in comparison of fibrin and thrombin generation based on dialysis access, it should be stressed that patients with a different vascular dialysis access often have different patient profiles. For example, all catheter-patients in our population suffered from atrial hypertension, while this occurred in 79% of the fistula population (p=0.02). Moreover, catheter patients were significantly older (fistula, 70 years (54-79 years); catheter, 83 years (71-87 years); p=0.0002).

Comparing citrate to heparin catheter locks in the catheter population of the M1 cohort

To limit the risk of thrombosis in the catheter lumen, an anticoagulant lock is provided between dialysis sessions. In our dialysis population, this lock is either based on citrate or on heparin as functional anticoagulant. We suspected this lock to be the cause of the difference in coagulation potential when evaluating the influence of dialysis access on coagulation. Subsequently, we examined the thrombin and fibrin generation profile of the heparin lock (n=12) in comparison to the citrate lock (n=16) in the M1 catheter group (n=28). When mapping the thrombin formation over time, a first glance indicates average thrombin formation to be lower, slower and less steep in patients with a heparin catheter lock (Figure 7b). Further statistical analysis on the TG variables was performed to validate these indications. The substantial delay between citrate and heparin was confirmed in comparison of medians of the lag time up until first thrombin formation. Patients on citrate form the first thrombin molecule 4.2 minutes (3.9-4.4 min) after addition of tissue factor, while this takes 6.5 minutes (4.2-6.7 min) in case of heparin catheter locks (p=0.007) (Figure 8c).

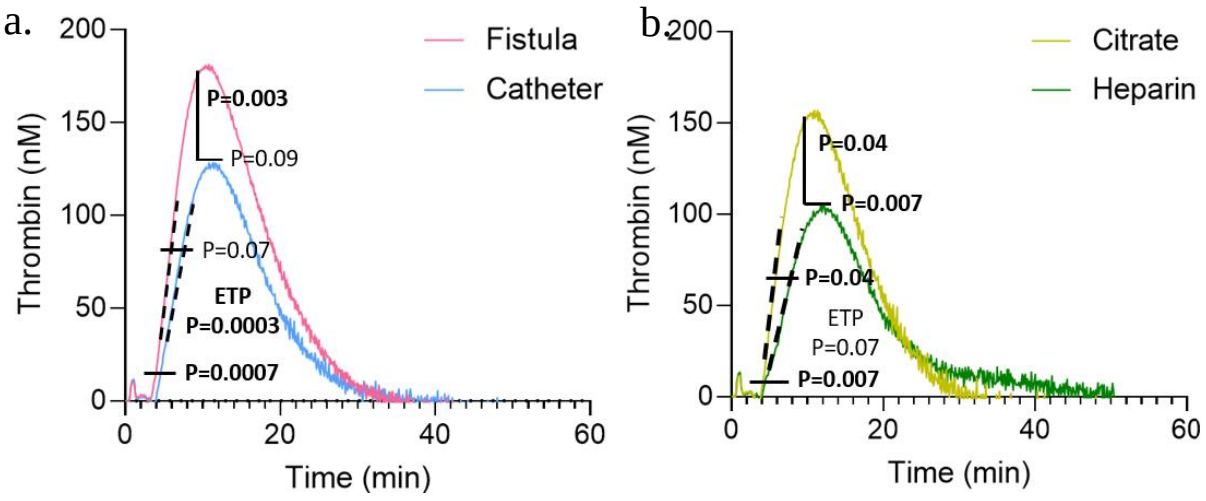

Figure 7a,b: Overall amount of thrombin generated over time after triggering by tissue factor, measured every 10 seconds and presented in averages of all measurements per subpopulation*. a. Comparison of patients with a fistula dialysis access to patients with a catheter dialysis access. Comparison of all patients with a catheter dialysis access, divided based on type of catheter lock, either heparin or citrate. nM, nanomolar; min, minutes; ETP, endogenous thrombin potential.*

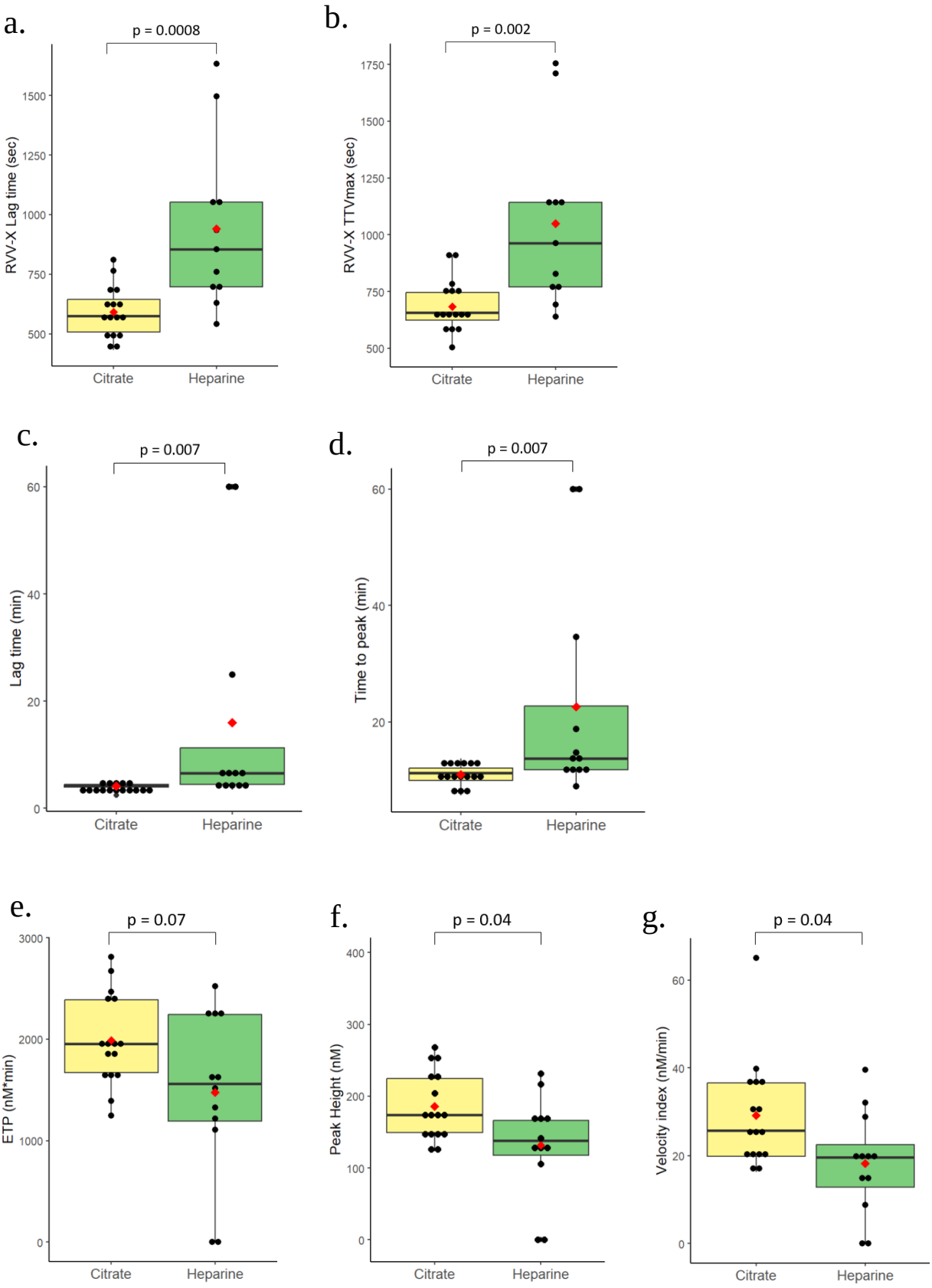

Figure 8a-g: Comparison of fibrin and thrombin generation in RVV-X and thrombin generation assays between citrate catheter locks (n=16) and heparin catheter locks (n=12)*. The group average is displayed as a red dot. a. Time until the first fibrin formation after RVV-X administration. b. Reaction rate of fibrin formation after RVV-X administration. c. Time until the first thrombin formation after triggering by tissue factor. d. Time needed to reach the peak amount of thrombin after triggering by tissue factor. e. Endogenous thrombin potential as the total amount of thrombin formed after triggering by tissue factor. f. Peak amount of thrombin formed after triggering by tissue factor. g. The peak amount of thrombin over the time needed to reach this peak after triggering by tissue factor. RVV-X, Russel Viper Venom-Factor X; TTVmax; time to Vmax; sec, seconds; min, minutes; nM, nanomolar.*

The time needed to reach the thrombin peak is significantly delayed in case of heparin lock (citrate, 11.1 minutes (9.8-12.0 min); heparin, 12.9 minutes (11.2-18.8 min); p=0.007) (Figure 8d). The peak amount of active thrombin decreased from 185.8 nM (±47.1 nM) in the citrate group to 131.8 nM (±71.7 nM) in the heparin group (p=0.04) (Figure 8f). The TG VI decreased as well from 25.2 nM/min (19.5-36.5 nM/min) to 19.2 nM/min (8.8-20.4 nM/min) (p=0.04). In translation, samples from a heparin catheter lock had a lower efficiency to reach the thrombin peak due to the slower rate of thrombin formation (Figure 8g). All previously mentioned findings are correspondent with the indications expected from the overall thrombin generation assay (Figure 7b). However, the difference in total amount of thrombin formed did not prove to be significant. In specific, the decrease in total amount of thrombin formed, from 1991.4 nM*min (±449.8 nM*min) in citrate to 1475.3 nM*min (±827.6nM*min) in heparin, can only be observed in a tendency (p=0.07)(Figure 8e). A possible explanation could be the trail behind the TG curve in case of heparin, where the curve for the citrate group shows a more concise ending. Since thrombin generation appears to last longer in case of heparin, despite the lower VI, lag time and peak, this could possibly close the observed gap in total amount of thrombin formed.

Lastly, fibrin generation was also evaluated. The median time to form fibrin increased in the heparin group by approximately 1.5 fold in both lag time and time to Vmax after adding RVV-X to the sample (Figure 8a,b). Concrete, the formation of the first fibrin fibres takes significantly longer in the heparin group compared to citrate lock (citrate, 574 seconds (497-634 sec); heparin, 854 seconds (689-1063 sec); p=0.0008) (Figure 8a). In addition, heparin slows down the fibrin formation by postponing enzymatic saturation (citrate, 657 seconds (603-738 sec); heparin, 963 seconds (765-1152 sec); p=0.002) (Figure 8b). Thus, in addition to lowering the capacity for thrombin generation, heparin also decreases the ability to form fibrin for coagulation.

Pairwise comparison of citrate and heparin catheter locks

Although there were no significant differences observed in population characteristics between the two groups based on catheter lock, bias in results could be due to underlying medical interpersonal differences. In June 2022, the nephrology department was confronted with a shortage of urokinase, a thrombolytic agent used in catheter thrombosis or flow irregularities. Therefore, patients provided with a citrate-based catheter lock were obliged to switch to a heparin-based catheter lock after M2. The nephrologists expected better patency of catheters with heparin and thus lower need for additional administration of urokinase-based thrombolytic agents in case of impaired catheter flow. As such, we were able to perform a paired analysis between citrate (M1) and heparin (M3). For further elimination of bias, we excluded patients who differed in antiplatelet medication between M1 and M3 (n=4).

After changing catheter lock from citrate to heparin, median RVV-X lag time increased from 564 seconds (462-575 sec) up to 771 seconds (491-1524 sec) (Figure 9a) (p=0.04). Additionally, samples from the heparin-group took longer to reach enzymatic saturation in the reaction for fibrin generation, indicating a slower reaction rate (citrate, 630 seconds (567-657 sec); heparin, 795 seconds (600-1452 sec); p=0.03) (Figure 9b). There was a significant increase in time needed for the first thrombin generation as well when switching from citrate (4.2 minutes (3.5-4.4 min)) to heparin (6.0 minutes (4.1-60.0 min)) (p=0.005) (Figure 9c). Furthermore, time to peak was slower after changing catheter lock (citrate, 11.4 minutes (10.0-12.1 min)); heparin, 14.5 minutes (11.1-60.0 minutes); p=0.03) (Figure 9d). The thrombin peak tended to be lower on heparin, but this failed to reach statistical significance (citrate, 185.1 nM (±52.8 nM); heparin, 127.7 nM (±100.2 nM)) (p=0.05) (Figure 9f). A decrease in total amount of thrombin generated, shown in TG ETP, could not be significantly proven (p=0.19). Lastly, the TG velocity index lowered from 24.6 nM/min (17.9-30.7 nM/min) to 19.0 nM/min (0.0-23.9 nM/min), indicating a decreased efficiency of thrombin generation after changing to a heparin catheter lock (p=0.004) (Figure 9g).

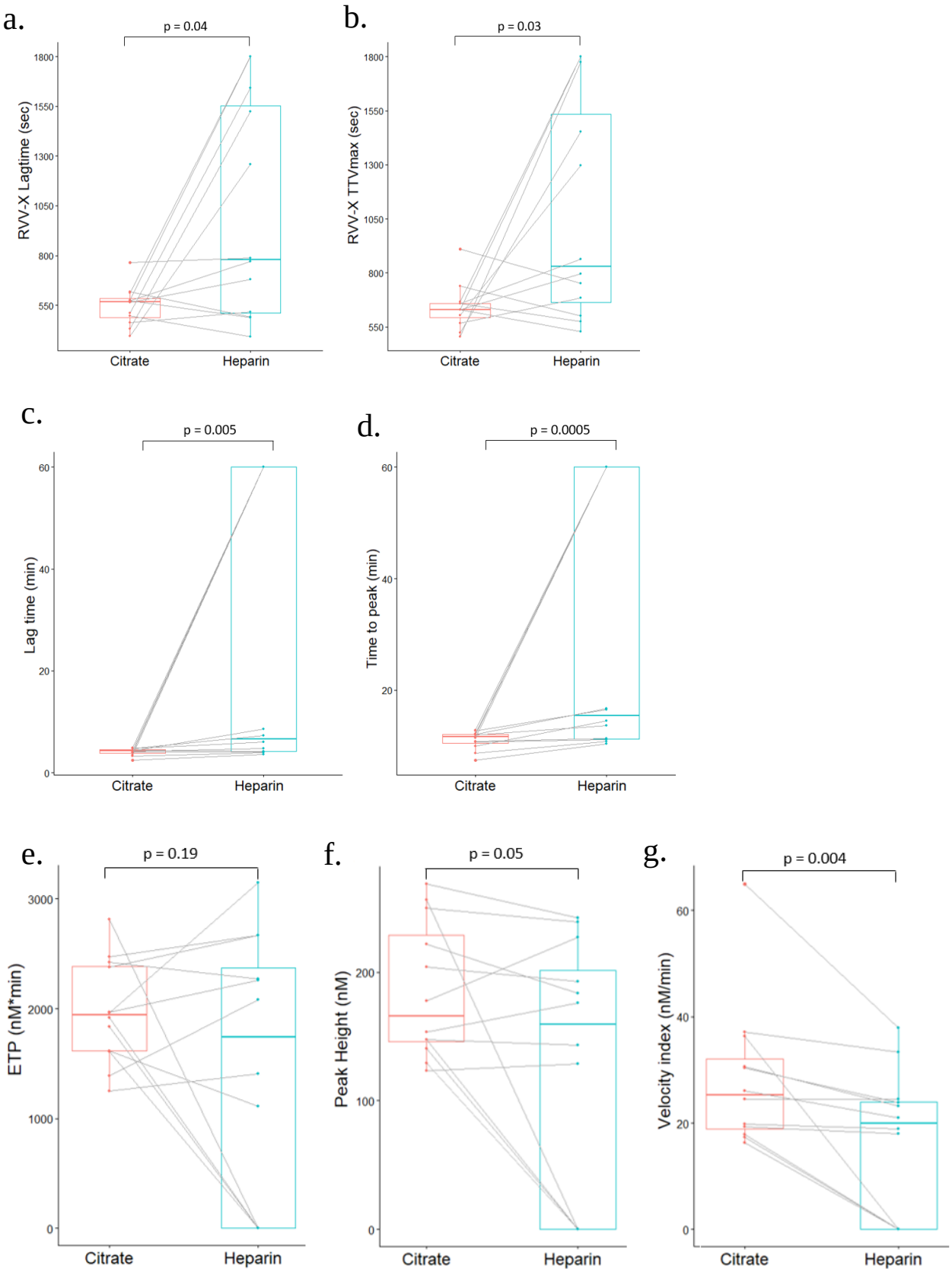

Figure 9a-g: Fibrin and thrombin generation in RVV-X and thrombin generation assays before and after changing citrate to heparin catheter lock due to urokinase shortage in June 2022 (n=12)*. The group average is displayed as a red dot. a. Time until the first fibrin formation after RVV-X administration. b. Reaction rate of fibrin formation after RVV-X administration. c. Time until the first thrombin formation after triggering by tissue factor. d. Time needed to reach the peak amount of thrombin after triggering by tissue factor. e. Endogenous thrombin potential as the total amount of thrombin formed after triggering by tissue factor. f. Peak amount of thrombin formed after triggering by tissue factor. g. The peak amount of thrombin over the time needed to reach this peak after triggering by tissue factor. RVV-X, Russel Viper Venom-Factor X; TTVmax; time to Vmax; sec, seconds; min, minutes; nM, nanomolar.*

Despite the difference in significances between the paired and unpaired analyses, the overlap in trends support the expectations of a stronger anticoagulant effect on thrombin and fibrin formation. In specific, previous analysis confirms the increase in RVV-X lag time, RVV-X TTV, TG lag time and TG TTP. Therefore, we can conclude that heparin specifically delays thrombin and fibrin formation. The effect on the amount of active thrombin, both in ETP and peak height, only appeared as tendencies and indicate a less decisive effect of heparin in comparison to citrate. However, the paired analysis only included 12 patients. Subsequently, a larger sample size should indicate whether the effect of heparin on thrombin peak height is significant.

Fistula patients as control group to evaluate the effect of catheter locks

The stronger anticoagulant effect of heparin compared to citrate can also be observed when using the fistula-group as a control during analysis of M1. When comparing patients with a heparin lock (n=12) to the fistula control group (n=42), heparin significantly affected all RVV-X and TG assay-results. Heparin delayed fibrin formation in RVV-X lag time (fistula, 552 seconds (515-624 sec); heparin, 854 seconds (689-1063 sec); p=0.00006) and slowed reaction saturation (fistula, 630 seconds (567-702 sec); heparin, 963 seconds (765-1152 sec); p=0.00008). Furthermore, heparin increased time to peak from 10.7 minutes (10.1-12.2 min) to 12.9 minutes (11.2-18.8 min) (p=0.003). Thrombin generation lag time was slower in patients with a heparin catheter lock compared to control (fistula, 3.8 minutes (3.4-4.2 min); heparin 6.5 minutes (4.2-6.7 min); p=0.0002). Heparin limited the endogenous thrombin potential with a decrease from 2394.5 nM*min (±618.6 nM*min) to 1475.3 nM*min (±827.6 nM*min) (p=0.003). Samples from patients with a heparin lock had a lower peak (fistula, 207.3 nM (±50.2 nM); heparin 131.8 nM (±71.7 nM); p=0.004) and a decreased velocity index (fistula, 29.4 nM/min (21.7-36.7 nM/min); heparin 19.2 nM/min (8.8-20.4 nM/min); p=0.007) (Table 2).

When comparing citrate catheter locks (n=16) to the fistula control group (n=42), the anticoagulant effects were limited. In specific, citrate only decreased the amount of total thrombin formed from 2394.5 nM*min (±618.6 nM*min) to 1991.4 nM*min (±449.8 nM*min) (p=0.01). An indication of a delay in TG lag time was observed when comparing median citrate locks to control, but this did not prove to be significant (fistula, 3.8 minutes (3.4-4.2 min); citrate, 4.2 minutes (3.9-4.4 min); p=0.07). There were no other significant effects of citrate observed on all other analysed assay results (Table 2). The more prominent significance of heparin to citrate on control groups could indicate heparin to have a stronger anticoagulation effects on thrombin and fibrin generation assays. This is in accordance with our previous findings.

Table 2: Statistical comparison of coagulation in citrate and heparin catheter locks to fistula dialysis access as control group. *TRAP-6, Thrombin Receptor Activator for Peptide-6; RLU, Relative Light Units; RVV-X, Russel Viper Venom-Factor X; TG, Thrombin Generation; ETP, Endogenous Thrombin Potential; TTP, Time To Peak; nM, nanomolar; sec, seconds; min, minutes*

Assay variable	Fistula (n=42)	Citrate (n=16)	P-value	Heparin (n=12)	P-value
RVV-X Lag time (sec)	552 (515-624)	574 (497-634)	0.43	854 (689-1063)	0.00006
RVV-X TTVmax (sec)	630 (567-702)	657 (603-738)	0.34	963 (765-1152)	0.00008
TG TTP (min)	10.7 (10.1-12.2)	11.1 (9.8-12.0)	0.99	12.9 (11.2-18.8)	0.003
TG Lag time (min)	3.8 (3.4-4.2)	4.2 (3.9-4.4)	0.07	6.5 (4.2-6.7)	0.0002
TG ETP (nM*min)	2394.5 ± 618.6	1991.4 ± 449.8	0.01	1475.3 ± 827.6	0.003
TG Peak (nM)	207.3 ± 50.2	185.8 ± 47.1	0.14	131.8 ± 71.7	0.004
TG Velocity index (nM/min)	29.4 (21.7-36.7)	25.2 (19.5-36.5)	0.68	19.2 (8.8-20.4)	0.007

Lastly, 29 samples over all four measurements (M1, 2/90; M2, 4/89; M3, 11/92; M4, 12/102) of our 116 included patients were not able to start the TG process at all, despite triggering by TF, and were assigned replacement values to be included in our analysis. The inability to generate thrombin did only occur in the heparin group and was fully absent in both the fistula-group or in the catheter-group with a citrate-based lock. Out of the 12 patients in the paired analysis, 4 were able to start thrombin generation on citrate and were not able to anymore after changing to heparin locks. The specificity of total inhibition of thrombin generation for heparin reveals an interesting tendency for further investigation.

The effect of citrate and heparin on platelet activation through a TRAP-6 assay

All samples were in addition analysed on platelet activation through the TRAP-6 assay to assess the role of platelet activation in the haemostatic disbalance of dialysis patients. After administration of TRAP-6 to the sample, which mimics thrombin, the amount of thrombin-like induced platelet activation is evaluated. When comparing maximal luminescence, the amount of observed RLU is proportionate to the amount of ATP used in platelet activation. In contrary to significances based on fibrin and thrombin, difference in dialysis access was not associated with differences in activation of platelets, based on results of the TRAP-6 assay (fistula, 0.21± 0.09 RLU; catheter, 0.20 ± 0.06 RLU; p=0.71). Moreover, both groups had an indistinguishable velocity in the activation of platelets as well (fistula, 0.0016±0.0006 RLU/sec; catheter, 0.0016±0.0005 RLU/sec; p=0.89). Subsequently, there is no difference in either the amount or rate of platelet activation based on type of dialysis access (Table 3).

The same analysis was performed when comparing patients with a citrate catheter lock to patients with a heparin lock in the M1 cohort. The citrate group appeared to have a lower amount of activated platelets (0.19±0.08 RLU) than heparin (0.22±0.05 RLU), but this did not prove to be significant (p=0.20). The velocity of platelet activation was almost equal between both groups (citrate, 0.0016±0.0006 RLU/sec; heparin 0.0016±0.0004 RLU/sec; p=0.90). However, when comparing patients' first measurement on citrate locks to the measurement after changing to heparin locks, different trends appear. The amount of activated platelets remained very similar (M1 citrate, 0.21±0.04 RLU; M3 heparin, 0.20±0.12 RLU; p=0.89). Therefore, there is no significant correlation between changing catheter lock and platelet activation. Nevertheless, the rate of platelet activation decreased from 0.0017 RLU/sec (±0.0006 RLU/sec) to 0.0013 RLU/sec (±0.0006 RLU/sec) after changing catheter lock. In translation, when on heparin, patients have a slower platelet activation than on citrate. Regardless, this decrease was observed as a trend and not as a significant difference (p=0.05) (Table 3). The variating results in analyses cause difficulty in forming a concise conclusion and could be explained by the low sample size (citrate, n=16; heparin, n=12; paired, n=12). Larger sample sizes might provide further insights in the effect of catheter lock on platelet activation in HD patients.

Table 3: Statistical comparison fistula to catheter and citrate to heparin to determine the effect on platelet activation through the TRAP-6 assay. *TRAP-6, Thrombin Receptor Activator for Peptide-6; RLU, Relative light units; sec, seconds.*

	TRAP-6 maximal luminescence		TRAP-6 velocity	
	RLU	P-value	RLU/sec	P-value
Fistula (n=42)	0.21 ± 0.09	0.71	0.0016 ± 0.0006	0.89
Catheter (n=28)	0.20 ± 0.06		0.0016 ± 0.0005	
Citrate (n=16)	0.19 ± 0.08	0.20	0.0016 ± 0.0006	0.90
Heparin (n=12)	0.22 ± 0.05		0.0016 ± 0.0004	
Citrate, M1 (n=12)	0.21 ± 0.04	0.89	0.0017 ± 0.0006	0.05
Heparin, M3 (n=12)	0.20 ± 0.12		0.0013 ± 0.0006	

DISCUSSION

Purpose statement

Haemodialysis patients are confronted with a severe disbalance in coagulation factors already present in ESRD patients. On top, this disbalance is aggravated through additional shear stress by the extracorporeal circulation and by contact with artificial materials (30). The COTCH study aims to provide deeper insights in the coagulation profile of haemodialysis patients for a more optimal treatment in prevention of thrombosis. During our exploratory analysis, we observed a significant difference in thrombotic tendencies between patients with a fistula or graft dialysis access and patients with a catheter dialysis access.

The difference in coagulation of patients on fistula access vs catheter access

Fistulas are the most preferred type of dialysis access due to the lowest infection rate and lowest amount of procedures required to maintain flow patency (48). A fistula is a high-resistance circuit between the arterial limb, through vessel dilatation, and the venous limb, through vessel restriction. However, the largest disadvantage to fistulae is the inability to mature. In specific, excessive thickening of the intimal layer in the venous limb after fistula placement can cause narrowing of the vessel and, subsequently, lead to stenosis. Stenosis, and the associated risk for thrombosis development, can complicate dialysis access and have adverse effects on the patients' health (49). Therefore, not all patients are provided with a fistula due to vascular structure; a meta-analysis performed on 13 studies showed that vascular calcification and endothelial dysfunction of older patients cause a 50-65% higher risk of primary fistula failure (50). This was confirmed in our patient population, with a significantly younger fistula population compared to the older patients with a catheter access. According to the KDIQO guidelines, a catheter can form a replacement dialysis access if the estimated dialysis period is below a year, if fistula creation is not possible or in case of patient's preference (51). The limited recommendations towards catheter use is consequential to the high risks for thrombosis and infections associated with the catheter itself. Thrombus formation gives rise to 30-40% of all catheter malfunctions and 2/3th of catheter failures (52). Moreover, catheter related right atrial thrombosis leads to an 18.3% mortality in the catheter population (53). This complication of thrombosis is most often caused by FXII interacting with the catheter surface, activating the intrinsic pathway, or contact between the catheter and the subendothelial layer. Exposure of the subendothelial layer to the catheter material activates FVII to bind TF in the extrinsic pathway. Subsequently, propagation and amplification of activated coagulation reactions lead to platelet activation and expression of glycoproteins for thrombin generation. Further research showed patients with four months of dialysis therapy through a catheter access to have higher thrombin generation compared to patients with a catheter for one month and compared to controls. In translation, patients with a longer dialysis period on catheter access had a higher activation of platelets and coagulation. Furthermore, the catheter favours stimulation of TF through focus on the intrinsic coagulation pathway, rather than the extrinsic pathway. The TG time to peak was slower in patients who already experiences a venous thromboembolism, indicating a possible recurrent thrombosis (52). Lastly, the increased amount of fibrin, with consequential fibrin deposits, promotes biofilm formation and can give rise to sepsis. In conclusion, patients with a catheter dialysis access show a higher thrombotic tendency (51).

However, our findings indicate that blood samples obtained through a catheter have a lower thrombotic tendency compared to samples obtained through a fistula, instead of a higher tendency. This was specifically shown in a significantly shorter time period for fibrin and thrombin generation (RVV-X lag time, RVV-X TTV, TG lag time) in the fistula population, compared to a slow generation in the catheter population. Additionally, the catheter population showed a significant lower peak in the maximal amount of thrombin and total amount of thrombin generated, as opposed to the expected higher amount of thrombin. Although our results might seem to contradict previous literature, the sample site can explain the observed differences; blood samples in previous literature were drawn from the peripheral vein of dialysis patients, while our samples were taken through the catheter access to limit the amount of punction for the patient. Additionally, literature showed the potential for thrombin generation to be lower when samples were taken directly from the catheter lumen instead of the peripheral vein (52). This confirmed our expectation for the catheter to have

an anticoagulant influence on our samples. In specific, the catheter is provided with an anticoagulant lock, either heparin-based or citrate-based, for prevention of thrombosis as complication. We expected this lock to be an explanation behind anti-thrombin tendencies of catheters in our analysis.

The effect of type of dialysis lock on fibrin and thrombin generation in blood samples through catheter access

Heparin exerts its anticoagulant effect through inactivation of FXa, therefore inhibiting the FXa-dependant conversion of prothrombin to thrombin. This inactivation is achieved through high-affinity binding to antithrombin. Moreover, heparin enhances the anticoagulant effects of antithrombin by 1000-fold. Subsequently, since coagulation is a self-enhancing cascade, the inhibition of thrombin also leads to limiting thrombin-induced activation of FV and FVIII (54, 55). The heparin-based lock is easily accessible and works very effectively in prevention of thrombosis and maintenance of catheter potency (15). However, its high efficiency is associated with an increased risk for bleeding and thrombocytopenia, in which the platelet count decreases notably. Additionally, heparin administration with a concentration higher than 5000 U/ml is correlated with an increased risk for bleeding complications. Lastly, high concentrations of heparin show to enhance biofilm production stronger than low heparin concentrations (51, 56). On contrary, citrate exerts its anticoagulant effect through chelation of ionized calcium to limit clotting in the catheter. Subsequently, it inhibits calcium-dependant steps in the coagulation system and prevents platelet activation. On top of its anticoagulant properties, it also exerts an antimicrobial effect, aiding in reduction of inflammatory complications associated with catheters (51). When citrate from the catheter lumen would leak into the peripheral blood flow, it is quickly metabolized by the liver and skeletal muscles. Therefore, leakage does not cause systemic anticoagulation, preventing the increased risk of bleeding as complication (56). Overall, citrate catheter locks are associated with a lower risk for excessive bleeding, demonstrated in a significant lower incidence of bleeding episodes in citrate compared to heparin (51). Lastly, patients with a citrate lock required fewer catheter replacements consequential to flow-related issues and had a decreased need for additional administration of anticoagulant medication compared to heparin (56).

The expectation for heparin to have stronger anticoagulant effects compared to citrate, based on previous literature, was confirmed in our findings. We observed heparin to have a delayed formation of fibrin and thrombin, shown in higher RVV-X lag time, RVV-X TTV, TG lag time and TG TTP. This sensitivity of RVV-X to anti-Xa and antithrombin drugs, e.g., heparin, has already been established with an increase of clotting time in a dose-dependent manner (43, 47). Furthermore, the amount of formed thrombin was more limited in case of a heparin catheter lock, both in total amount (cfr. TG ETP) and maximal amount (cfr. TG peak) of thrombin. Additionally, the efficiency to reach the thrombin peak, shown in velocity index, decreased in the heparin group, indicating an overall inhibiting effect. Lastly, this inhibiting effect was confirmed in further investigation of samples who were not able to generate thrombin at all; all samples were taken from patients with a catheter dialysis access and a heparin-based catheter lock. The inability to generate thrombin was not observed in either patients with a fistula or a citrate catheter lock. The decreased amount and slower formation of coagulation factors translate into a limited clotting potential.

We expect the difference in coagulation between citrate and heparin to be dependant of their target factor and pathway of inhibition. In explanation, administration of tissue factor in TG and RVV-X for fibrin generation are both in close interaction with factor Xa. The high affinity of heparin on the FXa pathway immediately neutralizes stimulation of FXa by stimulators RVV-X and TF. On contrary, citrate has a more regional anticoagulant activity by inhibiting calcium. The decreased activity of co-stimulator calcium in coagulation is then counteracted by TF and RVV-X administration, which activate factor X nevertheless (57). The expectance of factor X to be at the base of the significant difference can be confirmed by the absence of significant results in the TRAP-6 assays. Specifically, TRAP-6 is a thrombin-like molecule for activation of platelets and lack of difference between citrate and heparin, and catheter and fistula overall, can indicate that TRAP-6 administration compensates for the heparin-based inhibition of endogenous thrombin (42).

Limitations and future perspectives

To our knowledge, this is the first study to evaluate the effect of citrate and heparin catheter locks on thrombin and fibrin based coagulation assays. However, our study has several limitations. Firstly, due to unavailability of data of a healthy population, we were not able to provide a context of values from the haemodialysis population compared to controls. Therefore, we were only able to provide a comparison between patients with a fistula dialysis access and patients with a catheter dialysis access, on one hand, and within the catheter population on the other hand. Second, most analyses were based on small sample sizes. For the catheter population in particular this small sample size can be explained by our exclusion criteria; since the majority of patients excluded based on treatment with either vitamin K antagonists or low molecular weight heparins on interdialytic days, belonged to the catheter population. This, again, is associated with the differences in population characteristics between fistula and catheter, since patients with a catheter access are in general older and in a more severe medical condition. Subsequently, the catheter population has a higher need for more extensive medication. Lastly, the haemodialysis population is associated with a large range and close interaction of comorbidities. For a clear understanding concerning the influence of access and type of catheter lock on the thrombotic tendencies of the patient, a larger statistical model is needed. Hence, based on our results, we only discuss the influence of dialysis access and type of catheter lock on the results of the drawn blood sample. A next step in further investigation of the influence of catheter and catheter lock on coagulation assays could be to compare the coagulation profile of samples drawn from a peripheral vein to samples drawn through the catheter access, ideally of the same patient.

CONCLUSION

Our results indicate an effect of vascular access on thrombin generation in haemodialysis patients. More specifically, a heparin lock showed to have the strongest anticoagulant effect of the two types catheter lock, up until total inhibition of thrombin generation in some samples. As such, drawing a patient's blood sample through the catheter, especially when provided with a heparin catheter lock and despite adequate removal of the lock and flushing before sampling, still seems to be contaminated by the lock which might not represent the actual thrombotic tendencies of the patient. This should be accounted for in further research of coagulation in haemodialysis patients.

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Author contributions – **Who did what**? L.H. was responsible for study set-up, i.e., study design, requesting ethical approval and start-up of the study. Starting from 28/11/2022, B.W. was in charge of organisation of sample collections, monitoring patients for documentation of events and overseeing possible new inclusions. The Synapse Research Institute, with B.d.L. and M.N. in specific, was responsible for processing of blood samples and providing L.H. and B.W. with sample results. B.W. performed statistical analysis on these results and presented results and conclusions in this master's thesis.

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